UNIVERSIDADE ESTADUAL DE MONTES CLAROS

Otávio Cardoso Filho

Efeitos do resveratrol na caquexia associada ao câncer: ensaios *in vivo* no modelo tumoral murino singênico de melanoma cutâneo em camundongos C57BL/6.

Montes Claros
2018

Otávio Cardoso Filho

Efeitos do resveratrol na caquexia associada ao câncer: ensaios *in vivo* no modelo tumoral murino singênico de melanoma cutâneo em camundongos C57BL/6.

Exame de Defesa de Doutorado apresentado ao Programa de Pós-graduação em Ciências em Saúde da Universidade Estadual de Montes Claros - Unimontes, como parte das exigências para a obtenção do título de Doutor em Ciências da Saúde.

Área de Concentração: Mecanismos e Aspectos Clínicos das Doenças

Orientador: Prof. Dr. Alfredo Mauricio Batista de Paula

Coorientadora: Profa. Dra. Ludmilla Regina Souza

Montes Claros
2018

Cardoso Filho, Otávio.
C268e Efeitos do resveratrol na caquexia associada ao câncer [manuscrito] : ensaios *in vivo* no modelo tumoral murino singênico de melanoma cutâneo em camundongos C57BL/6 / Otávio Cardoso Filho. – 2018.
111f. : il.

Inclui Bibliografia.
Tese (doutorado) - Universidade Estadual de Montes Claros - Unimontes,

Programa de Pós-Graduação em Ciências da Saúde/PPGCS, 2018.

Orientador: Prof. Dr. Alfredo Mauricio Batista de Paula.
Coorientadora: Profa. Dra. Ludmilla Regina Souza.

1. Melanoma cutâneo. 2. Terapias. 3. Peso corporal. 4. Sobrevida. 5. Músculo esquelético. I. Paula, Alfredo Mauricio Batista de. II. Souza, Ludmilla Regina. III. Universidade Estadual de Montes Claros. IV. Título. V. Título: Ensaios *in vivo* no modelo tumoral murino singênico de melanoma cutâneo em camundongos C57BL/6.

Catalogação: Biblioteca Central Professor Antônio Jorge.

UNIVERSIDADE ESTADUAL DE MONTES CLAROS-UNIMONTES

Reitor(a): Professor João dos Reis Canela

Vice-reitor(a): Professor Antônio Alvimar Souza

Pró-reitor(a) de Pesquisa: Professor Virgílio Mesquita Gomes

Coordenadoria de Acompanhamento de Projetos: Karen Lafetá

Coordenadoria de Iniciação Científica: Leonardo Monteiro Ribeiro

Coordenadoria de Inovação Tecnológica: Dario Alves de Oliveira

Pró-reitor(a) de Pós-graduação: Professor Hercilio Martelli Junior

Coordenadoria de Pós-graduação Lato-sensu: Romulo S. Barbosa

Coordenadoria de Pós-graduação Stricto-sensu: Ildenílson Meireles Barbosa

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

Coordenador: Alfredo Batista Mauricio de Paula

Subcoordenadora: Marise Fagundes Silveira

ALUNO: OTÁVIO CARDOSO FILHO

UNIVERSIDADE ESTADUAL DE MONTES CLAROS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

Unimontes

CANDIDATO: OTÁVIO CARDOSO FILHO

TÍTULO DO TRABALHO: "Efeitos do Resveratrol na Caquexia associada ao câncer: ensaios in vivo no modelo tumoral murino singênico de Melanoma cutâneo em camundongos C57BL/6"

ÁREA DE CONCENTRAÇÃO: Mecanismos e Aspectos Clínicos das Doenças

LINHA DE PESQUISA: Clínica, Diagnóstico e Terapêutica das Doenças

BANCA (TITULARES)	**ASSINATURAS**
PROF. DR. ALFREDO MAURÍCIO BATISTA DE PAULA- ORIENTADOR	
PROFª. DRª LUDMILLA REGINA SOUZA-COORIENTADORA	
PROF. DR. EDUARDO ROBSON DUARTE	
PROF. DR. RICARDO CARDOSO CASSILHAS	
PROF.ª DR.ª ADRIANA BOZZI	
PROF. DR. ANDRÉ LUIS SENA GUIMARÃES	

BANCA (SUPLENTES)	**ASSINATURAS**
PROF. DR. SÉRGIO HENRIQUE SOUSA SANTOS	
PROFª. DRª LUCYANA CONEIÇÃO FARIAS	
PROF. DR. MARCOS VINÍCIUS MACEDO DE OLIVEIRA	

[X] **APROVADO(A)** [ ] **REPROVADO(A)**

Dedico este trabalho primeiramente aos meus pais, Otávio Cardoso dos Santos e Maria Edna dos Santos, pelo amor, incentivo e exemplo a mim sempre demonstrado. Dedico também ao meu tio, Gedeon Cardoso dos Santos (*in memórian*) que tanto nos entristeceu com a sua partida em consequência da caquexia associada ao câncer.

AGRADECIMENTOS

Agradeço a Deus, por permitir que eu chegasse até aqui, por não me deixar desistir e estar sempre presente em minha vida. Isso me fortalece!

Agradeço aos meus pais, Otávio Cardoso dos Santos e Maria Edna dos Santos, que através de exemplos me mostraram que superar as dificuldades faz parte do "crescimento". Sei que o orgulho neste momento também é de vocês. Obrigado!

Ao meu orientador, professor Alfredo Mauricio Batista de Paula, que com muita sabedoria, conduz a sua vida profissional de forma ética e íntegra. Obrigado pela confiança, pelos ensinamentos e pela oportunidade concedida para realização desta pesquisa. A sua orientação me transformou em uma pessoa melhor e mais madura. Serei imensamente grato à sua disponibilidade e dedicação.

A minha coorientadora, professora Ludmilla Regina de Souza David, um ser humano abençoado! Sou imensamente grato pelo carinho, companheirismo, presteza e orientação durante toda a conduta experimental. Sua companhia durante essa trajetória me fez compreender a importância da ética na produção científica.

A todos os membros do grupo de pesquisa NUPEMOC. Com vocês aprendi que a boa convivência e o trabalho coletivo são fundamentais para garantia do sucesso nos resultados.

Aos meus irmãos e sobrinhos, por suportar comigo todos os momentos difíceis dessa caminhada e me incentivar sempre que necessário. Obrigado por acreditarem e valorizarem o meu esforço. DEUS abençoe sempre essa família!

Ao meu amado afilhado, Pedro, e a minha querida afilhada, Maria Fernanda, por me proporcionarem carinho e momentos de descontração.

Ao amigo Roberto Rodney Ferreira Junior, por viabilizar alguns procedimentos laboratoriais. Obrigado por acreditar e se importar com o avanço científico!

Obrigado a todos os colegas e amigos do biotério e do laboratório de Pesquisa em Saúde, em especial a Daniela Fernanda, Victor Hugo, Lilian, Emisael, Jaciara, Pablo e Keila, que contribuíram com momentos de “crescimento” pessoal e científico.

Agradeço a todos os professores membros da banca, pela disponibilidade e atenção na leitura de meu trabalho.

Aos meus amigos titios, que, assim como uma família, torcem, acreditam e ficam felizes com o sucesso do outro. Obrigado meus amigos pelo apoio e companheirismo sempre demonstrado. Sou feliz por vocês fazerem parte de minha vida e por acrescentar alegria aos meus dias.

À funcionária do programa de pós-graduação, Du Carmo, sempre zelosa no auxílio aos trâmites administrativos.

Agradeço aos auxílios financeiros recebidos da Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), da Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) e do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

RESUMO

A caquexia é uma síndrome paraneoplásica associada à progressão do câncer, caracterizada pela anorexia e perda ponderal involuntária, sobretudo de massa esquelética e adiposa, com consequente impacto negativo sobre a resposta terapêutica antineoplásica, a qualidade de vida e o tempo de sobrevida de indivíduos com câncer. O presente estudo avaliou os aspectos metodológicos relacionados à caquexia experimental associada ao modelo tumoral murino singênico de melanoma cutâneo em camundongos C57Bl/6. Adicionalmente, investigar potenciais efeitos anticaquéticos promovidos pelo resveratrol nesse modelo de caquexia associado ao câncer. Por meio de uma revisão sistemática da literatura, contemplaram-se análises metodológicas de indução da caquexia em modelo murino, abordagens farmacológicas e os principais achados relacionados ao diagnóstico da doença. Para isso, títulos e palavras-chave foram consultados em bases de dados eletrônicas (MEDLINE, PUBMED, EMBASE, Scopus, SciELO e Web of Science). Cada estudo selecionado foi avaliado individualmente e a qualidade metodológica dos estudos usando a escala de Jadad. Inicialmente, foram selecionadas 57 publicações, sendo que sete destes preencheram os critérios de inclusão. O modelo mais comum de MC em camundongos tem sido estabelecido com inoculação subcutânea de células de melanoma murino B16F10 na região dorsal dos animais fêmeas C57BL6. A caquexia relacionada com o CM ocorre com a progressão do tumor e o diagnóstico de caquexia baseia-se principalmente no peso corporal associado na maioria dos estudos com massa do músculo gastrocnêmio ou tecido adiposo visceral. A abordagem terapêutica anticaquética relacionada ao melanoma cutâneo (MC) tem sido realizada apenas com anti-inflamatórios. Os tratamentos que promovem efeitos anticaquéticos são aqueles que também reduzem o volume do tumor concomitantemente. Em conclusão, poucos parâmetros são usados para definir a caquexia no modelo de camundongos e o efeito de anti-inflamatórios no modelo de MC de camundongo, associada à caquexia, está condicionado à redução do tumor. Com objetivo de investigar os efeitos anticaquéticos promovidos pelo uso do resveratrol (Resv.) em tecidos alvos da síndrome e na sobrevida de camundongos C57Bl/6 submetidos ao modelo tumoral singênico de caquexia associada ao melanoma cutâneo experimental. Inocularam-se células B16F10 murinas no flanco de cinquenta e oito camundongos fêmeas C57BL/6. O diagnóstico de CRC foi estabelecido individualmente considerando perda de peso $\geq$ 5%. O resv. foi administrado a 200 e 400mg /Kg de peso corpóreo usando gavagem oral em camundongos controles e experimentais durante 12 dias. O consumo de água e alimentos, peso corporal e tamanho do tumor foram mensurados diariamente. Os níveis séricos de albumina e proteína C-reativa (PCR) foram avaliados por imuno-ensaios enzimáticos. Força muscular esquelética, volume e massa foram avaliados usando um medidor de força de preensão, um dispositivo de ultrassom de alta frequência e uma balança analítica, respectivamente. As amostras de músculo esquelético, tecido adiposo branco e marrom, foram coletadas e submetidas à análise morfométrica e de expressão gênica. Camundongos tratados com resv. promoveram um atraso no estabelecimento da CRC, com ganho de peso corporal, de músculo esquelético (ME) e melhora na sobrevida de camundongos com CRC ($p < 0,01$, $p < 0,001$ e $p < 0,18$ respectivamente). Aumentou o volume do ME, a força muscular e preservou a perda de fibras musculares (200mg /Kg, $p < 0,001$ e 400mg /Kg, $p < 0,01$). Reduziu o peso do TAB e a área de adipócitos ($p < 0,01$). Promoveu diminuição na inflamação crônica de baixo grau (200mg /Kg, $p < 0,001$, e 400mg /Kg, $p < 0,01$). Constatou-se que a administração de resv. em camundongos C57BL/6 em modelo de caquexia relacionada ao MC singênico, apresenta efeitos anticaquéticos e melhora a sobrevida.

Palavras-chave: melanoma cutâneo; terapias; peso corporal; sobrevida; músculo esquelético.

ABSTRACT

Cachexia is a paraneoplastic syndrome associated with cancer progression, characterized by anorexia and involuntary weight loss, especially skeletal and adipose mass, with a consequent negative impact on the antineoplastic therapeutic response, the quality of life and the survival time of individuals with cancer . The present study evaluated the methodological aspects related to the experimental cachexia associated with the syngeneic murine tumoral model of cutaneous melanoma in C57Bl / 6 mice. Additionally, investigate the potential antico-ectic effects promoted by resveratrol in this cancer-associated cachexia model. Through a systematic review of the literature, we contemplated methodological analyzes of cachexia induction in murine model, pharmacological approaches and the main findings related to the diagnosis of the disease. For this, titles and keywords were consulted in electronic databases (MEDLINE, PUBMED, EMBASE, Scopus, SciELO and Web of Science). Each selected study was evaluated individually and the methodological quality of the studies using the Jadad scale. Initially, 57 publications were selected, of which seven met the inclusion criteria. The most common model of MC in mice has been established with subcutaneous inoculation of B16F10 murine melanoma cells in the dorsal region of female C57BL6 animals. CM-related cachexia occurs with tumor progression and the diagnosis of cachexia is based primarily on body weight associated with most studies with gastrocnemius muscle mass or visceral adipose tissue. The anticancer therapeutic approach related to cutaneous melanoma (MC) has been performed only with anti-inflammatory drugs. Treatments that promote anticoagulant effects are those that also reduce tumor volume concomitantly. In conclusion, few parameters are used to define cachexia in the mouse model, and the effect of anti-inflammatory drugs on the mouse CM model associated with cachexia is conditioned to tumor reduction. The objective of this study was to investigate the anticoagulant effects promoted by the use of resveratrol (Resv.) In target tissues of the syndrome and in the survival of C57B1 / 6 mice submitted to the syngeneic tumor model of cachexia associated with experimental cutaneous melanoma. Murine B16F10 cells were inoculated into the flank of fifty-eight female C57BL / 6 mice. The diagnosis of CRC was established individually considering weight loss $\geq$ 5%. The resv. was administered at 200 and 400 mg / kg body weight using oral gavage in control and experimental mice for 12 days. Water and food intake, body weight and tumor size were measured daily. Serum levels of albumin and C-reactive protein (CRP) were assessed by enzyme immunoassays. Skeletal muscle strength, volume and mass were evaluated using a grip force gauge, a high frequency ultrasound device and an analytical balance, respectively. Samples of skeletal muscle, white and brown adipose tissue were collected and submitted to morphometric analysis and gene expression. Mice treated with resv. ($p < 0.01$, $p < 0.001$ and $p < 0.18$, respectively) were associated with a delay in the establishment of CRC, with body weight gain, skeletal muscle (ME) and improvement in survival. Increased ME volume, muscle strength and preserved the loss of muscle fibers (200mg / kg, $p < 0.001$ and 400mg / kg, $p < 0.01$). Reduced TAB weight and adipocyte area ($p < 0.01$). It promoted a decrease in chronic inflammation of low grade (200mg / kg, $p < 0.001$, and 400mg / kg, $p < 0.01$). It was found that the administration of resv. in C57BL / 6 mice in a cachexia model related to the syngeneic MC, has anticoagulant effects and improves survival.

Keywords: cutaneous melanoma; therapies; body weight; survival; skeletal muscle.

SUMÁRIO

1 INTRODUÇÃO

1.1 Melanoma cutâneo

O melanoma cutâneo é um tipo de câncer de pele com baixa incidência, entretanto com elevado índice de letalidade. É responsável por 75% das mortes relacionadas ao câncer de pele devido à sua tendência em desenvolver metástase linfática e hematogênica (1). Apesar de ainda ser considerada de baixa incidência, a partir do século XX tem sido uma das neoplasias que mais aumentou o número de casos e índices de mortalidade (2, 3). Segundo a Organização Mundial da Saúde, a cada ano, estima-se a incidência de 2-3 milhões de casos de câncer de pele e, apesar do melanoma representar apenas 132.000 desses casos, a maioria das mortes é provocada por esse tipo de neoplasia.

As características fenotípicas e ambientais são consideradas fatores de risco, e neste contexto populações de pele clara são suscetíveis, o que favoreceu o maior aumento da incidência de melanoma nas últimas décadas em algumas regiões (4). Populações, como as europeias, norte-americanas e oceânicas, representam quase 82% da incidência global e aproximadamente 64% de mortalidade associada à doença (5). Austrália e Nova Zelândia são os países com maior número de casos novos no mundo (6). Segundo o Instituto Nacional de Câncer, no Brasil estima-se, para o biênio 2018-2019, que sejam diagnosticados cerca de 6.260 novos casos de melanoma cutâneo por ano.

O melanoma cutâneo é um tumor maligno de origem neuroectodérmica (7), considerado uma doença multi-fatorial decorrente da interação entre fatores genéticos e a exposição ambiental (8). Originado nos melanócitos, células dendríticas derivadas da crista neural, que migram na embriogenese para a epiderme (9), sendo encontrados principalmente na camada basal da pele, entre a epiderme e a derme (10). Essa célula tem a função de produzir e transferir para os queratinócitos a melanina, responsável pela proteção do núcleo contra possíveis danos no DNA, causados pela absorção da radiação ultravioleta (UV)(9). Seu crescimento não canceroso resulta na formação de nevos melanocíticos benignos (11). Entretanto, a célula pode sofrer transformações e apresentar desenvolvimento canceroso e se tornar um melanoma (12) (figura 1).

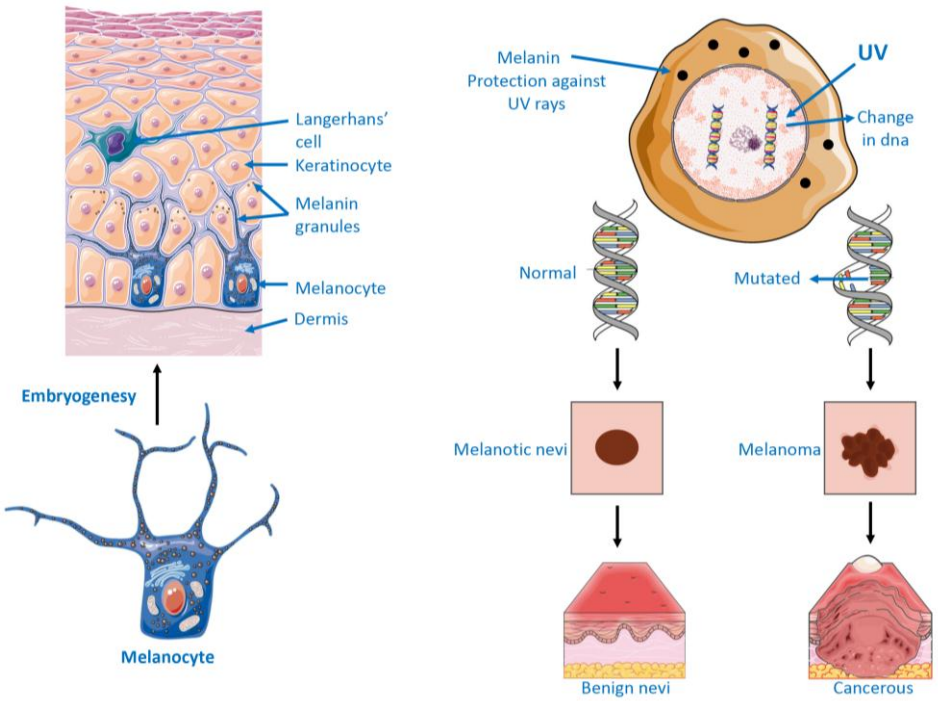


Figura 1: Origem e desenvolvimento do melanoma cutâneo maligno.
Fonte: Elaboração do autor.

Isso acontece devido a mutações sofridas pelo melanócito, em decorrência de danos não reparados no DNA ou outras alterações genéticas e exposição contínua ao carcinógeno, radiação UV (13, 14).

1.1.2 Diagnóstico do Melanoma Cutâneo

No que diz respeito a características clínicas, histológicas e a progressão do tumor, o melanoma cutâneo tipicamente se manifesta em diferentes subtipos, melanoma disseminativo superficial, melanoma nodular, melanoma lentiginoso acral e melanoma lentigo maligno (15, 16). Melanoma disseminativo superficial começa com fase intraepidérmica de crescimento horizontal ou radial com disseminação pagetóide de melanócitos malignos claros ao longo da epiderme (10). O melanoma nodular é exofítico marrom-preto, muitas vezes erodido ou sangramento tumoral, caracterizado pela agressividade do crescimento vertical (10). Lentigo maligna é um melanoma de crescimento lento *in situ*, geralmente em áreas expostas aos raios UV (17). Melanomas lentiginosos acral, mede de 2 a 3 cm é multifocal com discrepâncias entre as margens clinicamente e histopatológica (18, 19). Em sua fase intraepidérmica inicial, ocorre pigmentação irregular e mal circunscrita com posterior crescimento invasivo Em sua

fase intraepidérmica inicial, ocorre pigmentação irregular e mal circunscrita com posterior crescimento invasivo (20).

No diagnóstico, um melanoma tem um estágio numérico baseado em quão profundamente cresceu na pele e se espalhou para outras partes do corpo. Estágio 0, as células do melanoma ainda estão ligadas a epiderme; estágio 1, fase em que o melanoma pode chegar a 2 mm de espessura e alguns casos apresentar ulceração; estágio 2, o melanoma atinge 4 mm de espessura; estágio 3, células cancerosas atingem profundidade na pele, vasos linfáticos ou glândulas linfáticas; estágio 4, fase avançada em que o câncer se espalha para outras partes do corpo, como o pulmão, fígado, cérebro, ossos, gânglios linfáticos ou outros áreas da pele (21).

Para confirmação do diagnóstico, torna-se relevante a avaliação histopatológica considerando a espessura do tumor em mm, presença ou ausência de ulceração, número de mitoses por mm^2 e presença de microssatélites. Além disso, devem-se incluir informações da fase de crescimento (horizontal ou vertical), nível de invasão, presença ou ausência de regressão estabelecida, presença ou ausência de tumor denso infiltrado, embolia linfática, envolvimento vascular ou perineural. Caso necessário, as colorações imuno-histoquímicas podem ser úteis para a confirmação da natureza melanocítica do tumor (22).

1.1.3 Tratamento do Melanoma Cutâneo

Após confirmação histológica, é realizada uma excisão mais extensa para permitir a retirada de possível tumor residual e diminuir as taxas de recorrência tumoral (7, 24). A quimioterapia consiste na aplicação de drogas citotóxicas e representa a forma primária de abordagem do melanoma metastático. As drogas mais utilizadas atualmente são representadas pela Dacarbazina (DTIC), Cisplatina (CDDP), Nitrosouréias (Carmustina e Lomustina) e agentes que atuam sobre os microtúbulos (Alcalóides da Vinca e Taxanes). O principal e mais ativo quimioterápico no tratamento do melanoma é representado pela Dacarbazina, agente alquilante que isoladamente proporciona taxas de resposta de 14 a 20% com duração mediana de resposta de quatro a seis meses (25, 26).

O interferon alfa 2b é uma citocina que apresenta maior controvérsia para o uso adjuvante em pacientes com melanoma. Apesar de não existir consenso, a terapia com

Interferon alfa 2b administrado em altas doses pode aumentar o tempo livre de doença bem como proporcionar um discreto aumento de sobrevida (27, 28).

O melanoma é um tumor resistente à radioterapia, sendo indicada para casos de melanoma malignos inoperáveis e pode ser utilizado de forma paliativa em casos de metástases principalmente ósseas (29).

Estudos já demonstraram que o desenvolvimento de tumores singênicos de melanoma cutâneo (B16F10) induz caquexia em camundongos, promovendo um estado catabólico provavelmente em resposta a um ambiente pró-inflamatório (30-32). No entanto, existe uma carência de informações sobre a relação do tumor de melanoma cutâneo B16F10, especialmente em relação a interação entre o tratamento do tumor e a melhora nos componentes da caquexia associada ao câncer.

1.2 Caquexia associada ao câncer

1.2.1 Progressão tumoral, síndrome paraneoplásica e caquexia associada ao câncer

As inúmeras alterações moleculares ocorridas durante a progressão do câncer tendem a dotá-lo com uma maior agressividade biológica e clínica. Durante a progressão tumoral, é percebido que células cancerosas podem expressar genes que deveriam se encontrar reprimidos e/ou inativar genes que deveriam ser regularmente expressos, gerando distúrbios em vias de sinalização moleculares diversas, com efeitos locorregionais e sistêmicos. Com a progressão da doença neoplásica maligna, portanto, há uma tendência para a geração de células cancerosas muito proliferativas e/ou com baixa atividade apoptótica (resultando em tumores de maior dimensão clínica em um curto espaço de tempo); células cancerosas que crescem exibindo menor formação de complexos adesivos entre si e com a matriz extracelular (MEC), e adquirem mobilidade graças às modificações sofridas em seu citoesqueleto; além daquelas que se tornam capazes de promover degradação enzimática dos componentes da MEC (resultando em tumores recorrentes e com capacidade de originar focos metastáticos locais e à distância) (33-36).

A caquexia associada ao câncer é uma síndrome complexa caracterizada como síndrome multifatorial com redução progressiva de peso, anorexia, perda muscular e fraqueza (33-39). É causada principalmente por alteração morfofuncional progressiva do tecido muscular (33, 40, 41) e sua maior influência ocorre em fibras musculares esqueléticas, promovendo uma drástica hipotrofia muscular e, consequentemente,

debilidade generalizada, determinada pelo aumento do catabolismo proteico ou ainda pela diminuição na síntese proteica no tecido muscular (42, 43). Levando a um comprometimento progressivo da capacidade de trabalho (33).

Aproximadamente metade de todos os pacientes com câncer apresenta caquexia (44, 45). Trata-se de uma síndrome que não só provoca um impacto negativo na qualidade de vida do paciente, mas também está associada a respostas precárias à quimioterapia e à sobrevida (46, 47). A morte geralmente ocorre quando há 30% de perda de peso (48). É responsável pela morte de 22% dos pacientes com câncer (49), com a prevalência de aproximadamente 86% nas últimas 1-2 semanas de vida (50, 51).

A inflamação crônica é um dos principais causadores da caquexia, pois afeta a função de vários tecidos, como músculo esquelético, tecido adiposo, cérebro e fígado (52). Ocorre um elevado aumento de interleucinas inflamatórias durante a progressão do câncer e a inflamação sistêmica é uma característica da caquexia associada ao câncer, indicada pela produção de proteínas como resposta na fase aguda, como por exemplo, a proteína C-reativa (PCR) (53, 54). A PCR é considerada uma medida precisa da atividade das citocinas pró-inflamatórias (55) que tem sido implicada na perda de massa muscular (56). A perda de massa muscular durante o desenvolvimento do tumor pode modular significativamente a força muscular e a capacidade locomotora em portadores de tumores (57) (figura 02).

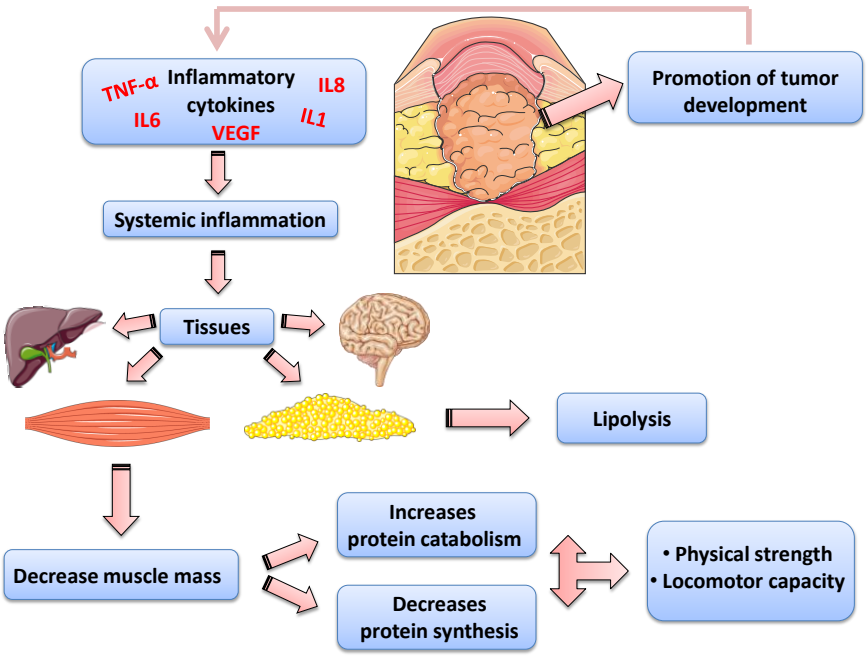


Figura 2: Influência da inflamação crônica no surgimento da caquexia associada ao câncer.
Fonte: Elaboração do autor.

A etiologia e a patogênese do CRC ainda possuem muitos aspectos a serem esclarecidos. As ocorrências de variações no fenótipo do tumor ou no genótipo dos pacientes parecem contribuir para o desenvolvimento do CRC. A participação de mediadores químicos pró-caquéticos liberados por células cancerígenas e/ou células imunes residentes e infiltrantes parece ser essencial na montagem de muitas alterações metabólicas nos órgãos-alvo (tecido muscular esquelético, tecido adiposo, cérebro, fígado e outros) durante a caquexia. Numerosos mediadores pró-caquéticos influenciam a redução dos estoques lipídicos nos tecidos adiposos, mas, principalmente, suas maiores influências ocorrem nas fibras musculares esqueléticas, que promovem a drástica hipotrofia. Consequentemente, uma fraqueza muscular generalizada que parece ser determinada muito mais pelo aumento do catabolismo proteico do que pela diminuição da síntese de proteínas no tecido muscular. Além disso, importantes alterações metabólicas no tecido muscular são observadas, o que promove um aumento no consumo de energia em repouso (35, 36, 52, 58).

1.2.2 Caquexia associada ao melanoma

A caquexia é um achado clínico comum nos pacientes oncológicos, associada inicialmente à ação natural da doença ou, mais tardiamente, ao crescimento tumoral e presença de metástases. Assim como ocorre com os demais tipos de cânceres humanos, estudos experimentais e humanos têm demonstrado que, com a progressão do melanoma cutâneo, nota-se a ocorrência de caquexia nos pacientes com esse tipo de câncer (39-44).

No modelo experimental de desenvolvimento tumoral com uso de células de melanoma B16 inoculadas em camundongos C57BL/6 verifica-se que durante a progressão tumoral ocorrem tanto uma progressiva perda de tecido adiposo branco nos animais, quanto uma redução acentuada da musculatura esquelética estriada, caracterizando um quadro de caquexia associada ao câncer (45). Nesses modelos, a presença de um fator conhecido como inibidor da lipoproteína lípase derivada de melanoma (LPL) ou o fator inibitório de leucemia parece desempenhar um papel importante no quadro caquético nos camundongos imunocomprometidos com melanoma induzido com células imortalizadas de melanoma B16. A inibição farmacológica dessas lipases parece contribuir para a reversão do quadro de caquexia associada a esse tipo de câncer (46-48, 50).

Tem sido também observado o papel da molécula melanocortina na ocorrência do quadro caquético associado ao desenvolvimento do melanoma cutâneo induzido. Essa molécula é responsável pela ativação de neurônios hipotalâmicos com o objetivo de integrar sinais periféricos que controlam negativamente o apetite e a sede e, indiretamente, o desempenho físico dos animais. O bloqueio deste sinal resulta na normalização da ingestão de alimentos e de bebidas e uma melhora do desempenho físico. Esses dados sugerem que este sistema de sinalização mediado pela melanocortina parece desempenhar um papel importante na mediação da caquexia associada ao melanoma cutâneo (49).

1.2.3 Os sistemas de degradação proteolíticos intracelulares

Proteínas intracelulares e extracelulares são renovadas em todas as nossas células/tecidos por meio de um processo contínuo de síntese, degradação e substituição. No que se refere ao fenômeno da degradação proteica (proteólise) realizada por sistemas proteolíticos intracelulares, esses apresentam ações complexas, estritamente coordenadas, que tem a missão de regular os níveis de diversas classes de proteínas, com diferentes períodos de meia-vida e ações sem, no entanto, promover alterações nos mecanismos moleculares que garantem a integridade estrutural e funcional celular. Em células eucariotas, os principais sistemas proteolíticos são: o sistema ubiquitina-proteassoma, o sistema de cascata de caspases, o sistema de proteases lisossomais (catepsinas), as calpaínas cálcio-dependentes e o sistema autofágico (51-55).

1.2.5 Diagnóstico da caquexia

Clinicamente, a caquexia associada ao câncer apresenta-se como um processo contínuo composto por três estágios: a *pré-caquexia*, a *caquexia*, e a *caquexia refratária*. Contudo, nem todos os pacientes passam por todo esse espectro. Na pré-caquexia, sinais clínicos e metabólicos precoces (por exemplo, anorexia e intolerância à glicose) podem preceder a substancial perda de peso (PP) involuntária (≤ 5% do PP regular do indivíduo). O risco de progressão varia e depende de fatores como o tipo de câncer e de seu estadiamento, a presença de inflamação sistêmica, a baixa ingestão de alimentos e a falta de resposta à terapia antineoplásica. Os pacientes que apresentam PP > 5% nos últimos 6 meses, ou um Índice de Massa Corporal (IMC) inferior a 20 kg/m^2

são considerados caquéticos. A caquexia refratária ocorre em indivíduos com câncer com estadiamento avançado ou de rápida progressão e que não responde à terapia antineoplásica. Nesse estágio nota-se catabolismo ativo promovido pela doença neoplásica e não há possibilidade de recuperação da PP demonstrada pelo paciente (39, 59).

1.2.6 Tratamento da caquexia

Além de ser uma causa direta da morte por câncer, a caquexia também limita as opções terapêuticas, já que os pacientes caquéticos normalmente são menos tolerantes a radioterapia e quimioterapia devido à fraqueza geral e desconforto (57, 60, 61). A melhor estratégia de gestão da caquexia associada ao câncer é tratamento do câncer subjacente, pois isso reverterá a síndrome de caquexia. Infelizmente, esta continua sendo uma conquista pouco frequente com cânceres avançados. Uma segunda opção poderia ser contrabalançar a perda de peso aumentando a ingestão nutricional, mas como na maioria dos pacientes caquéticos a anorexia é apenas uma parte do problema, a nutrição como terapia não é capaz de reverter completamente o desgaste estabelecido (62).

O tratamento adequado deve incluir medicamentos que abordem as seguintes condições: estado inflamatório, distúrbio nutricional, desarranjos metabólicos, defeitos imunológicos, baixa qualidade de vida e, fadiga (63). Assim, o tratamento para tem como desfechos variáveis, que foram recentemente identificadas como chave na caquexia: aumento na massa corporal magra e atividade funcional (força muscular e atividades locomotoras); diminuição do gasto energético de repouso; e melhora da fadiga (33).

Os agentes antineoplásicos regulares têm capacidade de tratar o câncer, porém em alguns casos pioram a caquexia. Daí a importância da realização estudos através do desenvolvimento de agentes de tratamento com capacidade de afetar a progressão do câncer, bem como melhorar a qualidade de vida do paciente, nesse contexto são apresentadas algumas drogas candidatas a esse fim.

O sorafenibe apresenta um efeito anticancerígeno no tratamento de tumores sólidos, além de aumentar melhoria na sobrevida de pacientes com câncer (64). A curcumina é um polifenol, vulgarmente conhecida como açafrão, tem sido usada

extensivamente na medicina, por ser não tóxica e possuir uma variedade de propriedades terapêuticas, incluindo atividade antioxidante, anti-inflamatória, anticancerígeno (65). Pornalrestat, um inibidor da redutase da aldose, ativa a atividade da lipoproteína lipase (LPL) no tecido adiposo e alivia os sintomas caquéticos induzidos pelo melanoma B16 em camundongos (66). O ácido linoléico conjugado (CLA) está atraindo interesse devido a seus efeitos na composição corporal, especificamente uma redução na massa de gordura corporal. Outros efeitos benéficos relacionados à saúde do CLA incluem propriedades anticarcinogênicas (67). Dentre as citadas destaca-se o resveratrol.

1.3 Resveratrol

O resveratrol é uma fitoalexina, sintetizado naturalmente na planta sob duas formas isômeras: trans-resveratrol (trans-3,5,4'-trihidroxiestilbeno) e cis-resveratrol (cis-3,5,4'-trihidroxiestilbeno) (68), porém o isômero trans-isômero é o mais estável (69). A forma (3,5,4'-tri-hidroxi-trans-estilbeno) é um composto estilbenoide e polifenólico (70), pertencente ao grupo dos flavonoides (71, 72). É produzido por mais de 70 espécies de plantas (73) através da via fenilpropanóide e é derivado do ácido *p*-oceânico, que é um intermediário formado durante a produção de lignina. É encontrado naturalmente, especialmente em amendoim, uvas e algumas bagas (70).

1.3.1 Resveratrol e câncer

É amplamente estudado e apresenta diversas atividades biológicas e benefícios, o que o torna uma substância importante para uso nas indústrias farmacêutica, alimentícia e cosmética (74). Estudos *in vitro* com células de câncer, mostram ações do resveratrol na indução de apoptose (75) e na inibição da proliferação celular ao interferir no ciclo celular, a fim de prejudicar a duplicação do DNA (76). Dentre os estudos *in vivo* que foram relatados os seus numerosos efeitos biológicos e farmacológicos, incluem, efeito anti-inflamatório (77, 78), anticancerígeno (79-81), aumento na sobrevida de portadores de câncer (81) efeito antioxidante (82), efeito protetor - anti catabólico do músculo (83-86), hepatoprotetor (87, 88) e cardioprotetor (89, 90).

O resveratrol pode alterar o ciclo celular e a maquinária apoptótica (91), atuando como um agente antiproliferativo de alguns tipos de tumores (92). Vários estudos têm mostrado que isso acontece devido ao fato de inibir a migração de células de câncer de

EMT associados e a invasão através da inibição da via de sinalização de PI-3K/ Akt/ NF-kB, inibição de TGF-B1 e a inibição da sinalização da via hedgehog (93). Induz a apoptose de um modo dependente da p53 (94). Ou através da supressão de sinais extracelulares regulada de sinalização da cinase p53, Rb, / E2F, ciclinas e CDKs (68). Há ainda grandes evidências que o resveratrol tenha um forte efeito inibitório sobre a fosforilação do EGFR (95). Pode também ter ação na atividade da PFK, portanto, perturba o metabolismo da glicose e reduz a viabilidade em células cancerosas (96).

Estudo recentes, apontam ainda a proteína Tigar como um importante alvo de resveratrol, diminuindo a proteína independente da linhagem celular utilizada. Essa proteína regulada desencadeia uma queda nos níveis de glutationa reduzida, o que resulta em ROs, responsável pela apoptose e autofagia. Em alguns trabalhos ainda demonstra inibição de enzimas metabólicas importantes, incluindo PKM2 (97).

1.3.2 Estudos animais

A suplementação do resveratrol em modelos animais portadores de câncer tem demonstrado que sua eficiência depende da rota de administração, da dose, do modelo tumoral, dentre outros fatores. Dentro de um tipo de câncer específico, existe uma variabilidade entre os estudos com relação à estirpe, idade e sexo do animal utilizado (98).

Estudos mostraram que o pré-tratamento de pele de camundongos com resveratrol impediu vários efeitos induzidos por TPA, incluindo o aumento da expressão da ciclooxigenase (COX) -1, COX-2, c-myc, c-fos, c-Jun, fator de crescimento transformante-β1, e fator de necrose tumoral-α (99).

O resveratrol foi significativamente eficaz na inibição da taxa de formação na redução no número tumores cutâneos de animais induzidos por DMBA, através da indução de apoptose, caracterizado por indução da liberação de citocromo c, expressão de p53, e inibição da proteína Bcl-2 (100).

O resveratrol, inibiu a síntese de DNA, aumentou a apoptose e suprimiu novas vascularização dos tumores, quando testado em camundongos portadores de carcinoma do pulmão de Lewis, altamente metastático, reduzindo significativamente o volume e o peso do tumor e metástases de pulmonar (101).

Ao administrar o resveratrol em camundongos, portadores de tumores intestinais. O estudo ocorreu durante sete semanas, a partir da quinta semana de idade

(102). O resveratrol impediu a formação de tumores do cólon e reduziu a formação de pequenos tumores intestinais em 70%. Isso possivelmente ocorreu em função da regulação de vários genes que estão envolvidos na ativação de células imunes e na inibição do processo carcinogênico. Esses dados demonstram a complexidade dos eventos associados com a tumorigenese intestinal e isso potencializa a idéia da utilização do resveratrol como um agente quimiopreventivo no tratamento da carcinogênese.

2 OBJETIVOS

2.1 Objetivo geral

Avaliar os aspectos metodológicos, abordagens farmacológicas e parâmetros utilizados para definir caquexia associada ao melanoma cutâneo; bem como investigar o efeito do resveratrol na caquexia associada ao modelo tumoral murino singênico de melanoma cutâneo em camundongos C57Bl/6.

2.2 Objetivos específicos

- Avaliar os aspectos metodológicos, abordagens farmacológicas e parâmetros utilizados para definir o diagnóstico da caquexia associada ao melanoma cutâneo em modelo singênico de camundongos C57BL/6.

- Investigar o efeito do resveratrol na caquexia associada ao melanoma singênico B16F10 em modelos de camundogos C57BL/6.

3 PRODUTOS

3.1 Produto 1: *Anticachectic Effects of Pharmacological Agents on Mouse Models of Cutaneous Melanoma-Related Cachexia: a Systematic Review.*

3.2 Produto 2: *Resveratrol decreases low-degree chronic inflammation, inhibits body weight and skeletal muscle mass losses, decreases pro-cachectic myokines and adipokines expression, and improves cancer-related survival in C57BL/6 mice bearing syngeneic tumor.*

3.1 PRODUTO 1

Title: Anticachectic Effects of Pharmacological Agents on Mouse Models of Cutaneous Melanoma-Related Cachexia: a Systematic Review.

Article type Review Article

Abstract

Aim: Update the knowledge about the main methodological aspects, and pharmacological approaches from mouse Cutaneo Melanoma (CM)-related cachexia models. Methods: A systematic literature search was conducted using MESH headings and keywords in electronic databases (MEDLINE, PUBMED, EMBASE, Scopus, SciELO, Web of Science). Two reviewers independently assessed selected articles according to the PRISMA protocol. The methodological quality of clinical trial studies was performed using the Jadad scale. Effect size studies were carried. Results: The systematic research yielded 57 publications, seven of these met the inclusion criteria. The most common mouse CM model was established with subcutaneous inoculation of murine B16F10 melanoma cells into the back of the C57BL6 mice. The diagnosis of cachexia was mainly based on body weight monitoring. Conclusion: Few parameters are used to define the cachexia in mouse model. The effect of anti-inflammatory drugs in CM-related cachexia mouse model is associated with a concomitant reduction of tumor volume.

Keyword: cancer-related cachexia, cutaneous melanoma; mouse cancer model; mouse cutaneous melanoma-related cachexia model; therapy.

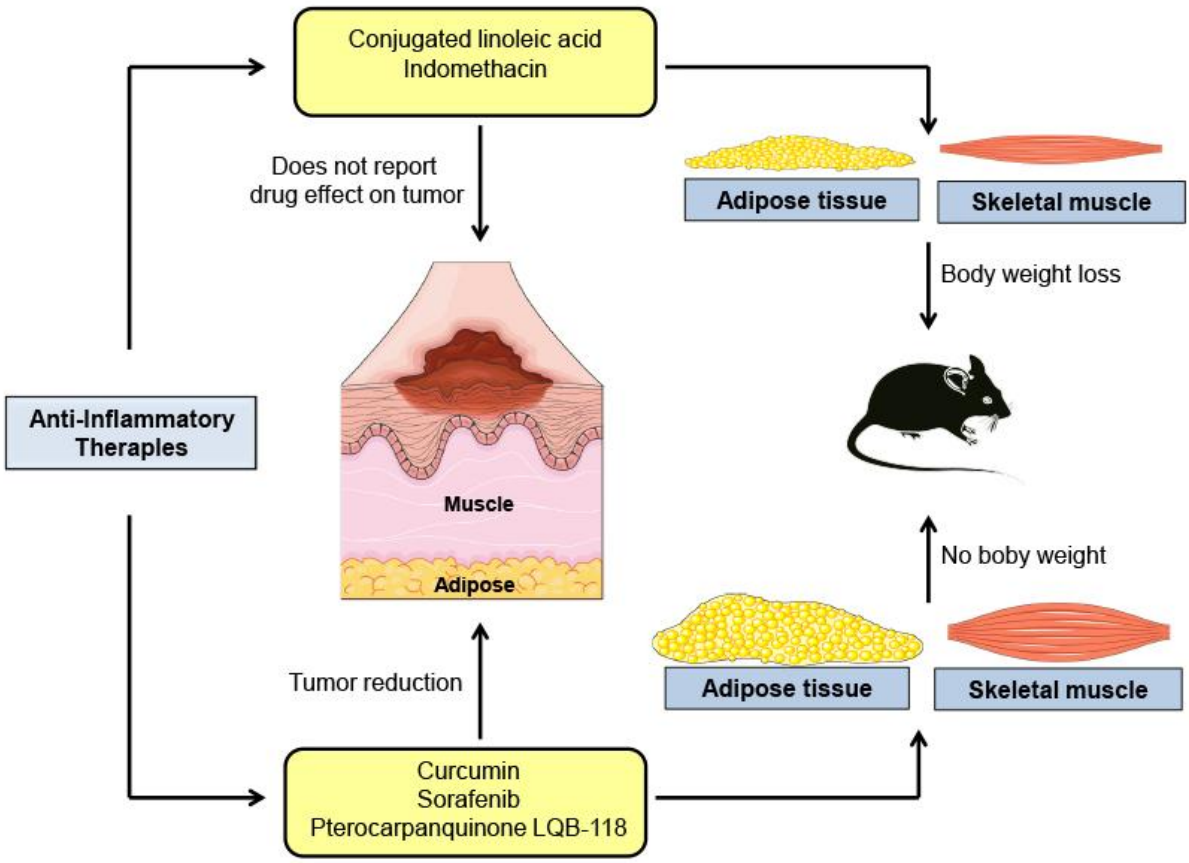
Manuscript category: Oncology

Corresponding Author: Alfredo Mauricio Batista de paula

Corresponding Author's/Institution:
Nucleus of Epidemiological and Molecular Research Catrumano. Health Research Laboratory. Health Science Post-graduate Programme. Department of Dentistry. Universidade Estadual de Montes Claros, 39401-001, Montes Claros, MG, Brazil. e-mail: ambpatologi@gmail.com

Order of Authors: OTAVIO Cardoso Filho, João Lucas Rodrigues dos Santos, Andréia de Souza Brito, Amanda Souto Machado, Amanda Rodrigues Santos, Magda Mendes Vieira, Erivelton Pereira Santos, Marcos Vinícius Oliveira, Renato Monteiro Junior, Ludmilla Souza, Alfredo Mauricio Batista de Paula

Graphical Abstract file:



Anticachectic Effects of Pharmacological Agents on Mouse Models of Cutaneous Melanoma-Related Cachexia: a Systematic Review.

1. Introduction

Cancer-related cachexia (CRC) is defined as a multifactorial syndrome marked by a continuous loss of skeletal muscle mass (with or without loss of fat mass). CRC cannot be fully reversed by conventional nutritional supports and leads to progressive functional impairment in individuals with cancer (1). Clinically, CRC is characterized by an involuntary, progressive physical consummation that occurs mainly in those individuals who present advanced tumor staging (2). The progressive weight loss experienced by individuals with CRC is mainly due to a variable combination of involuntary reduced food intake (anorexia), presence of a low-intensity chronic systemic inflammatory state, intense protein catabolism in skeletal muscle fibers, and a negative energy balance that occurs in adipose tissues (3, 4). Although CRC is a prevalent pathological systemic condition associated with the progression of many types of cancer, it is not readily recognized, thoroughly assessed, and successfully managed. Notably, CRC management represents one of the greatest challenges to health professionals dealing with cancer patients. The great difficulty in establishing efficient treatment protocols for CRC is mostly explained by its complex pathogenesis. From the clinical point of view, CRC is independently responsible for poor outcomes to patients with cancer, such as: drastic decrease of life quality, lower tolerance and reduced response to standard antineoplastic therapies, and a shorter overall survival due to respiratory or cardiac failures (5-8).

The cutaneous melanoma (CM) is the deadliest skin cancer that originates from both genetic and epigenetic disturbances in melanocytes located on the basal layer of epidermis (2, 9, 10). The global incidence of CM is about 160.000 new cases per year, with about 50.000 deaths occurring worldwide, notably due to its early capacity to generate local and distant metastatic dissemination. Nowadays, CM is considered an important public health problem in many countries around the world (11-13). The risk of CM is linked to chronic exposure to ultraviolet light radiation A (UVA) and B (UVB), most notably in fairskinned and red-haired individuals. Moreover, a higher susceptibility to develop CM risk is also influenced by other inheretable and

environmental factors such as socioeconomic status and previous history of heritable or acquired melanocytic skin lesions (e.g., dysplastic nevus, atypical mole syndrome, giant congenital melanocytic nevi, or even a primary melanoma) (14, 15). Despite recent advances in CM diagnosis and therapeutics, prognosis of individuals with CM remains poor, with a five-year overall survival rate for about 20% for patients that presented distant metastases at the time of diagnosis (16). Typically, CM is characterized by its highly aggressive clinical and biological behavior, especially due to the early occurrence of dissemination of metastatic cancer cells (10, 17, 18). However, the occurrence of CRC in individuals with advanced CM, as occurs for other human solid tumors at advanced stages, appears to contribute to its lethality (19-21). Notably, only a few human studies have explored the occurrence of CRC in individuals with CM and the clinical consequences of this paraneoplastic syndrome in these individuals.

In researching human cancer, murine models allow for better understanding of the malignancy without the added risk of harming an actual human being. Mouse CM models have been used in preclinical studies in order to better uncover characteristics of tumor biology, especially the molecular mechanisms responsible for determining the clinical behavior of CM (22). Remarkably, there is a paucity of information regarding diagnosis, pathogenesis, therapeutic approaches, and outcomes related to CRC from murine melanoma models (21, 23, 24). Although presenting limitations that typically occur in study designs with animal models, they can potentially contribute to a better understanding about cachexia that occurs with MC progression. Additionally, they offer the advantage of facilitating the development of new pharmacological and non-pharmacological therapeutic approaches for CRC treatment. However, the main findings from studies points to different, and sometimes controversial, results regarding the treatment effectiveness on CRC. This is probably due to the different types of therapies adopted, the treatment period, the drug administration route, among other factors (25-27).

In this systematic review study, we aimed to update the knowledge about the main findings related to diagnosis, methodological aspects, and pharmacological approaches from mouse CM-related cachexia models.

2. Methods

The present study is a systematic literature review that investigated anticachectic pharmacological approaches performed in murine CM-related cachexia models. Main steps were conducted according to the methodology established by the *Preferred Reporting Items is Systematic Reviews and Metanalyses* (28).

2.1 Resources

Data collection occurred from February, 2017 to March, 2018 and the papers, written in the English language exclusively, were retrieved by online search on databases related to *International Literature on Health Sciences* (MEDLINE) through online database PubMed, *Scientific Electronic Library Online* (SciELO), bibliographic database containing abstracts and citations of academic articles (Scopus), and ISI Web of Knowledge. Moreover, a few studies identified in others resources (e.g. list of references, and gray literature, as Google Scholar) were also evaluated if they reached potential eligibility.

2.3 Search strategy

A preliminary exploratory search was conducted to track the descriptors to be used in the survey of thematic references in the structured vocabulary of the *Descriptors in Health Sciences* (DeCS) (http://decs.bvs.br/) using the tool "Descriptor accurate" and in *Medical Subject Headings* (MeSH) by searching them in the keywords, title and/or summary of the studies. Search terms used were “melanoma” OR “experimental melanoma” AND “pharmacological approach” OR “therapeutic” OR “treatment” OR “drug” AND “cachexia” OR “muscle wasting disorder”. Additional resources were used according to the database availability (Booleans AND, OR, Title/Abstract). The purpose of this procedure was not only to filter the results, but also to cross the main terms in order to obtain the maximum number of possible studies. The author reviewers of this present study attempted to identify all terms and their synonyms in the titles or abstracts of the papers for relevance to the defined review question. If it was not clear from the abstract whether the paper might contain relevant data, the full paper was assessed.

2.4 Eligibility criteria

Original randomized controlled trials (RCT) that investigated any pharmacological approaches for treatment of mouse CM-related cachexia were considered as potential papers to be included in this study. Duplicated papers found on the databases or reference lists were excluded.

2.5 Study selection

We screened all retrieved records and included studies in which any pharmacological approach for treatment of mouse CM-related cachexia models (syngeneic and genetically engineered mouse cancer-related cachexia models) was performed. Two reviewers (Cardoso-Filho, O and Souza, LR) performed the first screening of titles and abstracts to select eligible studies, and then, independently evaluated the records. Three steps were followed: 1) title reading; 2) abstract reading; 3) full text reading. In the first step, we selected the studies that presented at least two terms (or synonyms) identified in the MeSH, as possible searched. In the second step, only papers with enough data regarding the performed intervention (anticachectic pharmacological approach) and analyzed outcomes (experimental cancer-related cachexia) were selected. In the final step, during full text reading, the main results were considered. Two articles studied met all inclusion criteria from the reading of the abstract, although the full articles were not available in the databases or when requested by email, being nevertheless included in the present review for presenting important information. However, they are not covered in all analyzes due to the absence of complete information.

We excluded studies that investigated MC mouse models when induction occurred with cancer cell lines of human CM. A flowchart showing the numbers of papers identified and included or excluded at each stage is presented in Figure 1.

2.6 Data extraction

The following information from studies was extracted: data presenting direct association with therapies to prevent or treat CM, as well as a relation with the development of cachexia during experimental CM progression. A standardized form

was used to extract data related to: melanoma cell line (origin; primary or immortalized melanoma cell; quantity used for tumor induction), mouse CM model (age and gender of mice; sample size; presence of control group; local of CM cell inoculation, post-therapeutic clinical findings, survival of mice), and anticachectic drug (type; chemical categorization; route of administration; dose and period of treatment) were included in this systematic review. When data were unclear, there was a further meeting with the three reviewers to establish a consensus.

2.7 Risk of bias in individual studies

The risk of bias in individual studies was evaluated according to their quality. For this purpose, we used Jadad scale to classify methodological quality of each study (29). Two reviewers were invited to conduct this procedure. In case of divergence in the assessments, a third reviewer participated of the decision. Any disagreement was discussed and resolved by consensus. Notably, one of the issues was the Jaddad scale not applying to animal studies, we then made an adaptation of the scale, defining the higher score as four. A study was considered of poor quality if it received two points or less after its evaluation, while a score greater than two was considered high quality. In this present review, classification criteria were adapted considering some cut-off points as follows: level A = 4 points; level B = 3-2 points; level C $\leq$ 2 points. Studies with scores lower than three points were considered by referred scale as "poor quality". The "A" score means "high quality" and "B" and "C" scores mean "low quality" of the study. Moreover, a funnel plot was applied to observe any publication bias of selected studies.

2.8 Data synthesis and analysis

Effect size was calculated for study relation according to Hopkins et al. criteria (30). Effect size was carried out to evaluate the effect of experimental cancer-related cachexia treatment, especially the effects on body weight and animal gastrocnemius skeletal muscle weight. The experiments included in the analysis had descriptions of 1) body weight of control and experimental group; 2) gastrocnemius muscle weight of control and experimental groups; 3) sample size, mean, and standard deviation of each group and statistical comparison (30). Effect size was used to classify the results as

follows: trivial (< 0.2), small (≥ 0.2 - < 0.6), moderate (≥ 0.6 - < 1.2), large (≥ 1.2 - < 2), very large (≥ 2 - < 4), almost perfect (= 4), and perfect (> 4).

3. Results

3.1 Characteristics and methodological quality of selected studies

The first step was to screen the titles and abstracts and remove duplicated studies. Bibliographic search resulted in 57 papers identified in the databases. Among them, eight (14.03%) studies were excluded as they were in duplicates, 42 (73,68%) studies did not meet the inclusion criteria, in 28 (49,12%) there were no drug-based treatments, nine (15,78%) did not investigate the influence of the antitumor treatment on cachexia and five (8,77%) of the studies did not use drugs. In this sense, seven papers were fully analyzed and included in the present review (Figure 1), two of which were analyzed based on the abstract information (Kawamura et al., 1999a and Kawamura et al., 1999b).

The Cohen k value for inter-reviewers agreement was 0.82, which is considered an excellent value.

All the selected studies showed quality scores above two, evidencing a good quality of these studies included in the systematic review. The selected studies included publications from 1999 to 2016.

3.2 Characterization of mouse CM-related cachexia model

Regarding the experimental design, in the studies included for evaluation, the use of 5 x 10^5 cells B16F10 (80.0%), with subcutaneous inoculation (71.4%) close to back was the most usual. Animal strain used in all the studies was C57BL/6 females (100%) (Table 1). Daily gavage and inclusion in the diet were the most used therapies for melanoma. Treatment period of cutaneous melanoma tumors ranged from 10 to 17 days (Table 2).

3.3 Characterization of mouse CM-related cachexia model and pharmacological therapies

The anticachectic drugs used in the selected studies were ponalrestat ($C_{17}H_{12}BrFN_2O_3$, molecular weight: 391.196 g/mol), conjugated linoleic acid ($C_{18}H_{32}O_2$, molecular weight: 280.452 g/mol), bezafibrate ($C_{19}H_{20}ClNO_4$, molecular weight: 361.822 g/mol) and ibrolipim ($C_{19}H_{20}BrN_2O_4P$, molecular weight: 451.257 g/mol), pterocarpanquinone ($C_{19}H_{12}O_4$, molecular weight: 304.301 g/mol), indomethacin ($C_{19}H_{16}ClNO_4$, molecular weight: 357.79 g/mol), curcumin ($C_{21}H_{20}O_6$, molecular weight: 368.379 g/mol) and sorafenib ($C_{21}H_{16}ClF_3N_4O_3$, molecular weight: 464.829 g/mol).

Ponalrestat is an aldose reductase inhibitor. Linoleic acid is an acid with anti-inflammatory action. Bezafibrate is an antilipemic agent and anti-inflammatory. Ibrolipim is a lipoprotein lipase (LPL) activator with antihyperlipidemic activity. Pterocarpanquinone is an antineoplastic agent and exhibits an anti-inflammatory effect. Indomethacin is a nonsteroidal anti-inflammatory drug that acts as a cyclooxygenase inhibitor. Curcumin is a yellow-orange pigment that possesses anti-inflammatory properties. Sorafenib is a synthetic compound that blocks tumor angiogenesis and has anti-inflammatory properties.

3.4 Diagnosis of mouse CM-related cachexia

In 71.4% of the studies, the variation of the body weight was considered to define CRC promoted by mouse CM (Table 2). However, in none of these studies the amount or percentage of weight loss was mentioned in order to define the diagnosis of CRC. Moreover, it was not established a mean time to CRC diagnosis. Only the study conducted by Salustiano et al., 2016 included the monitoring of animal body weight during the treatment period. The other authors considered as cachectic those CM animals that presented lower body weight in comparison to the control animals without tumor. In these cases, the influence of treatment on body weight was evaluated only in the end of the experiment. In 83% of the selected studies, decrease of certain skeletal muscles (soleus, tibial, plantaris and gastrocnemius muscles) mass or white adipose tissue (epididymal adipose, carcass and total lipid) were parameters associated to body weight loss and were used for CRC diagnosis. Only the studies of Kawamura et al.

1999 did not evaluate body weight, and the parameters adipose tissue weight and lipid dosage were evaluated for CRC diagnosis. The gastrocnemius muscle mass represented the second most used parameter (57.1% of the studies) for CRC diagnosis.

3.5 Anticachectic effects of the investigated drugs

All antitumor treatments used in the studies of our systematic review were through drugs with anti-inflammatory properties. Treatments with curcumin (Beckett et al., 2008), sorafenib (Toledo et al., 2014) and pterocarpanquinone (Salustiano et al., 2016) showed antineoplastic effects, once they promoted decreased tumor volume. In parallel, these drugs also did not induce body weight loss, despite having promoted loss of muscle mass after sorafenib and curcumin treatments (Table 2). Interestingly, the treatment with sorafenib increased white adipose tissue mass, improved physical performance of animals and increased food consumption. Only in the Salustiano et al., 2016 study, the treatment effect on survival of the animals was investigated, and it was noticed that the pterocarpanquinone treatment did not promote increase survivability.

Ponalrestat (Kawamura et al, 1999a) administration showed an effect considered as partial because it promoted a positive effect on locomotor restoration of mice with CRC and recuperation of body adipose tissue (Table 2). Similarly, the pharmacological association of bezafibrate and ibrolipim (Kawamura et al, 1999b) also attenuated adipose weight loss. On the order hand, conjugated linoleic acid (McCarthy & Graves, 2006) and indomethacin (Graves et al, 2006) did not preserve muscle mass or promote body weight loss. In all of these studies, no antitumor effect was reported due to the pharmacological agent treatments.

3.6 Effect of treatment on body weight and muscle mass

A total of five and four studies met the criteria for the implementation of the effect analysis of the treatment on body weight and muscle mass, respectively. Effect size analysis related to body weight monitored during the antitumor treatment period showed that treatment effects were positively classified in 80% of the studies (Figure 2A). Treatment effect on body weight was classified as perfect and very large after pterocarpanquinone treatment. In this study, the treatment performed with pterocarpanquinone LQB-118 reversed cachexia considerably. A limitation of this

research relies on the fact that it evaluated only the body weight to define cachexia. Considering the sorafenib treatment, it was verified that it did not promote body weight loss (large effect on size) but caused reduction of muscle mass although the effect size on muscle mass has been considered large (Figure 2B). The therapy with curcumin preserved body weight (large effect size), but with impaired gastrocnemius muscle mass, whose classification of effect size was trivial. Effect size obtained with conjugated linoleic acid treatment on skeletal muscle mass and body weight of mice was small, and there was loss of body weight and skeletal muscle mass. The use of indomethacin showed the lowest effect size on body weight and gastrocnemius muscle mass. The negative effect noted for body weight measurement showed that the therapy with indomethacin promoted an opposite effect.

4. Discussion

CRC is considered a paraneoplastic syndrome in which an involuntary body weight loss occurs due to progressive skeletal muscle wasting, with or without adipose tissue loss. This pathological catabolic systemic condition is consequence of molecular disturbances that affect metabolic molecular pathways in individuals with cancer. Clinically, cachexia represents an independent prognostic factor in these individuals and is one important factor that causes resistance to antineoplastic therapies (32-35). The murine models for induction of cachexia provoked by melanoma and the parameters for the definition of the cachectic framework are highly variable throughout the literature. To the best of our knowledge, this is the first systematic review that assembled general information about mouse CM-related cachexia models, highlighting parameters used to diagnose the mouse model for CM-related cachexia. This study is also the first description of the antineoplastic and anticachectic effects obtained with different drug treatments.

According to findings in this study, the most common mouse MC model was established with subcutaneous inoculation of murine B16F10 melanoma cells between scapulae of the C57BL6 mice. Mouse CM-related cachexia seems to occur as a consequence of tumor progression. According to our findings, the diagnosis of cachexia was mainly based on body weight monitoring (mean loss weight between groups), followed by measurement of the gastrocnemius muscle and visceral adipose tissue. Therapeutic approaches against CM-related cachexia in mouse models were performed

with anti-inflammatory drugs exclusively. Treatments that showed anticachectic effects were those that also reduced the mass and tumor volume concomitantly (Figure 3).

Oral administration with gavage is the most frequent drug administration route. It is also the least invasive administration form of the drug and the most clinically relevant to test the efficacy of potential drugs for the treatment of human diseases, since orally administered drugs provide a broad systemic biodistribution (31).

The most frequent syngeneic mouse MC-related cachexia model is performed with tumor induction through subcutaneous inoculation of the B16 cell lines (subclones F1 and F10) in C57BL/6 (36-42). Usually, after three days post-inoculation of murine melanoma cells, the spontaneous formation of the tumor occurs (43). These syngeneic mouse models best suit CRC studies since they preserve many of the immunological aspects that usually occur during CM progression (44). Typically, CRC progression is associated with an onset of low-grade, systemic chronic inflammatory state which seems to contribute to the occurrence of disturbed catabolic conditions in a number of target tissues, most notably the striated skeletal muscle (45). The maintenance of an immune response to cancer cells is pivotal to verify immunological effects obtained with different anticancer therapies in preclinical studies using animal models (46).

The subcutaneous inoculation of murine melanoma cells between scapulae represent the most used anatomical site for experimental CM induction. That anatomic region frequently makes the experimental CM easier to monitor (47). In the current study, we identified that parameters used for CRC diagnosis are rather variable in the published studies. However, in general, the studies herein selected showed that mouse CM model seems to be adequate to investigate the CRC phenomenon. According to selected studies, parameters used to CRC diagnosis in mouse CM model were loss of body weight (71.4%) and gastrocnemius muscle mass (57.1%). A combination between epididymal adipose tissue mass and total and carcass lipid dosage was evaluated in 28.5% of studies. CRC diagnosis is commonly based on loss of body weight, sarcopenia, and decrease of white adipose tissue storages assessed by simple devices, such as analytical balance and metric tape. Overall, the measurements obtained with these devices have been considered reliable. The gold standard to evaluate morpho functional alterations in internal organs (area, volume, and changes in the composition of the original tissue), which are paramount for studies with CRC, is reached with the use of magnetic resonance and computerized tomography imaging devices (48, 49). However, especially for those research centers that do not have sophisticated equipment

for the analysis of weight and volume of internal organs (e.g., the evaluation of skeletal muscle tissues in experimental CRC studies), the combination of more than one evaluation parameter for CRC diagnosis seems to be the most adequate for establishing a reliable diagnosis of the syndrome. Interestingly, about 40-60% of individuals with cancer may present an overweight condition combined with loss of muscle mass (50, 51). However, there is no definition in the published work of the mean time to diagnose cachexia or clinical aspects observed in treated and untreated cachectic animals.

Fat tissue wasting is diagnosed on early stages of CRC and seems to precede the skeletal muscle loss in individuals with cancer (52). Notably, the severity of fat loss in individuals with cancer cannot be attributable to food restriction alone. Although parenteral nutrition may favor the reestablishment of adipose tissue and therefore the gain of body weight, with improvement of the life quality and survival outcomes in individuals with cancer, it does not reverse CRC (53). Plasma total lipid dosage is another indicator parameter that was identified for CRC diagnosis in the studies herein selected. However, in our point of view, this parameter should be used in conjunction with other parameters, such as loss of body weight and muscle mass. It has been evidenced that increased lipid mobilization has a central role in adipose tissue wasting during CRC, while inhibition of adipocyte development and lipid deposition may also contribute (54). Enhanced lipolysis is regulated by adipose triglyceride lipase and hormone-sensitive lipase, once both promote triacylglycerol degradation (55). Moreover, local and systemic lipolysis during CRC is also stimulated by zinc-α2-glycoprotein (ZAG) adipokine that is expressed and secreted by adipocytes and cancer cells (56).

Although certain signs and symptoms related to experimental CRC are relevant to being observed and followed, such as occurrence of fatigue, pain, anorexia, depression, low physical performance (measurement of muscle strength), and biochemical abnormalities (plasma levels of albumin and hemoglobin), they were not considered in the studies selected in this present review (57, 58).

Only one study herein selected did not perform oral administration (gavage or mixing into the diet) as delivery route to anti-inflammatory drugs used for mouse CM-related cachexia models. Findings from these studies showed that drugs exhibited both antineoplastic and anticachectic effects. In the studies where no drug effect was observed on tumor reduction, no effect on CRC was noted. Although etiology of CRC has not been fully unveiled, individuals with CRC typically maintain an ongoing low-

grade systemic chronic inflammatory state (59). Therapeutic interventions using nonselective anti-inflammatory agents have potential to exert antitumor effects and also ameliorate CRC (60). Apparently, the efficiency of the anti-inflammatory drug treatments for mouse MC-related cachexia models also primarily depended on the antitumor effects of these drugs (57, 61). Some studies have shown that therapies with antitumor properties, after promoting the decrease in the development of the tumor, consequently trigger an anti-cachexia effect (62, 63). As previously mentioned, mouse cancer models that preserve or simulate both humoral and cellular immune response characteristics that occur during molecular and cellular interaction between host and cancer cells are fundamental to uncover the aspects of tumor biology. Hence, it is also possible to optimally develop efficacious anticancer therapies in preclinical research (64).

The analysis of effect size shows that body weight is the parameter that has the greatest influence over the treatment, being consistent with the statistical difference presented throughout the studies. The use of the effect size approach adds information to the statistical significance concept once it allows for measurement of the real significance aggregated by the intervention, through the observed effect description, which is independent of a possible mistaken effect generated in function of the sample size (65, 66).

In conclusion, our review showed that the most common mouse CM model for CRC investigation is represented by subcutaneous inoculation of B16F10 cells into C57BL/6 animals. CRC in that mouse cancer model is predominantly defined by monitoring the animal body weight, and secondarily by measuring skeletal muscle mass and epididymis adipose tissue mass. Moreover, it was noted from studies herein selected that anticachectic effect promoted by the antinflammatory drugs was more efficient when the reduction of tumor volume occurred concomitantly. According to the effect size analysis, the anti-cachectic effect of drugs is better perceived when the parameter loss of body weight is used to experimental CRC diagnosis. Signals and symptoms-related to cachexia, physical performance status, imaging of internal target organs, and molecular disturbance investigations are poorly explored or nonexistent in the mouse CM-related cachexia studies. Despite its unquestionable relevance to the poorer outcome in individuals with late stage of cancer, including CM, CRC is still underdiagnosed and seldom treated. Our findings highlight a need of future studies that

delineate the contribution of mouse CM-related cachexia model for CM treatment in human beings.

Conflict of interest

The authors declare that there is no conflict of interests that could influence the impartiality of the research reported.

Acknowledgements

We would like to thank the Foundation for Research Support of the Minas Gerais State (FAPEMIG; grant numbers: PPM-00029-17 and DEG-00010-16); National Council for Scientific and Technological Development (CNPq, grant number: 150038/2017-8 and 430759/2016-9); and the Coordination of Improvement of Higher Education Personnel (CAPES) for the support on carrying out this study. AMB De-Paula is research productivity fellow of the CNPq.

5. References

1. Fearon K, Strasser F, Anker SD, Bosaeus I, Bruera E, Fainsinger RL, et al. Definition and classification of cancer cachexia: an international consensus. The Lancet Oncology. 2011 May;12(5):489-95. PubMed PMID: 21296615. Epub 2011/02/08. eng.
2. Iranzo CC, Rubia-Ortí JEDL, Castillo SS, Firmino-Canhoto J. Lesões cutâneas malignas e pré-malignas: conhecimentos, hábitos e campanhas de prevenção solar. Acta Paulista de Enfermagem. 2015;28:2-6.
3. Argiles JM, Lopez-Soriano FJ, Busquets S. Mechanisms to explain wasting of muscle and fat in cancer cachexia. Current opinion in supportive and palliative care. 2007 Dec;1(4):293-8. PubMed PMID: 18685378. Epub 2008/08/08. eng.
4. Chopard A, Hillock S, Jasmin BJ. Molecular events and signalling pathways involved in skeletal muscle disuse-induced atrophy and the impact of countermeasures. Journal of cellular and molecular medicine. 2009 Sep;13(9B):3032-50. PubMed PMID: 19656243. Pubmed Central PMCID: PMC4516463. Epub 2009/08/07. eng.
5. Fearon KC, Glass DJ, Guttridge DC. Cancer cachexia: mediators, signaling, and metabolic pathways. Cell metabolism. 2012 Aug 8;16(2):153-66. PubMed PMID: 22795476. Epub 2012/07/17. eng.
6. Davis MP, Dickerson D. Cachexia and anorexia: cancer's covert killer. Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer. 2000 May;8(3):180-7. PubMed PMID: 10789957. Epub 2000/05/02. eng.
7. Kalantar-Zadeh K, Rhee C, Sim JJ, Stenvinkel P, Anker SD, Kovesdy CP. Why cachexia kills: examining the causality of poor outcomes in wasting conditions. Journal of cachexia, sarcopenia and muscle. 2013 Jun;4(2):89-94. PubMed PMID: 23749718. Pubmed Central PMCID: PMC3684705. Epub 2013/06/12. eng.
8. Houten L, Reilley AA. An investigation of the cause of death from cancer. Journal of Surgical Oncology. 1980;13(2):111-6.
9. Wick MR. Cutaneous melanoma: A current overview. Seminars in diagnostic pathology. 2016 Jul;33(4):225-41. PubMed PMID: 27229301. Epub 2016/05/28. eng.
10. Eggermont AM, Spatz A, Robert C. Cutaneous melanoma. Lancet (London, England). 2014 Mar 1;383(9919):816-27. PubMed PMID: 24054424. Epub 2013/09/24. eng.
11. Baroudjian B, Pages C, Lebbe C. [Melanoma, from diagnosis to treatment]. Revue de l'infirmiere. 2016 Mar(219):16-8. PubMed PMID: 26944639. Epub 2016/03/06. Melanomes, du diagnostic a la prise en charge. fre.
12. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA: a cancer journal for clinicians. 2017 Jan;67(1):7-30. PubMed PMID: 28055103. Epub 2017/01/06. eng.
13. Brehmer F, Ulrich M, Haenssle HA. Strategies for early recognition of cutaneous melanoma-present and future. Dermatology practical & conceptual. 2012 Jul;2(3):203a06. PubMed PMID: 23785608. Pubmed Central PMCID: PMC3663361. Epub 2013/06/21. eng.
14. McCarthy SW, Scolyer RA. Pitfalls and Important Issues in the Pathologic Diagnosis of Melanocytic Tumors. The Ochsner Journal. 2010 Summer;10(2):66-74. PubMed PMID: PMC3096206.
15. Clark WH, Jr., Reimer RR, Greene M, Ainsworth AM, Mastrangelo MJ. Origin of familial malignant melanomas from heritable melanocytic lesions. 'The B-K mole syndrome'. Archives of dermatology. 1978 May;114(5):732-8. PubMed PMID: 646394. Epub 1978/05/01. eng.

16. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA: a cancer journal for clinicians. 2015 Jan-Feb;65(1):5-29. PubMed PMID: 25559415. Epub 2015/01/07. eng.
17. Lee JE, Mansfield PF, Ross MI. Cutaneous melanoma metastases. The New England journal of medicine. 1998 Mar 26;338(13):922-3. PubMed PMID: 9518292. Epub 1998/03/28. eng.
18. Schoenewolf NL, Belloni B, Simcock M, Tonolla S, Vogt P, Scherrer E, et al. Clinical implications of distinct metastasizing preferences of different melanoma subtypes. European journal of dermatology : EJD. 2014 Mar-Apr;24(2):236-41. PubMed PMID: 24721680. Epub 2014/04/12. eng.
19. Das SK, Eder S, Schauer S, Diwoky C, Temmel H, Guertl B, et al. Adipose triglyceride lipase contributes to cancer-associated cachexia. Science (New York, NY). 2011 Jul 8;333(6039):233-8. PubMed PMID: 21680814. Epub 2011/06/18. eng.
20. Voltarelli FA, Frajacomo FT, Padilha CdS, Testa MTJ, Cella PS, Ribeiro DF, et al. Syngeneic B16F10 Melanoma Causes Cachexia and Impaired Skeletal Muscle Strength and Locomotor Activity in Mice. Frontiers in Physiology. 2017 09/2905/12/received09/05/accepted;8:715. PubMed PMID: PMC5626871.
21. Kawamura I, Yamamoto N, Sakai F, Yamazaki H, Naoe Y, Inami M, et al. Activation of lipoprotein lipase and inhibition of B16 melanoma-induced cachexia in mice by ponalrestat, an aldose reductase inhibitor. Anticancer research. 1999 Jan-Feb;19(1A):341-8. PubMed PMID: 10226565. Epub 1999/05/05. eng.
22. Becker JC, Houben R, Schrama D, Voigt H, Ugurel S, Reisfeld RA. Mouse models for melanoma: a personal perspective. Experimental dermatology. 2010 Feb;19(2):157-64. PubMed PMID: 19849715. Epub 2009/10/24. eng.
23. Benny Klimek ME, Aydogdu T, Link MJ, Pons M, Koniaris LG, Zimmers TA. Acute inhibition of myostatin-family proteins preserves skeletal muscle in mouse models of cancer cachexia. Biochemical and biophysical research communications. 2010 Jan 15;391(3):1548-54. PubMed PMID: 20036643. Epub 2009/12/29. eng.
24. Khatib MN, Shankar A, Kirubakaran R, Gaidhane A, Gaidhane S, Simkhada P, et al. Ghrelin for the management of cachexia associated with cancer. Cochrane Database of Systematic Reviews. 2016 (6). PubMed PMID: CD012229.
25. McCarthy DO, Graves E. Conjugated linoleic acid preserves muscle mass in mice bearing the Lewis lung carcinoma, but not the B16 melanoma. Research in nursing & health. 2006 Apr;29(2):98-104. PubMed PMID: 16532476. Epub 2006/03/15. eng.
26. Salustiano EJ, Dumas ML, Silva-Santos GG, Netto CD, Costa PR, Rumjanek VM. In vitro and in vivo antineoplastic and immunological effects of pterocarpanquinone LQB-118. Investigational new drugs. 2016 Oct;34(5):541-51. PubMed PMID: 27189479. Epub 2016/05/18. eng.
27. Graves E, Ramsay E, McCarthy DO. Inhibitors of COX activity preserve muscle mass in mice bearing the Lewis lung carcinoma, but not the B16 melanoma. Research in nursing & health. 2006 Apr;29(2):87-97. PubMed PMID: 16532483. Epub 2006/03/15. eng.
28. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ. 2009;339.
29. Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? Controlled clinical trials. 1996 Feb;17(1):1-12. PubMed PMID: 8721797. Epub 1996/02/01. eng.

30. Selya AS, Rose JS, Dierker LC, Hedeker D, Mermelstein RJ. A Practical Guide to Calculating Cohen's f(2), a Measure of Local Effect Size, from PROC MIXED. Frontiers in Psychology. 2012 04/1702/22/received03/27/accepted;3:111. PubMed PMID: PMC3328081.
31. Parks G, Director. Manual of Animal Technology. The Canadian Veterinary Journal. 2009;50(4):374-. PubMed PMID: PMC2657517.
32. Delmonico MJ, Harris TB, Lee JS, Visser M, Nevitt M, Kritchevsky SB, et al. Alternative definitions of sarcopenia, lower extremity performance, and functional impairment with aging in older men and women. Journal of the American Geriatrics Society. 2007 May;55(5):769-74. PubMed PMID: 17493199. Epub 2007/05/12. eng.
33. Martin L, Birdsell L, Macdonald N, Reiman T, Clandinin MT, McCargar LJ, et al. Cancer cachexia in the age of obesity: skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013 Apr 20;31(12):1539-47. PubMed PMID: 23530101. Epub 2013/03/27. eng.
34. Bachmann J, Heiligensetzer M, Krakowski-Roosen H, Buchler MW, Friess H, Martignoni ME. Cachexia worsens prognosis in patients with resectable pancreatic cancer. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 2008 Jul;12(7):1193-201. PubMed PMID: 18347879. Epub 2008/03/19. eng.
35. Deans C, Wigmore SJ. Systemic inflammation, cachexia and prognosis in patients with cancer. Current opinion in clinical nutrition and metabolic care. 2005 May;8(3):265-9. PubMed PMID: 15809528. Epub 2005/04/06. eng.
36. Danciu C, Oprean C, Coricovac DE, Andreea C, Cimpean A, Radeke H, et al. Behaviour of four different B16 murine melanoma cell sublines: C57BL/6J skin. International journal of experimental pathology. 2015 Apr;96(2):73-80. PubMed PMID: 25664478. Pubmed Central PMCID: PMC4459798. Epub 2015/02/11. eng.
37. Fang L, Lee VC, Cha E, Zhang H, Hwang ST. CCR7 regulates B16 murine melanoma cell tumorigenesis in skin. Journal of leukocyte biology. 2008 Oct;84(4):965-72. PubMed PMID: 18519742. Pubmed Central PMCID: PMC2538602. Epub 2008/06/04. eng.
38. Wang HH, McIntosh AR, Hasinoff BB, MacNeil B, Rector E, Nance DM, et al. Regulation of B16F1 melanoma cell metastasis by inducible functions of the hepatic microvasculature. European journal of cancer (Oxford, England : 1990). 2002 Jun;38(9):1261-70. PubMed PMID: 12044514. Epub 2002/06/05. eng.
39. Kotieva IM, Kit OI, Frantsiyants EM, Kaplieva IV, Trepitaki LK, Bandovkina VA, et al. 1724PSome mechanisms of increasing malignancy of B16/F10 melanoma in female mice with chronic pain. Annals of Oncology. 2017;28(suppl_5):mdx391.023-mdx391.023.
40. Baird JR, Byrne KT, Lizotte PH, Toraya-Brown S, Scarlett UK, Alexander MP, et al. Immune-Mediated Regression of Established B16F10 Melanoma by Intratumoral Injection of Attenuated Toxoplasma gondii Protects against Rechallenge. The Journal of Immunology. 2013;190(1):469.
41. Overwijk WW, Restifo NP. B16 as a Mouse Model for Human Melanoma. Current protocols in immunology / edited by John E Coligan [et al]. 2001;CHAPTER:Unit-20.1. PubMed PMID: PMC2763508.
42. Zhang X-H, Qiao E-Q, Gao Z, Yuan H-Q, Cai P-F, Li X-M, et al. Efficacy of combined axitinib with dacarbazine in a B16F1 melanoma xenograft model. Oncology Letters. 2013 05/1412/20/received04/30/accepted;6(1):69-74. PubMed PMID: PMC3742768.

43. Fidler IJ, Nicolson GL. Organ selectivity for implantation survival and growth of B16 melanoma variant tumor lines. Journal of the National Cancer Institute. 1976 Nov;57(5):1199-202. PubMed PMID: 1003551. Epub 1976/11/01. eng.
44. Stei MM, Loeffler KU, Holz FG, Herwig MC. Animal Models of Uveal Melanoma: Methods, Applicability, and Limitations. BioMed Research International. 2016;2016:9.
45. Jo E, Lee SR, Park BS, Kim JS. Potential mechanisms underlying the role of chronic inflammation in age-related muscle wasting. Aging clinical and experimental research. 2012 Oct;24(5):412-22. PubMed PMID: 22717404. Epub 2012/06/22. eng.
46. Talmadge JE, Singh RK, Fidler IJ, Raz A. Murine models to evaluate novel and conventional therapeutic strategies for cancer. Am J Pathol. 2007 Mar;170(3):793-804. PubMed PMID: 17322365. Pubmed Central PMCID: PMC1864878. Epub 2007/02/27. eng.
47. Hatakeyama S, Yamamoto H, Ohyama C. Tumor formation assays. Methods in enzymology. 2010;479:397-411. PubMed PMID: 20816179. Epub 2010/09/08. eng.
48. Gray C, MacGillivray TJ, Eeley C, Stephens NA, Beggs I, Fearon KC, et al. Magnetic resonance imaging with k-means clustering objectively measures whole muscle volume compartments in sarcopenia/cancer cachexia. Clinical nutrition (Edinburgh, Scotland). 2011 Feb;30(1):106-11. PubMed PMID: 20727625. Epub 2010/08/24. eng.
49. Prado CM, Birdsell LA, Baracos VE. The emerging role of computerized tomography in assessing cancer cachexia. Current opinion in supportive and palliative care. 2009 Dec;3(4):269-75. PubMed PMID: 19667996. Epub 2009/08/12. eng.
50. Ryan AM, Power DG, Daly L, Cushen SJ, Ni Bhuachalla E, Prado CM. Cancer-associated malnutrition, cachexia and sarcopenia: the skeleton in the hospital closet 40 years later. The Proceedings of the Nutrition Society. 2016 May;75(2):199-211. PubMed PMID: 26786393. Epub 2016/01/21. eng.
51. Bonaldo P, Sandri M. Cellular and molecular mechanisms of muscle atrophy. Disease models & mechanisms. 2013 Jan;6(1):25-39. PubMed PMID: 23268536. Pubmed Central PMCID: PMC3529336. Epub 2012/12/27. eng.
52. Kliewer KL, Ke JY, Tian M, Cole RM, Andridge RR, Belury MA. Adipose tissue lipolysis and energy metabolism in early cancer cachexia in mice. Cancer biology & therapy. 2015;16(6):886-97. PubMed PMID: 25457061. Pubmed Central PMCID: PMC4622729. Epub 2014/12/03. eng.
53. Porporato PE. Understanding cachexia as a cancer metabolism syndrome. Oncogenesis. 2016 02/22/online;5:e200.
54. Bing C, Trayhurn P. Regulation of adipose tissue metabolism in cancer cachexia. Current opinion in clinical nutrition and metabolic care. 2008 May;11(3):201-7. PubMed PMID: 18403913. Epub 2008/04/12. eng.
55. Bing C. Lipid mobilization in cachexia: mechanisms and mediators. Current opinion in supportive and palliative care. 2011 Dec;5(4):356-60. PubMed PMID: 21934502. Epub 2011/09/22. eng.
56. Mracek T, Stephens NA, Gao D, Bao Y, Ross JA, Ryden M, et al. Enhanced ZAG production by subcutaneous adipose tissue is linked to weight loss in gastrointestinal cancer patients. British journal of cancer. 2011 Feb 1;104(3):441-7. PubMed PMID: 21245862. Pubmed Central PMCID: PMC3049573. Epub 2011/01/20. eng.
57. Penet MF, Bhujwalla ZM. Cancer cachexia, recent advances, and future directions. Cancer journal (Sudbury, Mass). 2015 Mar-Apr;21(2):117-22. PubMed PMID: 25815852. Pubmed Central PMCID: PMC4910156. Epub 2015/03/31. eng.

58. Schwarz S, Prokopchuk O, Esefeld K, Groschel S, Bachmann J, Lorenzen S, et al. The clinical picture of cachexia: a mosaic of different parameters (experience of 503 patients). BMC cancer. 2017 Feb 14;17(1):130. PubMed PMID: 28193264. Pubmed Central PMCID: PMC5307774. Epub 2017/02/15. eng.
59. Tsoli M, Robertson G. Cancer cachexia: malignant inflammation, tumorkines, and metabolic mayhem. Trends in endocrinology and metabolism: TEM. 2013 Apr;24(4):174-83. PubMed PMID: 23201432. Epub 2012/12/04. eng.
60. MacDonald N. Cancer cachexia and targeting chronic inflammation: a unified approach to cancer treatment and palliative/supportive care. The journal of supportive oncology. 2007 Apr;5(4):157-62; discussion 64-6, 83. PubMed PMID: 17500503. Epub 2007/05/16. eng.
61. Rahman M, Halade GV, El Jamali A, Fernandes G. Conjugated linoleic acid (CLA) prevents age-associated skeletal muscle loss. Biochemical and biophysical research communications. 2009 Jun 12;383(4):513-8. PubMed PMID: 19393220. Pubmed Central PMCID: PMC2893570. Epub 2009/04/28. eng.
62. Beluzi M, Peres SB, Henriques FS, Sertie RA, Franco FO, Santos KB, et al. Pioglitazone treatment increases survival and prevents body weight loss in tumor-bearing animals: possible anti-cachectic effect. PloS one. 2015;10(3):e0122660. PubMed PMID: 25807446. Pubmed Central PMCID: PMC4373945. Epub 2015/03/26. eng.
63. Martins HA, Sehaber CC, Hermes-Uliana C, Mariani FA, Guarnier FA, Vicentini GE, et al. Supplementation with L-glutamine prevents tumor growth and cancer-induced cachexia as well as restores cell proliferation of intestinal mucosa of Walker-256 tumor-bearing rats. Amino acids. 2016 Dec;48(12):2773-84. PubMed PMID: 27539646. Epub 2016/08/20. eng.
64. Day C-P, Merlino G, Van Dyke T. Preclinical Mouse Cancer Models: A Maze of Opportunities and Challenges. Cell. 2015;163(1):39-53.
65. Roger EK. Practical Significance: A Concept Whose Time Has Come. Educational and Psychological Measurement. 1996 1996/10/01;56(5):746-59.
66. Selya AS, Rose JS, Dierker LC, Hedeker D, Mermelstein RJ. A Practical Guide to Calculating Cohen's f(2), a Measure of Local Effect Size, from PROC MIXED. Front Psychol. 2012;3:111. PubMed PMID: 22529829. Pubmed Central PMCID: PMC3328081. Epub 2012/04/25. eng.

LEGEND OF FIGURES

Figure 1. Flowchart containing information about the full procedures from screening to analysis of studies, according to recommendations of the PRISMA methodology (LIBERATI, 2009).

Figure 2. Effect size according to parameters considered to diagnosis of cancer-related cachexia in mouse tumor model. A) Effect size of treatment on body weight as a parameter to monitoring of cancer-related cachexia. B) Effect size of treatment on gastrocnemius muscle mass to monitoring of cancer-related cachexia. Classification of effect size: trivial (< 0.2), small (≥ 0.2 - < 0.6), moderate (≥ 0.6 - < 1.2), large (≥ 1.2 - < 2), very large (≥ 2 - < 4), almost perfect (= 4), and perfect (> 4) (Hopkins, 2009).

Figure 3: Action of anti-inflammatory drugs in melanoma-related cachexia, with focus on tumor and the consumption of components that form the adipose tissue and muscular tissue (Adapted from Servier Medical Art, www.servier.com).

FIGURES

Figure 1.

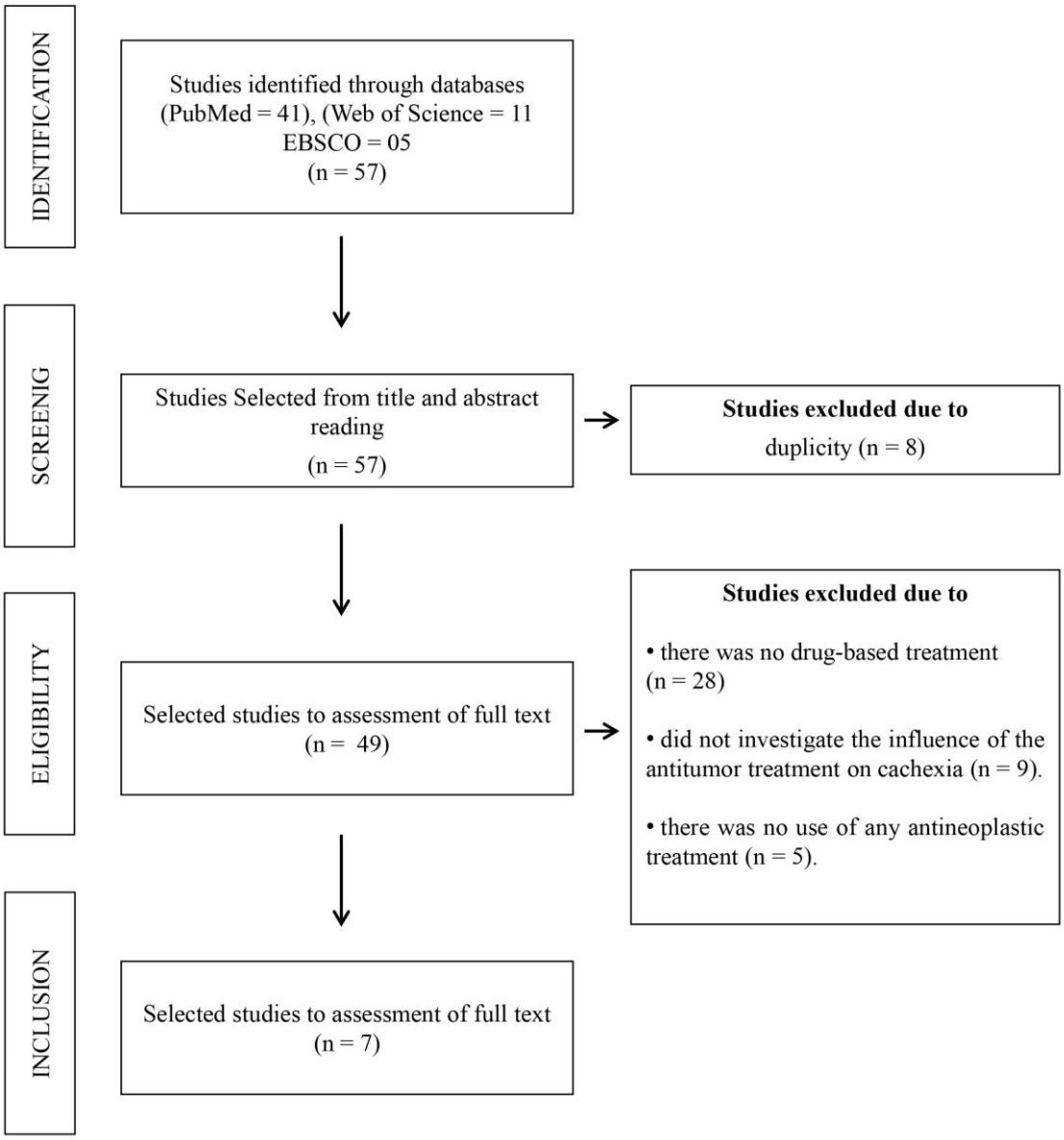


Figure 2.

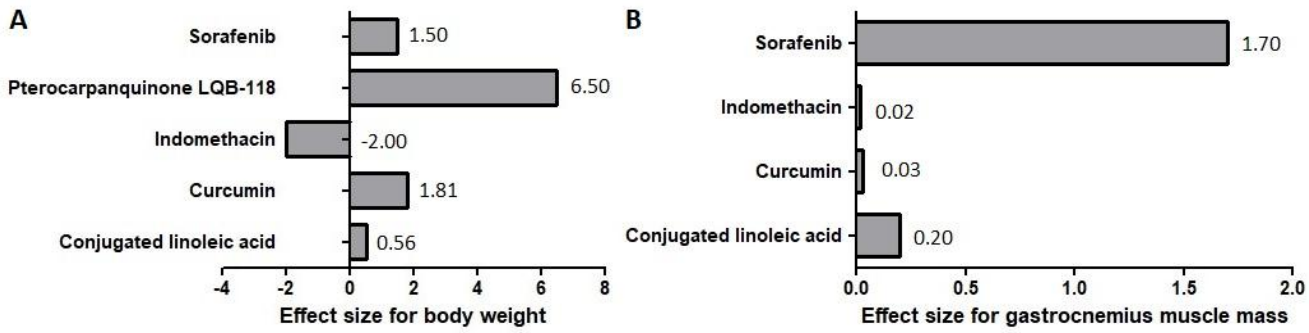
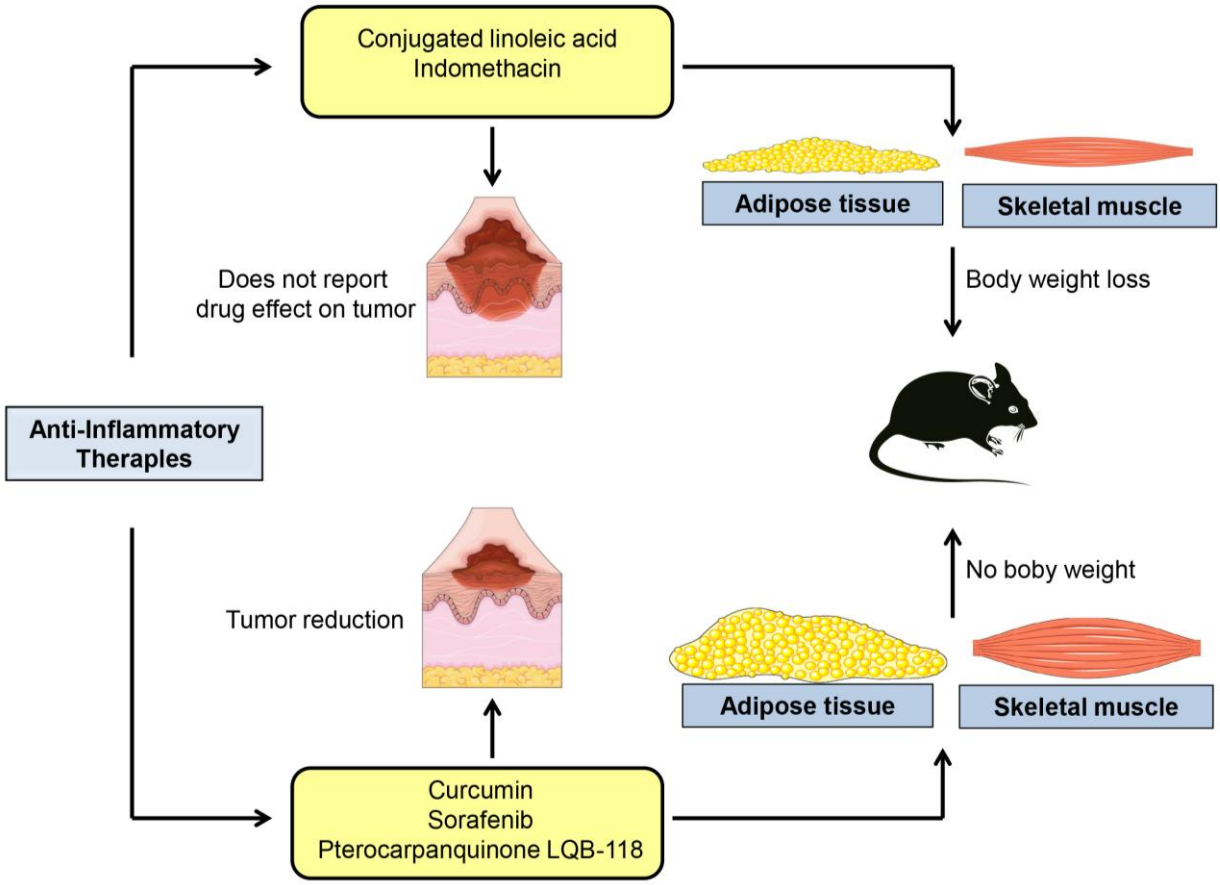

A
Sorafenib
Pterocarpanquinone LQB-118
Indomethacin
Curcumin
Conjugated linoleic acid
1.50
6.50
-2.00
1.81
0.56
-4
-2
0
2
4
6
8
Effect size for body weight
B
Sorafenib
Indomethacin
Curcumin
Conjugated linoleic acid
1.70
0.02
0.03
0.20
0.0
0.5
1.0
1.5
2.0
Effect size for gastrocnemius muscle mass

Figure 3.


Conjugated linoleic acid
Indomethacin
Adipose tissue
Skeletal muscle
Does not report
drug effect on tumor
Body weight loss
Anti-Inflammatory
Theraples
Tumor reduction
No boby weight
Adipose tissue
Skeletal muscle
Curcumin
Sorafenib
Pterocarpanquinone LQB-118

TABLES

Table 1. Characteristics cutaneous melanoma cells and mouse models of cutaneous melanoma-related cachexia found in the studies included in this review.

Author/Year	CM cell type	Quantity of inoculated murine CM cells	Mouse/Gender/(n)	Control group	Anatomical site of CM cells inoculation
Kawamura et al., 1999a	B16F10	Not reported	Not reported	Yes	Intraperitoneal
Kawamura et al., 1999b	B16F10	Not reported	Not reported	Yes	Intraperitoneal
McCarthy & Graves, 2006	B16F10	5×10^5	C57BL/6 – Female ($n_T = 24$; n = 6/group)	Yes	Subcutaneous between the Scapulae
Graves et al., 2006	B16F10	5×10^5	C57BL/6 – Female ($n_T = 30$; n = 6/group)	Yes	Subcutaneous between the scapulae
Beckett et al., 2008	B16F10	$5x10^5$	C57BL/6 – Female ($n_T = 30$; $n_{NTu} = 6$/group; $n_{Tu} = 9$/group)	Yes	Subcutaneous between the scapulae
Toledo et al., 2014	B16F10	$5x10^5$	C57BL/6 – Female ($n_T = 22$; $n_{NTu} = 6$/group; $n_{Tu} = 8$/group)	Yes	Subcutaneous
Salustiano et al., 2016	B16F10	1×10^5	C57BL/6 – Female ($n_T = 30$; n = 10/group)	Yes	Subcutaneous between the scapulae

* n_T = Total sample; number; n_{NTu} = Sample number of Non-Tumor groups; n_{Tu} = Sample number of Tumor groups

Table 2. Characteristics of anti-cachectic drugs and treatments of the murine CM-related cachexia model found in studies included in this review.

Author, Year	Pharmacological agent	Classification of pharmacological agent	Treatment frequency and route of administration	Drug effects in the tumor	Diagnosis of CRC	Main effects of treatment on CRC
Kawamura et al., 1999a	Ponalrestat	Aldose reductase inhibitor Anti-inflammatory	Not reported	Not reported	Weight of epididymal adipose, carcass and total lipid, dosage of triglycerides and non-esterified fatty acids, locomotor activity.	It attenuated the adipose weight loss and improved locomotor activity. It increased levels of triglycerides and non-esterified fatty acids.
Kawamura et al., 1999b	Bezafibrate and Ibrolipim (NO-1886)	Anti-lipemic agent Anti-inflammatory	Not reported	Not reported	Weight of epididymal adipose, carcass and total lipid, dosage of triglycerides and non-esterified fatty acids.	It attenuated the adipose weight loss. It increased levels of triglycerides and non-esterified fatty acids.
McCarthy & Graves, 2006	Conjugated linoleic acid	Anti-inflammatory	17 days 0.5% drug mixed in the diet	Not reported	Body weight, and weight of gastrocnemius muscles.	It did not show preservation of muscle mass. Body weight loss.
Graves et al., 2006	Indomethacin	Anti-inflammatory	17 days 5 mg/kg/day 1 mg/kg/day Mixed in the diet	Not reported	Body weight, and gastrocnemius muscle weight.	Reduced muscle mass. Body weight loss.
Beckett et al., 2008	Curcumin	Antioxidant Anti-inflammatory	17 days 150 mg/kg/day Mixed in the diet	Decreased tumor	Body weight, and weight of soleus, plantaris and gastrocnemius muscles.	There was not body weight loss; Skeletal muscle mass loss.
Toledo et al., 2014	Sorafenib	Antineoplastic Anti-inflammatory	14 days 90 mg/kg/day Gavage	Decreased tumor	Body weight, and weight of the gastrocnemius and tibial muscles, White adipose tissue weight, Total physical activity, Food consumption.	There was not body weight loss. Mass muscle were reduced. Increased white adipose tissue. Improvement in total physical activity. Increased food consumption.
Salustiano et al., 2016	Pterocarpanquinone LQB-118	Antineoplastic Anti-inflammatory	10 days 0.36 mg/kg/day Intraperitoneal	Decreases tumor	Body weight.	There was not body weight loss.

Table 3. Quality of evidence (adapted Jadad Scale).

Author (year)	Score	Evidence Level
Kawamura et al., 1999a	2	B
McCarthy & Graves, 2006	3	B
Kawamura et al., 1999b	2	B
Salustiano et al., 2016	4	A
Graves et al., 2006	3	B
Beckett et al., 2008	2	B
Toledo et al., 2014	4	A

A = 4 points; B = 2 - 3 points; C < 2 points.

3.2 PRODUTO 2

Resveratrol decreases low-degree chronic inflammation, inhibits body weight and skeletal muscle mass losses, decreases pro-cachectic myokines and adipokines expression, and improves cancer-related survival in C57BL/6 mice bearing syngeneic tumor

Running-title: Anti-cachectic effects of resveratrol in mouse cancer-related cachexia model.

Otávio Cardoso-Filho[1]; Magda Mendes Vieira[1], Andréia de Souza Brito[1]; João Lucas Rodrigues dos Santos[1]; Amanda Rodrigues da Silva[1]; Valéria Couto Quintão[1], Lorrane Katherine Martins Pereira[1], Maria Isabela Alves Bernardo[1], Erivelton Pereira dos Santos[1], Amanda Souto Machado[1]; Vinicius Dias Rodrigues[1,2], Gefter Thiago Batista Corrêa[3]; Sérgio Henrique Sousa Santos[1,4], Lucyana Conceição Farias[1,5], André Luiz Sena Guimarães[1,5], Ludmilla Regina de Souza David[1], Alfredo Mauricio Batista De-Paula[1,5]

[1] Nucleus of Epidemiologic and Molecular Research Catrumano (Nupemoc). Health Research Laboratory. Post-graduate Programme in Health Sciences. Universidade Estadual de Montes Claros, 39401-001, Montes Claros, Minas Geras, Brazil.

[2] Department of Physical Education. Universidade Estadual de Montes Claros, Unimontes, 39401-001, Montes Claros, Minas Gerais, Brazil.

[3] Department of Dentistry. Faculdades Independentes do Nordeste, 45055-030, Vitória a Conquista, Bahia, Brazil.

[4] Institute of Agricultural Sciences. Food Engineering College. Universidade Federal de Minas Gerais, 39404-547, Montes Claros, Minas Gerais, Brazil.

[5] Department of Dentistry. Universidade Estadual de Montes Claros, Unimontes, 39401-001, Montes Claros, Minas Gerais, Brazil.

Address correspondence to:

Alfredo Maurício Batista De-Paula.
Programa de Pós-graduação em Ciências da Saúde.
Hospital Universitário Clemente de Faria - Universidade Estadual de Montes Claros.
Avenida Cula Mangabeira, 562. Bairro Santo Expedito, Montes Claros.
Minas Gerais, Brazil.
CEP: 39401-001.
Phone: 55-21-38 32248380
e-mail: ambpatologi@gmail.com

Abstract

Background During tumor progression, might occur cancer-related cachexia (CRC), a paraneoplastic syndrome characterized as progressive, systemic physical consumption state of individual with cancer. Trans-resveratrol (3,4,5-trans-trihydroxystilbene, Resv) is a naturally occurring polyphenol. It has been evidenced modulatory roles of Resv on disturbed molecular pathways related to cancer progression. But, the influence of Resv on adipose and skeletal muscle tissues during CRC is still inconclusive.

Aim We investigated the effects of Resv on plasma inflammatory biomarker, skeletal muscle (SM), white adipose tissues (WAT), and survival from C57BL/6 mice bearing syngeneic cutaneous-melanoma (CM) model.

Material and Methods Murine B16F10 cells were injected into flank of the fifty-eight female C57BL/6 mice in order to establish a syngeneic mouse CM-related cachexia model. CRC diagnosis was individually established for each animal using as parameter weight loss $\geq$ 5%. Resv was administered in concentrations of 200 and 400mg/Kg body weight using oral gavage in both control and experimental mice. The consumption of water and food, body weight, and tumor size were daily measured. Albumin and C-reactive protein (CRP) serum levels were measured by enzyme immunoassays. SM strength, volume, and mass were assessed using a grip strength meter, a high-frequency ultrasound device, and an analytical balance, respectively. SM, WAT, and BAT samples were collected and submitted to morphometric and gene expression analysis. Control and experimental mice were submitted to cancer-related survival (CRS) analysis.

Results Mice treated with Resv significantly promoted a delayed in CRC occurrence, promoted gain of body weight, and improved survival of mice with CRC ($p = 0.038$, $p = 0,001$, and $p = 0.040$, respectively). In SM of mice, Resv administration increased SMT weight (Resv200mg/kg $p < 0.05$ e Resv400mg/kg, $p < 0.001$) and volume (Resv200mg/kg, $p < 0.01$ e Resv400mg/kg, $p < 0.05$), muscle strength (Resv200mg/kg $p < 0.05$), and quantity of muscle fibers (Resv200mg/kg, $p < 0.001$ e Resv400mg/kg, $p < 0.01$) in SMT ($p < 0.05$). Resv significantly decreased WAT weight and adipocyte area (Resv200mg/kg, $p < 0.01$ e Resv400mg/kg, $p < 0.001$). Rev treatment increase adiponectin gene expression ($p < 0,001$). Resv administration promoted reduced low-degree chronic inflammation in mice bearing tumor (Resv200mg/kg, $p < 0.001$ e Resv400mg/kg, $p < 0.01$). The most of these effects promoted by Resv were dose-dependent.

Conclusions Resv exhibits a plethora of anticachectic effects on plasma, SMT and WAT from C57BL/6 mice bearing tumor with CRC.

Key-words: cancer-related cachexia, C57BL/6 mice, B16F10 mouse melanoma cell, myokines, adipokines, cancer-related survival.

Introduction

Resveratrol (Resv; 3,4´,5-trihydroxystilbene) is a non-flavonoid, stilbene-based polyphenolic phytoalexin that naturally occurs in many plant sources. Resv molecule comprises two phenolic rings connected by a styrene double bond to produce 3,4´,5-trihydroxystilbene, which naturally occurs in both chemical trans- (major isoform) and cis-isoforms. (1, 2). Functionally, It has been frequently reported that Resv exhibits diverse molecular targets in in components from normal, as well as, disturbed signaling pathways, such as membrane and intracellular receptors and ligands, transcription factors, biogenesis enzymes, intracellular oxidative systems, and DNA-repair, cell proliferation, differentiation, and apoptosis mechanisms (3). Due to that pleiotropic functional characteristic, Resv has been therapeutically used for many human diseases, which exhibit a variety of pathogenesis related to immunological, inflammatory, infectious, metabolic, and neoplastic disturbances (4-9). Cancer-related cachexia (CRC) is a multifactorial paraneoplastic syndrome characterized for involuntary ongoing loss of the skeletal muscle (SMT) and white adipose tissue (WAT) mass, that cannot be fully reversed by conventional nutritional supports. Consequently, CRC frequently leads the individual with cancer to progressive functional impairments ad death (2, 10). CRC is a prevalent pathological systemic condition that occurs in individuals with certain types of cancer since early stages of tumorigenesis but it reaches more clinical significance during late stages of tumor progression. The progressive physical consummation experienced by individuals with CRC mainly occurs due to a combination of anorexia, intense protein catabolism in skeletal muscle fibers and a negative energy balance that occurs in adipose tissues and other tissues. Of note, as occurs in other chronic pathological conditions, individuals with CRC exhibits a typical background of a chronic low-grade inflammation state (11, 12). Frequently, CRC is not readily

recognized, thoroughly assessed, and successfully managed therapeutically. Because of these aspect, CRC has been associated to poor outcomes in individuals with cancer such as, drastic decrease of quality of life, lower tolerance/reduced response to standard antineoplastic therapies, and a shorter survival, usually due to respiratory or cardiac failures. These aspects turns CRC one of the great clinical challenges to health professionals that deal with oncological treatment of individuals with cancer (12, 13).

Because it presents diverse anticancer actions and, additionally, presents little or no cytotoxicity in normal cells, there is a great clinical expectation of the use of Resv as a strategic adjuvant or neoadjuvant agent against cancer. However, there are limitations of Resv as some of its biochemical characteristics that hinder its bioavailability in organism In to concern to anticancer properties, findings from *in vitro* and *in vivo* assays have showed that Resv promotes a set of modulatory roles on various disturbed molecular components that participates of signaling pathways relevant to tumorigenesis and tumor progression phenomenon (14-17).(18, 19). Importantly, a few studies have been evidenced that Resv administration is able to exert anti-cachectic effects in mouse CRC models (1, 17). However, molecular mechanisms triggered by Resv in its anti-cachectic effects on CRC in mouse CRC models have not yet been fully unraveled.

The aim of this study was to analyze the effects of Resv administration on CRC occurrence and cancer-related survival of C57BL/6 mice with syngeneic CRC model. Moreover, we investigated if Resv administration could influence skeletal muscle mass and strength in control and case mice hindlimbs. Moreover, we compared a set of histomorphometry parameters and catabolic gene expression in SMT and WAT. Finally, we compared the plasma expression of inflammatory biomarkers between control and Resv treated mice.

Material and methods

Reagents

All chemicals were purchased from Sigma-Aldrich (Poole, UK) unless otherwise stated. Trans-resveratrol ($C_{14}H_{12}O_3$, 501-36-0) was obtained from Zhejiang Chemicals (Hangzhou, CHI). High glucose Dulbecco's modified Dulbecco's medium (DMEM), Gibco™ RPMI 1640 (Thermo Fisher Scientific, Waltham, MA USA), fetal bovine serum (FBS, Euroclone, MI, ITA), 0.05% trypsine–EDTA, a penicillin-streptomycin mixture 100X (Lonza Walkersville, Inc., Basel, SWI), lipofectamine 2000, 3,4,5-dimethylthiazol-2,5 biphenyl tetrazolium bromide (MTT, Trizol reagent, and TaqMan gene expression assay and DNTP were obtained from Invitrogen, OligodT 15 primer and Random primer they were obtained Promega. Xylazine and ketamine 2% acquired from bayer laboratory.

Cell culture

Murine B16-F10 cutaneous melanoma (CM) cell line (ATCC® CRL-6475™) was obtained from Antitumor Substances Laboratory, Institute of Biological Sciences, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brazil. B16-F10 cells were cultured in DMEM supplemented with 10% FBS (v/v), 2 mM L-glutamine, 100 U/mL penicillin and 100 mg/mL streptomycin, and 50 mM β-mercaptoethanol. The culture medium was filtered with a 0.22 μm filter and stored at -4° C. The pH of this culture medium was adjusted to 7.4. In use, the culture medium will be preheated at 37°C in a 5% CO_2-humidified incubator. After reaching around of 90% cell confluence in the culture plate, the adhered cells were released from the plate after treatment with trypsin for 5 minutes, oven temperature of37° C, and 5% CO_2. Soon after, the trypsin was inactivated with FBS 10%. The detached cells were centrifuged (1000 rpm),

suspended in Gibco™ RPMI 1640 culture medium and the cell concentration measured using Neubauer's mirrored chamber (Grid Optics, OG-200, Hong Kong, CHI).

Ethical aspects

The *in vivo* study design addressed in this research project was analyzed and approved by the Ethics Committee in *Animal Well-being and Experimentation* of the State University of Montes Claros (protocol number: 131/2017), which follows the recommendations of the *Brazilian Code for Use of Laboratory Animals* (20).

Animals, syngeneic mouse tumor model, and treatment with Resv

Seventy-two C57BL/6 female mice, 8-10 weeks old, were obtained from the Animal Facility Center from Federal University of Minas Gerais (UFMG). Mice were maintained under controlled temperature (22 ± 2° C), light (12 h of light/12 h of darkness), relative air humidity (60 ± 5 %) and allowed free access to water and balanced food chow (Purina-Labina®, São Paulo, BRA). The food and water intake were manually measured three-times a week, calculating the difference between the weight of the received water and food and the reweight of the remained water and food, after 24 h after. All mice had body weight assessed daily. After one-week acclimatization, prior to syngeneic C57BL/6 CM model induction, the B16F10 cells were removed from culture flasks by adding 0.05% of trypsin solution, centrifuged, and ressuspended in sterile PBS. C57BL/6 mice were subcutaneously inoculated with 5×10^5 cells/animal (0.05 mL) into the flank using a 1-mL tuberculin syringe (Hamilton Co., Reno, NV, USA) with a 27-gauge hypodermic needle (21, 22).

Animals were randomly assigned into six groups, as follows: i. $Control_{non\text{-}tumor,}$ administration of PBS 0.1 mL (placebo), no tumor induction (n = 8); ii. $Control_{non\text{-}tumor/Resv200,}$ administration of Resv 200mg/Kg body weight, no tumor induction (n = 8); iii. $Control_{non\text{-}tumor/Resv400,}$ administration of Resv 400mg/Kg body weight, no tumor induction (n = 8); iv. $Control_{tumor,}$ administration of PBS 0.1 mL, tumor induction (n = 10); v. $Resv200_{tumor}$, administration of Resv 200 mg/Kg body weight, tumor induction (n = 10); vi. $Resv400_{tumor}$ administration of Resv 400 mg/Kg body weight, tumor induction (n = 10).

Administration of Resv and placebo (PBS) in mice was performed with oral gavage and using a curved, ball-ended metal 1-mL tuberculin needle. Treatments were performed daily for 12 days.

Assessment of tumor volume

To establish a melanoma growth curve, the animals were monitored daily from the onset of clinically palpable tumor. A trained examiner, blinded to the animal group, was responsible for the daily recording of tumor volume using a digital caliper, with resolution of 0.01mm (Mitutoyo CSX-B, São Paulo, BRA) (23). Measurement of tumor volume was determined in cephalad-to-cauda, left-to-right and height dimensions and posteriorly application of the standard formula a prolate ellipsoid, tumor volume (mm^3) = (π x length x width x height/ 6) (24, 25). We also performed the tumor measurement every 5 days after diagnosis of the tumor using a Doppler ultrasound imaging device (LOGIQ® e Pro, GE HealthCare Medical Systems, Inc. Wauwatosa, WI, USA) with electronic linear probe (L8-18i-RS). The gain, frequency, focus position, and depth parameters were adjusted appropriately to make sure that the tumor was displayed clearly on the screen. Tumor volume was scanned transversely, longitudinally and per

depth and image aspects of tumor, such as maximum diameter, margin and shape, were stored in the internal hard disk of the device for subsequent analysis (26).

Diagnosis of the syngeneic mouse CM-related cachexia

All mice were evaluated daily for body weight and water and food intake (Supplementary material. figure S1). Body weight and feed was recorded with the aid of an analytical balance precision digital scale (A.Cientifica EEQ9003E). Water consumption was recorded using a plastic beaker with volume scale (27).

In order that the tumor volume did not interfere with the changes in body weight, the daily tumor weight was subtracted from the daily body weight measurement. Then, a linear regression equation was used to define the relationship between tumor volume measured by the pachymeter and tumor weight. The equation was generated based on a 12-day experiment with tumor bearing animals (n = 12). Each day an animal was sacrificed and the tumor measures (volume and weight) were collected. At the end of the experiment, data were obtained from different tumor stages and the relation between weight and volume was defined in the linear equation (28) (Supplementary material. Figure S2). M-related cachexia was established as soon as the mice bearing syngeneic CM model show a loss of *at least 5% of body weight* during tumor progression (29). Mice were monitored for occurrence of signs of CRC progression, such as prolonged inactivity, dehydration, arched posture, occurrence of dry or color change of skin, coarse/rough coat, reddened eyes, and discharge of nasal secretion (30).

Assessment of skeletal muscle volume

Measurement of the skeletal muscle (SM) perimeter and volume from right hindlimb of C57BL/6 mice were assessed three times a week. SM perimeter was

performed using a digital caliper, with sensitivity of 0.01 mm, CSX-B model (Mitutoyo, São Paulo, BRA). SM volume measurement was performed using a Doppler ultrasound imaging device (LOGIQ® e Pro, GE HealthCare Medical Systems Inc., Wauwatosa, WI, USA) with dynamic range of 258 dB and frame rate of 1449 frames per second. The electronic linear probe with 11.1-mm x 34.8 mm footprint, and bandwidth of 6.7-18 MHz imaging frequency was used (L8-18i-RS). Gain, frequency, focus position, and depth parameters were adjusted appropriately to make sure that the SM tissues (gastrocnemius, tibialis, and quadriceps) were displayed clearly on the screen, scanned transversely and longitudinally. Sonographic images were stored in the internal hard disk of the device for subsequent analysis (31).

Skeletal muscle strength analysis

To perform the hindlimbs and hind limbhindlimbs grip strength analysis in all , we used an automated grip strength meter (Bonther®, São Paulo, BRA) device (32). The assessment of muscle strength in all groups occurred 7 days before inoculation on the day of cancer induction, and 10 and 15 days after. Mice were initially weighed, restarted the meter, chose grams as unit/scale of values, raised the animal by its tail to the height where both hindlimbs and hind limbhindlimbs are at the same height as the bar, moved the animal horizontally to the bar until reaching the reach, visually checked a symmetrical and tight grip with both hindlimbs and hindlimbsexerting a detectable resistance against the investigator pull, gently pulled the animal to away until its grip was separated. The pull was at a constant speed and slow enough to allow the mouse to increase resistance against it, turned backward during traction or left the bar without resistance. Sufficient time (at least one-minute minimum interval) between the measurements was allowed in order to allow the animal to recover and avoid habit

formation. Total peak force was measured and average of three attempts was registered. A normalized grip strength was obtained by dividing the muscle strength value by the body weight of animal.

Euthanasia and collection of biological material

Euthanasia was performed by decapitation on anesthetized animals with ketamine 100 mg/kg and xylazine 10mg/kg, diluted in 100 µl of saline solution. The tumor, blood, SM muscles and BAT and WAT adipose tissues were collected. Tissues were subjected to macroscopic examination, weighed an analytical balance and stored for further analysis.

Plasma inflammatory biomarkers measurement

Serum albumin levels were measured using the bromocresol green dye-binding method with a Roche Modular DP analyzer device (Roche Diagnostics, Basel, SWI) used the Labmax plenno automation (LabTest Diagnóstica, Lagoa Santa, MG, BRA). High-sensitive C-reactive protein (CRP) level was determined with a highly sensitive latex reagent immunoassay (33)

Tissues samples and histomorphometric studies

After euthanasia, hindlimb SM (gastrocnemius, quadriceps, and soleum), brown adipose tissue (BAT) from scapula, white adipose tissue (WAT) from epididymal were removed, weighted, properly fixed, and stored. A fragment of the samples was embedded in optimal cut temperature compound (Tissue-Tek®, Sakura Finetek, USA), frozen in liquid nitrogen-cooled isopentane, stored at -80°C, and posteriorly cut into 10 µm thick cryosections with a cryostat (Microm HM525, Thermo Fisher Scientific, Waltham, MA, USA). Another fresh fragment was kept in RNA holder for posterior gene expression assays. A third fragment was removed and placed

in buffered 10% formalin solution overnight and embedded in paraffin and submitted to five-micron-thick sections using a Leica RM2125 (Leica Biosystems, IL, USA) microtome. SM, WAT, and BAT sections were stained with H&E and Masson´s Trichrome. Tissue sections were observed with microphotographs from these tissues were taken with an Olympus FSX100 microscope (Tokyo, JPN). Histomorphometrical analyses were performed using ImageJ software (33, 34). SM tissue sections were made at 90^{o} and 180^{o} to the longitudinal axis of the muscle fibers. At least five distinct randomly chosen microscopic fields of the SM tissues *per* animal were photographed. We quantified the number of SM fibers. Additionally, we calculated the cross-sectional area (μm2) of the SM fiber as the smallest diameter across an ellipse, which corresponds to the narrowest aspect of the fiber (35). Measurements of quantity and area of the SM fiber were evaluated at final magnification of 200x. A minimum number of fibers (n = 30, *per* animal) were counted to ensure measurements representative of the overall specimen. The presence of lipid droplets in SM tissues (perivascular, interfibrillar, and intramyocellular lipid spaces) was measured through optical density (OD) at 520 nm (36). Morphological images from epididymal WAT and scapular BAT sections were stained with H&E and Oil red O. At least three distinct randomly chosen microscopic fields of the samples per animal were photographed at 20× optical magnification. The images were submitted to morphometric analyses to estimate the number, diameter (μm), and area (μm^2) of the adipocytes. In WAT, in addition to the previously mentioned parameters, the number of intracellular lipid droplets was measured (37).

Initially, microscopic image calibration was performed to recognize the sample size based on the number of pixels and magnification of the microscope. The pixel ratio was set to 1, the unit length in μm and the "Global" box was selected to maintain the

settings of all subsequent image analyzes. To determine these parameters the image was taken with the same magnification of the images of interest (38). Limit image adjustment was performed with the objective of providing defined areas consisting of membrane material and void space identified by black and white, respectively. After defining the areas of interest, the image was converted into a binary format to allow analysis. Each membrane was uniformly augmented to define the cell membrane and to identify the individual area of each cell. Using the "Measure and label macro" tool of ImageJ, the cells were defined by means of a yellow trace in their interior and by a unique number in the center (39). Later the tool "Wand" was in charge of counting, being that the cells that touched the edge of the image were not counted. After the quantification and measurement of the areas, the recorded data were copied, followed by the statistical analysis (40). All histomorphological and histometric analyses were carried out by two authors (de-Paula, AMB and Cardoso-Filho, O) who were blinded to the mice group distribution.

RNA isolation, cDNA synthesis, and quantitative real time-polimerase chain reaction (qPCR) gene expression analysis

Samples were purified using Trizol according to the manufacturer's protocol. RNA was isolated with DNase I (Deoxyribonuclease I, AMBION) to remove genomic DNA contamination. Reverse transcription reaction was performed using transcriptase reversa (M-MLV Reverse Transcriptase – Invitrogen, USA), OligodT (15) primer (PROMEGA), Random Primers (PROMEGA) and Rnase OUT (Invitrogen).The RNA concentration was measured by UV absorbance. RNA samples with an A260/280 ratio between 1.8 to 2.0 were used. RT-PCR reactions were set up in triplicate using TaqMan gene assays and amplified in an QuantStudio^{TM} 6 Flex (Thermofisher). The

amplification reactions werw conducted according to the manufacturer's instructions with the aid of taqman assays. Primers evaluated on muscle tissue samples were GAPDH (Mm99999915_g1), Igf2 (Mm00439564_m1), Myog (Mm00446194_m1), Igf1 (Mm00439560_m1), FBXO32 (Mm00499523_m1), TRIM63 (Mm01185221_m1), TRIM55 (Mm01292969_m1), Srebf (Mm00550338_m1), PPAR-gamma (Mm00440940_m1) were evaluated on dipose tissue samples. Internal template controls (GAPDH) run under the same conditions.

For each gene, the number of cycles required for exponential amplification was determined on 40 cycles. Gene expression levels were normalized to the level of GAPDH expression. Paired t-tests were conducted on relative quantity (RQ) values for each group to determine their significance.

Survival analysis

Groups of $control_{tumor}$ (n = 10) and experimental ($Resv_{200tumor}$ and $Resv_{400tumor}$, n = 10, in each group) mice were submitted to survival analysis between study groups during follow-up of 18 days after CRC diagnosis at most. Kaplan-Meier survival curves were estimated for each event and the curves of the different groups were compared using the log-rank test. However, any mice were euthanized as soon as they become moribund, exhibiting severe impairment of bodily functions or behavior due to extensive necrosis, ulceration, and growth of the tumor mass.

Statistical analysis

All the data collected were scanned in an electronic database. Subsequently, the data were analyzed statistically in the PASW® software (*Predictive Analytics Software*). Results were expressed as mean ± S.D. The confidence level adopted in all analyzes

was established in 95% ($p < 0.05$). Data from all groups were analyzed using Kruskal-Wallis one-way analysis of variance on ranks followed by Bonferroni correction multiple comparison test. The confidence level adopted in all analyzes will be set at 95% ($p < 0.05$).

Results

Resv promoted gain of body weight in the of C57BL/6 bearing-tumor mice.

To determine whether Resv administration could influence body weight, we assessed body weight daily in both control and C57BL/6 bearing-tumor mice. Body weight measurement of bearing-tumor mice was corrected with subtraction of the tumor weight per day. Figure 1A exhibits the evolution of body weight of all control and treated with Resv mice. According to our findings, the non-tumor mice treated with Resv 400 mg/kg ($Control_{non\text{-}tumor/Resv400}$) showed a tendency in exhibiting a greater body weight loss compared to control mice ($Control_{no\text{-}tumor}$). However, bearing-tumor mice treated with Resv ($Resv200_{tumor}$ and $Resv400_{tumor}$) exhibited a lower body weight loss when compared to $Control_{tumor}$ group from the fifth day of treatment. At the end of treatment, all groups treated with resveratrol had statistically different body weights from the $Control_{tumor}$ group (Figure 1A). At the twelfth day, the mean values of the body weight assessment for each studied group were $Control_{non\text{-}tumor}$ = 23.03 ± 1.53 g, $Control_{non\text{-}tumor/Resv200}$ = 21.40 ± 1.18 g, $Control_{non\text{-}tumor/Resv400}$ = 20.18 ± 1.09 g, $Control_{tumor}$ = 17.14 ± 1.51 g, $Resv200_{tumor}$ = 20.81 ± 1.06 g, and $Resv400_{tumor}$ = 18.38 ± 1.24 g (Figure 1C). The difference in body weight between them can be seen in the figure 1B.

Resv administration delayed CRC occurrence and improved CRS of the C57BL/6 bearing-tumor mice

Kaplan-Meier curves represent the time of diagnosis for cancer-related cachexia (CRC) and overall cancer-related survival (CRS) of the C57BL/6 bearing-tumor mice ($Control_{tumor}$, $Resv200_{tumor}$, and $Resv400_{tumor}$) (Figure 2). Mean time for CRC diagnosis in C57BL/6 bearing-tumor mice was $Control_{tumor}$ (5.12 ± 1.12 days), $Resv200_{tumor}$ (8 ± 2.77 days), and $Resv400_{tumor}$ (10.57 ± 2.31 days) (Figure 2A). Our previous findings showed that C57BL/6 mice with syngeneic CM model developed CRC in 6.8 ± 2.01 days in mean (data not shown). In this current study, CRC diagnosis occurred earlier in $Control_{tumor}$ mice compared to other experimental groups. In the seventh day, all mice from $Control_{tumor}$ had developed CRC, while about 50% mice from $Resv200_{tumor}$ and 70% of mice from $Resv400_{tumor}$ were still presenting a non-cachectic or pre-cachectic condition (body weight lesser than 5%). $Control_{tumor}$ group significantly exhibited an earlier CRC occurrence compared to $Resv200_{tumor}$ and $Resv400_{tumor}$ mice groups ($p = 0.018$ for both groups) (Figure 2B). In order to investigate CRS in this study, all mice were followed-up by a period of 18 days after tumor induction. When CRS data were compared using Log-rank test and Kaplan-Meier graphics, it was showed that mice from $Resv200_{tumor}$ and $Resv400_{tumor}$ groups exhibited a better CRS compared to mice from $Control_{tumor}$ group ($p = 0.038$ and $p = 0.040$, respectively) (Figures 2C-D).

Resv promoted gain of skeletal muscle volume and weight in control and bearing-tumor C57BL/6 mice

We investigated whether Resv administration could influence the skeletal muscle (SM) volume from mice. An ultrasound device was used to acquisition of the right hindlimb SM images for volume analysis in each animal. Ultrassonographic exam

was performed at three times during experiment (second, seventh, and eleventh days) (Figure 3A). Our findings showed that all bearing-tumors mice exhibited significant reduction of the SM volume compared to mice from $Control_{non\text{-}tumor}$ groups ($p < 0.001$, for seventh and eleventh days). Additionally, $Resv200_{tumor}$ and $Resv400_{tumor}$ groups exhibited a higher gain of SM volume compared to $Control_{tumor}$ mice on eleventh day of treatment ($p < 0.001$ and $p < 0.05$, respectively) (Figure 3B). Soon after mice sacrifice, right hindlimb MS was assessed macroscopically and then weighed using an analytical balance (Figure 4A). Among non-tumor mice, Resv400 administration promoted SM weight gain compared to $Control_{non\text{-}tumor}$ group ($p < 0.001$). In C57BL/6 mice bearing tumor, our findings showed that $Resv200_{tumor}$ and $Resv400_{tumor}$ mice groups exhibited a higher SM weight gain compared to $Control_{tumor}$ mice ($p < 0.05$ and $p < 0.001$, respectively) (Figure 4B). Posteriorly, the SM which make up hind limb (gastrocnemius, femoral quadriceps, and tibial) were dissected, weighed individually, and data were compared. When compared with $Control_{tumor}$ group, $Resv200_{tumor}$ mice exhibited higher gain of weight in quadriceps ($p < 0.001$) and tibial ($p < 0.05$) muscles while $Resv400_{tumor}$ mice exhibited higher weight gain for gastrocnemius ($p < 0.05$), quadriceps ($p = 0.001$), and tibial ($p = 0.001$) muscles (Figures 4C-E).

Resv promoted increasing of skeletal muscle strength of control and C57BL/6 mice bearing tumor

The SM strength of the forelimbs in control and C57BL/6 mice bearing tumor was performed with a handgrip strength meter. This assessment occurred one day before of starting treatment, and fourth, seventh, and twelfth days of treatment (Figure 5A). According to our findings, on twelfth day, with the exception of the mice of the $Resv400_{tumor}$ group, all the other mice groups showed significant increase of the grip

muscle strength compared to $Control_{tumor}$ mice ($p < 0.05$). Despite this, with seven days of treatment, resveratrol promoted an increase in strength of the animals of the $Resv400_{tumor}$ group. Mice from $Resv200_{tumor}$ group exhibited higher forelimb muscle strength compared to $Control_{tumor}$ mice after four and twelfth days of treatment ($p < 0.05$) (Figure 5A-B).

Resv promoted increase of number but not muscle fiber area of gastrocnemius from control and C57BL/6 mice bearing tumor

Mice from $Control_{tumor}$ exhibited lower number (unity) of SM fibers compared to $Control_{non\text{-}tumor}$, Resv200, Resv400, $Resv200_{tumor}$ ($p < 0.001$, for all associations) and $Resv400_{tumor}$ ($p < 0.01$) (Figure 6A). Assessment of the SM fiber area (μm^2) of the gastrocnemius not showed difference between study groups (Figure 6B). Representative microscopical tissue images of the gastrocnemius muscle from control and mice bearing tumor groups treated with Resv (Figure 6C).

Resv promoted lower adipocyte number and cell area in WAT from control and C57BL/6 mice bearing CM

We investigated whether Resv administration could influence the epididymal WAT weight of control and C57BL/6 mice bearing tumor. Overall, Resv administration promoted WAT weight loss in both healthy and mice bearing CM. $Control_{tumor}$, $Resv200_{tumor}$, and $Resv400_{tumor}$ groups not exhibited difference when WAT weight. Mice from both $Resv200_{non\text{-}tumor}$ and $Resv400_{non\text{-}tumor}$ groups exhibited significant WAT loss compared to $Control_{non\text{-}tumor}$ mice group ($p < 0.05$) (Figure 7A). Both control and mice bearing CM not treated with Resv also exhibited significant decreasing for number and area of adipocytes compared to control and mice bearing CM treated with Resv ($p <$

0.05, for all associations) (Figures 7B-D). Representative images of the WAT tissues from both control and experimental mice groups exhibit findings of the histomorphometrical parameters analyzed (Figure 7E).

Resv modulate the expression of the myokines related to CRC in control and mice bearing tumors.

We selected six myokines genes related to modulation of signaling pathways that modulate CRC pathogenesis, such as *MYOG, FBXO32, IGF1, IGF2, TRIM55*, and *TRIM63*. It was performed qPCR in order to investigate gene expression of the selected myokines in SM tissues from control and C57BL/6 mice bearing tumor. Mice treated with Resv were jointly grouped. In this way, Resv represented both Resv200 and Resv400 mice groups, and $Resv_{tumor}$ represented $Resv200_{tumor}$ and $Resv400_{tumor}$ mice bearing tumor groups. Treatment with Resv in animals without tumor did not alter the expression of the genes under study ($p > 0.05$ - $Control_{non\text{-}tumor}$ x $Control_{non\text{-}tumorResv}$). Comparison of gene expression between tumor animals ($Control_{tumor}$) and healthy animals ($Control_{non\text{-}tumor}$) revealed that the tumor promotes increased IGF2 and TRIM63 ($p < 0.05$). Resv significantly reduced gene expression of *MYOG* ($p < 0.001$), *IGF2* ($p < 0.05$), and *TRIM55* ($p < 0.05$) of the SM in $Resv_{tumor}$ mice group compared to $Control_{tumor}$. In the other hand, Resv significantly increased gene expression of *FBXO32* ($p < 0.01$), *IGF1* ($p < 0.05$), and *TRIM63* ($p < 0.05$) (Figure 8).

Resv administration decreases the expression of pro-cachectic adipokine sin WAT from control and mice bearing tumors.

We selected two adipokines genes related to modulation of signaling pathways that modulate CRC pathogenesis, such as *PPAR-γ and SREFB1*. It was performed qPCR

in order to investigate gene expression of the selected adipokines in WAT from control and C57BL/6 mice bearing tumor. As previously reported, mice treated with Resv were jointly grouped. Bearing-tumor animals showed significantly increased SREBF1 gene expression relative to non-tumor animals ($p < 0.05$ - $Control_{non\text{-}tumor}$ x $Control_{tumor}$). Resv treatment did not alter the expression of *PPAR-γ* and SREBF1 in healthy animals ($p > 0.05$ - $Control_{non\text{-}tumor}$ x $Control_{non\text{-}tumorResv}$). However, bearing-tumor mice treated with Resv exhibited the higher gene relative expression levels for both selected genes compared to $Control_{tumor}$ group ($p < 0.05$, for *PPAR-γ,* and $p < 0.001$ for SREBF1) (Figure 9).

Resv decreased CRP and increased albumin plasma levels in C57BL/6 mice bearing tumor.

We assessed the influence of Resv treatment on albumin and CRP plasma levels of the control and mice bearing tumors. Peripheral blood samples were collected in the end of treatment (twelfth day). Mice from $Control_{tumor}$ group exhibited lower albumin levels compared to mice from $Resv200_{tumor}$ group ($p < 0.05$) (Figure 10A). Conversely, mice from $Control_{tumor}$ group exhibited higher CRP levels compared to $Control_{non\text{-}tumor}$, $Resv200_{tumor}$, $Resv400_{tumor}$ ($p < 0.01$ for all associations) (Figure 10B).

Discussion

Our findings showed that mice treated with Resv significantly promoted a delayed in CRC occurrence, promoted gain of body weight, and improved survival of mice with CRC. Moreover, Resv administration promoted hypertrophic and hyperplasic effects on SMT, such as gain of weight, volume, and strength, as well as, higher quantity of muscle fibers. In WAT, Resv significantly decreased weight and adipocyte area, in both controls and mice bearing tumor. Rev treatment positively modulated the expression of anti-cachectic myokines and adiponectins. In a systemic point of view, Resv promoted a reduction of the low-degree chronic inflammation in mice bearing tumor.

In our study, resv. in addition to decreasing tumor volume, was responsible for making it difficult to establish the cachexia of animals treated with 200 mg/kg and 400 mg/kg. Similar to the result found in another study, mice bearing tumors treated with 200 mg/kg resveratrol promoted similar body weight gain (41) control.

Clinically, cachexia is represented by significant body weight loss, being this, important factor in determining the time of cachexia (42). Our data initially show weight loss in mice in the early stage of tumor development, possibly due to the nutritional needs of its establishment. However, treatment with resv reestablished the body weight of the animals.

In individuals with CRC, the loss of body weight is one of the most prevalent clinical findings (rates between 50% and 80%, depending on the type of cancer) related to cancer progression. Although this condition is more frequently diagnosed in individuals with advanced malignancy, it has been evidenced that the weight loss begins during early stages of cancer (pre-cancer), where the primary malignancy may not even be clinically noted (42-46).

Skeletal muscle loss appears to be the most essential component associated with a poor outcome in cancer cachexia (47). It is common for the anabolic and catabolic balance of skeletal muscle proteins to be disrupted during tumor progression (48, 49). Tumor tissue secrete specific factors for its development, thus promoting loss of muscle mass (50) and when mediated by the inflammatory condition play a significant role in the loss of skeletal muscle strength (51, 52). Resv decreases possible oxidative damage, and promotes reduction of the protein ubiquitinilação, indicating less protein degradation (53), however, our findings show the role of resveratrol in the preservation of skeletal muscle, considering the behavior of the expression of genes such as IGF1, TRIM55/MuRF2, TRIM63/MuRF1 and MYOG.

IGF1 is a key factor that regulates the development and growth of skeletal muscle capable of enhancing protein synthesis and inhibiting protein degradation and promotes muscle hypertrophy (54), more precisely in fast contraction fibers, because the MLC activator, active predominantly in fast II fibers (55, 56) this is possibly due to activation of the AKT pathway. Increased expression of IGF1 was observed in our study in bearing-tumor animals treated with resveratrol.

Over-expression of TRIM55/MuRF2 was shown to be important for the stability of microtubules along with other M-line proteins (56, 57). MuRF1 is the only member of the family that is associated with muscle atrophy and results in attenuation of muscle loss when excluded (58, 59). Our study showed effect of Resv as an inhibitor of TRIM63/MuRF1 and Myog gene expression, reinforcing again the importance of resveratrol in skeletal muscle hypertrophy. TRIM63/MuRF1 is involved in the binding of selective substrates for ubiquitination and subsequent degradation by the 26S proteasome. Thus, increased expression after an atrophy-inducing stressor is believed to be responsible for the change in protein balance from the liquid synthesis to the net

degradation, thereby inducing a loss of muscle mass (60). A study showed that resveratrol prevented an increase in the expression of TRIM63, preventing muscle atrophy induced by cachexia associated with chronic kidney disease (61). Our findings confirm the increase in muscle strength when resveratrol promoted a decrease in Myog expression. Myog is an important developmental regulator for the formation of skeletal muscles during embryonic and fetal development. In adults it is preferentially expressed in slow contracting muscle fibers (62). However, in cell culture, low doses of myogenin promote the formation of fast contracting fibers (63), such as the gastrocnemius muscle. In addition, a study shows that adult mice with Myog deletion exhibit 56% more exercise capacity, culminating with our results. In this sense, the role of myogenin in adult skeletal muscle seems to be different from its role in the embryonic and fetal muscle (64).

In our study, the resv suppressed white adipose tissue, depending on the presence of the tumor. Resv, compost protects against obesity induced (65-67), decreasing area (68) and the amount of adipocytes (69-71). Adipogenesis involving the proliferation of pre-adipocytes and the maturation of adipocytes is driven by important adipogenic transcription factors such as PPARγ. It is known that this transcription factor act coordinatedly in adipogenesis and therefore is believed to play central roles in modulating the whole process of differentiation (72, 73). After activation, PPARγ regulate each other to maintain high levels of expression, as well as to induce the expression of adipocyte-related proteins, including SREBF-1 (74). The low expression of SREBF-1 may decrease lipid synthesis, increase lipid oxidation (75). In our study, we found that resv significantly inhibited SREBF-1 expression, suggesting the anti-adipogenic effect of the traditional down-regulation of resveratrol in the expression

of transcription factors such as PPARγ and SREBF1 during adipocyte differentiation (76).

The improvement of effects related to cachexia in our study may have been due to the anti-inflammatory action exerted by resveratrol (77, 78). This can be explained when resv increased levels of albumin and decreased levels and CRP in the groups that showed improved symptoms of cachexia.

Albumin (ALB) can maintain colloid pressure and transport free fatty acids, bilirubin, and drug metabolites. Serum ALB may be low during acute inflammation, but it is also affected by nutritional status. A low ALB level is most often linked with chronic disease, frequently correlated with nutritional status (79, 80). Meanwhile, CRP/ALB ratio, a new inflammation-based prognostic score, has been demonstrated to show prognostic value in hepatocellular carcinoma, esophageal squamous cell carcinoma, and small cell lung cancer (81-84).

Resv was responsible for making it difficult to establish the cachexia of animals treated with 200 mg/kg and 400 mg/kg. Similar to the result found in another study, mice bearing tumors treated with 200 mg/kg resveratrol promoted similar body weight gain (41) control. The improvement of effects related to cachexia in our study may have been due to the anti-inflammatory action exerted by resveratrol (77, 78). This can be explained when resv increased levels of albumin and decreased levels and CRP in the groups that showed improved symptoms of cachexia.

Adipogenesis involving the proliferation of pre-adipocytes and the maturation of adipocytes is driven by important adipogenic transcription factors such as PPARγ. It is known that this transcription factor act coordinatedly in adipogenesis and therefore is believed to play central roles in modulating the whole process of differentiation (72, 73). After activation, PPARγ regulate each other to maintain high levels of expression,

as well as to induce the expression of adipocyte-related proteins, including SREBF-1 (74). The low expression of SREBF-1 may decrease lipid synthesis, increase lipid oxidation (75). In our study, we found that resv significantly inhibited SREBF-1 expression, suggesting the anti-adipogenic effect of the traditional down-regulation of resv in the expression of transcription factors such as PPARγ and SREBF1 during adipocyte differentiation (76).

Mouse CM models have been used in preclinical studies in order to better uncover characteristics of tumor biology, notably the molecular mechanisms responsible for determining the clinical behavior of CM (22). Notably, there is a paucity of information dealing with diagnosis, pathogenesis, therapeutic approaches, and outcomes related to CRC from mouse melanoma models (21-24). Although presenting limitations that typically occur in study designs with animal model, it can potentially contribute to a better understanding about CRC pathogenesis during MC progression. Additionally, they offer the advantage of facilitating the development of new pharmacological and non-pharmacological therapeutic approaches for CRC treatment (25-27).

In In conclusion, findings from this study showed that Resv administration promoted a plethora of anti-cachectic effects in C57BL/6 mice bearing CM-related cachexia model, with resulting improvement of CRS of animals.

Conflict of interest

None to declare.

Acknowledgements

We would like to thank the Foundation for Research Support of the Minas Gerais State (FAPEMIG, processes numbers: PPM-00029-17 and DEG-00010-16); National Council for Scientific and Technological Development (CNPq, processes number: 437311/2016-3 and 430759/2016-9); and the Coordination of Improvement of Higher Education Personnel (CAPES) for the support to carry out this study.

References

1. Ko JH, Sethi G, Um JY, Shanmugam MK, Arfuso F, Kumar AP, et al. The Role of Resveratrol in Cancer Therapy. International journal of molecular sciences. 2017 Dec 1;18(12). PubMed PMID: 29194365. Pubmed Central PMCID: PMC5751192. Epub 2017/12/02. eng.
2. Soleas GJ, Diamandis EP, Goldberg DM. Resveratrol: a molecule whose time has come? And gone? Clinical biochemistry. 1997 Mar;30(2):91-113. PubMed PMID: 9127691. Epub 1997/03/01. eng.
3. Pervaiz S, Holme AL. Resveratrol: its biologic targets and functional activity. Antioxidants & redox signaling. 2009 Nov;11(11):2851-97. PubMed PMID: 19432534.
4. Abba Y, Hassim H, Hamzah H, Noordin MM. Antiviral Activity of Resveratrol against Human and Animal Viruses. Advances in virology. 2015;2015:184241. PubMed PMID: 26693226. Pubmed Central PMCID: 4676993.
5. Giuseppina Pace Pereira Lima FV, Camila Renata Corrêa, Renê Arnoux da Silva Campos, Milena Galhardo Borguini. Polyphenols in Fruits and Vegetables and Its Effect on Human Health. Food and Nutrition 2014;Vol.5 No.11,.
6. Kursvietiene L, Staneviciene I, Mongirdiene A, Bernatoniene J. Multiplicity of effects and health benefits of resveratrol. Medicina. 2016;52(3):148-55. PubMed PMID: 27496184.
7. Poulsen MM, Fjeldborg K, Ornstrup MJ, Kjaer TN, Nohr MK, Pedersen SB. Resveratrol and inflammation: Challenges in translating pre-clinical findings to improved patient outcomes. Biochimica et biophysica acta. 2015 Jun;1852(6):1124-36. PubMed PMID: 25583116.
8. Santos JA, de Carvaho GS, Oliveira V, Raposo NR, da Silva AD. Resveratrol and analogues: a review of antioxidant activity and applications to human health. Recent patents on food, nutrition & agriculture. 2013 Aug;5(2):144-53. PubMed PMID: 23688141.
9. T. Ozcan AA-B, L. Yilmaz-Ersan, and B. Delikanli. Phenolics in Human Health. International Journal of Chemical Engineering and Applications 2014;vol. 5, no. 5:393-6.
10. Fearon K, Strasser F, Anker SD, Bosaeus I, Bruera E, Fainsinger RL, et al. Definition and classification of cancer cachexia: an international consensus. The Lancet Oncology. 2011 May;12(5):489-95. PubMed PMID: 21296615. Epub 2011/02/08. eng.
11. Argiles JM, Lopez-Soriano FJ, Busquets S. Mechanisms to explain wasting of muscle and fat in cancer cachexia. Current opinion in supportive and palliative care. 2007 Dec;1(4):293-8. PubMed PMID: 18685378. Epub 2008/08/08. eng.
12. Fearon KC, Glass DJ, Guttridge DC. Cancer cachexia: mediators, signaling, and metabolic pathways. Cell metabolism. 2012 Aug 8;16(2):153-66. PubMed PMID: 22795476. Epub 2012/07/17. eng.
13. Kalantar-Zadeh K, Rhee C, Sim JJ, Stenvinkel P, Anker SD, Kovesdy CP. Why cachexia kills: examining the causality of poor outcomes in wasting conditions. Journal of cachexia, sarcopenia and muscle. 2013 06/08
05/22/received
05/22/accepted;4(2):89-94. PubMed PMID: PMC3684705.
14. Aggarwal BB, Bhardwaj A, Aggarwal RS, Seeram NP, Shishodia S, Takada Y. Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. Anticancer research. 2004 Sep-Oct;24(5A):2783-840. PubMed PMID: 15517885. Epub 2004/11/03. eng.

15. Brakenhielm E, Cao R, Cao Y. Suppression of angiogenesis, tumor growth, and wound healing by resveratrol, a natural compound in red wine and grapes. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2001 Aug;15(10):1798-800. PubMed PMID: 11481234. Epub 2001/08/02. eng.
16. He L, Fan F, Hou X, Gao C, Meng L, Meng S, et al. Resveratrol suppresses pulmonary tumor metastasis by inhibiting platelet-mediated angiogenic responses. The Journal of surgical research. 2017 Sep;217:113-22. PubMed PMID: 28629815. Epub 2017/06/21. eng.
17. Oku N, Matsukawa M, Yamakawa S, Asai T, Yahara S, Hashimoto F, et al. Inhibitory effect of green tea polyphenols on membrane-type 1 matrix metalloproteinase, MT1-MMP. Biological & pharmaceutical bulletin. 2003 Sep;26(9):1235-8. PubMed PMID: 12951464. Epub 2003/09/03. eng.
18. Abdur Rauf MI, Masood Sadiq Butt, Muhammad, Nadeem DGPMSM. Resveratrol as an anti-cancer agent: A review. Critical Reviews in Food Science and Nutrition. 58:9:1428-47.
19. Varoni EM, Lo Faro AF, Sharifi-Rad J, Iriti M. Anticancer Molecular Mechanisms of Resveratrol. Frontiers in nutrition. 2016;3:8. PubMed PMID: 27148534. Pubmed Central PMCID: PMC4828556. Epub 2016/05/06. eng.
20. Marques RG, Morales MM, Petroianu A. Brazilian law for scientific use of animals. Acta cirurgica brasileira. 2009 Jan-Feb;24(1):69-74. PubMed PMID: 19169547. Epub 2009/01/27. eng.
21. Miranda-Hernandez DF, Franco-Molina MA, Mendoza-Gamboa E, Zapata-Benavides P, Sierra-Rivera CA, Coronado-Cerda EE, et al. Expression of Foxp3, CD25 and IL-2 in the B16F10 cancer cell line and melanoma is correlated with tumor growth in mice. Oncol Lett. 2013 Nov;6(5):1195-200. PubMed PMID: 24179494. Pubmed Central PMCID: PMC3813795. Epub 2013/11/02. eng.
22. Sahu RP, Turner MJ, DaSilva SC, Rashid BM, Ocana JA, Perkins SM, et al. The environmental stressor ultraviolet B radiation inhibits murine antitumor immunity through its ability to generate platelet-activating factor agonists. Carcinogenesis. 2012 Jul;33(7):1360-7. PubMed PMID: 22542595. Pubmed Central PMCID: PMC3405652. Epub 2012/05/01. eng.
23. Goh J, Enns L, Fatemie S, Hopkins H, Morton J, Pettan-Brewer C, et al. Mitochondrial targeted catalase suppresses invasive breast cancer in mice. BMC cancer. 2011 May 23;11:191. PubMed PMID: 21605372. Pubmed Central PMCID: PMC3123323. Epub 2011/05/25. eng.
24. Tomayko MM, Reynolds CP. Determination of subcutaneous tumor size in athymic (nude) mice. Cancer chemotherapy and pharmacology. 1989;24(3):148-54. PubMed PMID: 2544306. Epub 1989/01/01. eng.
25. Pettan-Brewer C, Morton J, Coil R, Hopkins H, Fatemie S, Ladiges W. B16 melanoma tumor growth is delayed in mice in an age-dependent manner. Pathobiology of aging & age related diseases. 2012;2. PubMed PMID: 22953040. Pubmed Central PMCID: PMC3424493. Epub 2012/09/07. eng.
26. Liao AH, Ma WC, Wu MF. Evaluation of ultrasound combined with chitosan for the control of weight and local fat in mice. Ultrasound in medicine & biology. 2013 Oct;39(10):1794-803. PubMed PMID: 23859895. Epub 2013/07/19. eng.
27. Talbert EE, Metzger GA, He WA, Guttridge DC. Modeling human cancer cachexia in colon 26 tumor-bearing adult mice. Journal of cachexia, sarcopenia and muscle. 2014 03/26
12/23/received

03/04/accepted;5(4):321-8. PubMed PMID: PMC4248405.
28. Honors MA, Kinzig KP. Characterization of the Yoshida sarcoma: a model of cancer cachexia. Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer. 2013 Oct;21(10):2687-94. PubMed PMID: 23689977. Pubmed Central PMCID: PMC3780404. Epub 2013/05/22. eng.
29. Voltarelli FA, Frajacomo FT, Padilha CdS, Testa MT, Cella PS, Ribeiro DF, et al. Syngeneic B16F10 Melanoma Causes Cachexia and Impaired Skeletal Muscle Strength and Locomotor Activity in Mice. Frontiers in physiology. 2017;8:715.
30. Toth LA. Defining the moribund condition as an experimental endpoint for animal research. ILAR journal. 2000;41(2):72-9.
31. Mele A, Fonzino A, Rana F, Camerino GM, De Bellis M, Conte E, et al. In vivo longitudinal study of rodent skeletal muscle atrophy using ultrasonography. Scientific reports. 2016 02/01/online;6:20061.
32. Wagner KR, McPherron AC, Winik N, Lee SJ. Loss of myostatin attenuates severity of muscular dystrophy in mdx mice. Annals of neurology. 2002;52(6):832-6.
33. Goode A, Gilbert B, Harkes J, Jukic D, Satyanarayanan M. OpenSlide: A vendor-neutral software foundation for digital pathology. Journal of pathology informatics. 2013;4:27. PubMed PMID: 24244884. Pubmed Central PMCID: PMC3815078. Epub 2013/11/19. eng.
34. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: an open-source platform for biological-image analysis. Nature methods. 2012 Jun 28;9(7):676-82. PubMed PMID: 22743772. Pubmed Central PMCID: PMC3855844. Epub 2012/06/30. eng.
35. Lawlor MW, Read BP, Edelstein R, Yang N, Pierson CR, Stein MJ, et al. Inhibition of activin receptor type IIB increases strength and lifespan in myotubularin-deficient mice. Am J Pathol. 2011 Feb;178(2):784-93. PubMed PMID: 21281811. Pubmed Central PMCID: PMC3069865. Epub 2011/02/02. eng.
36. Schrauwen-Hinderling VB, Hesselink MK, Schrauwen P, Kooi ME. Intramyocellular lipid content in human skeletal muscle. Obesity (Silver Spring, Md). 2006 Mar;14(3):357-67. PubMed PMID: 16648604. Epub 2006/05/02. eng.
37. Cinti S, Zingaretti MC, Cancello R, Ceresi E, Ferrara P. Morphologic techniques for the study of brown adipose tissue and white adipose tissue. Methods in molecular biology (Clifton, NJ). 2001;155:21-51. PubMed PMID: 11293073. Epub 2001/04/11. eng.
38. Sternberg SR. Biomedical Image Processing. Computer. 1983;16(1):22-34.
39. Girven M, Dugdale HF, Owens DJ, Hughes DC, Stewart CE, Sharples AP. l-glutamine Improves Skeletal Muscle Cell Differentiation and Prevents Myotube Atrophy After Cytokine (TNF-alpha) Stress Via Reduced p38 MAPK Signal Transduction. Journal of cellular physiology. 2016 Dec;231(12):2720-32. PubMed PMID: 26991744. Epub 2016/03/19. eng.
40. Parlee SD, Lentz SI, Mori H, MacDougald OA. Quantifying size and number of adipocytes in adipose tissue. Methods in enzymology. 2014;537:93-122. PubMed PMID: 24480343. Pubmed Central PMCID: PMC4069255. Epub 2014/02/01. eng.
41. Shadfar S, Couch ME, McKinney KA, Weinstein LJ, Yin X, Rodríguez JE, et al. Oral resveratrol therapy inhibits cancer-induced skeletal muscle and cardiac atrophy in vivo. Nutrition and cancer. 2011 06/09;63(5):749-62. PubMed PMID: PMC3623008.
42. Evans WJ, Morley JE, Argiles J, Bales C, Baracos V, Guttridge D, et al. Cachexia: a new definition. Clinical nutrition (Edinburgh, Scotland). 2008 Dec;27(6):793-9. PubMed PMID: 18718696. Epub 2008/08/23. eng.

43. Bosaeus I, Daneryd P, Svanberg E, Lundholm K. Dietary intake and resting energy expenditure in relation to weight loss in unselected cancer patients. International journal of cancer. 2001 Aug 1;93(3):380-3. PubMed PMID: 11433403. Epub 2001/07/04. eng.
44. Loprinzi CL, Schaid DJ, Dose AM, Burnham NL, Jensen MD. Body-composition changes in patients who gain weight while receiving megestrol acetate. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1993 Jan;11(1):152-4. PubMed PMID: 8418227. Epub 1993/01/01. eng.
45. Maltoni M, Nanni O, Scarpi E, Rossi D, Serra P, Amadori D. High-dose progestins for the treatment of cancer anorexia-cachexia syndrome: a systematic review of randomised clinical trials. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2001 Mar;12(3):289-300. PubMed PMID: 11332139. Epub 2001/05/03. eng.
46. Simons JP, Schols AM, Hoefnagels JM, Westerterp KR, ten Velde GP, Wouters EF. Effects of medroxyprogesterone acetate on food intake, body composition, and resting energy expenditure in patients with advanced, nonhormone-sensitive cancer: a randomized, placebo-controlled trial. Cancer. 1998 Feb 1;82(3):553-60. PubMed PMID: 9452274. Epub 1998/02/06. eng.
47. Fearon K, Strasser F, Anker SD, Bosaeus I, Bruera E, Fainsinger RL, et al. Definition and classification of cancer cachexia: an international consensus. The Lancet Oncology. 2011;12(5):489-95.
48. Bonaldo P, Sandri M. Cellular and molecular mechanisms of muscle atrophy. Disease models & mechanisms. 2013 Jan;6(1):25-39. PubMed PMID: 23268536. Pubmed Central PMCID: PMC3529336. Epub 2012/12/27. eng.
49. Cohen S, Nathan JA, Goldberg AL. Muscle wasting in disease: molecular mechanisms and promising therapies. Nature reviews Drug discovery. 2015 Jan;14(1):58-74. PubMed PMID: 25549588. Epub 2015/01/01. eng.
50. Porporato PE. Understanding cachexia as a cancer metabolism syndrome. Oncogenesis. 2016 02/22/online;5:e200.
51. Voltarelli FA, Frajacomo FT, Padilha CdS, Testa MTJ, Cella PS, Ribeiro DF, et al. Syngeneic B16F10 Melanoma Causes Cachexia and Impaired Skeletal Muscle Strength and Locomotor Activity in Mice. Frontiers in Physiology. 2017 09/29
05/12/received
09/05/accepted;8:715. PubMed PMID: PMC5626871.
52. Chen J-a, Splenser A, Guillory B, Luo J, Mendiratta M, Belinova B, et al. Ghrelin prevents tumour- and cisplatin-induced muscle wasting: characterization of multiple mechanisms involved. Journal of cachexia, sarcopenia and muscle. 2015 04/22
08/12/received
11/11/revised
02/13/accepted;6(2):132-43. PubMed PMID: PMC4458079.
53. Wilson WN, Baumgarner BL, Watanabe WO, Alam MS, Kinsey ST. Effects of resveratrol on growth and skeletal muscle physiology of juvenile southern flounder. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 2015 2015/05/01/;183:27-35.
54. Bodine SC, Stitt TN, Gonzalez M, Kline WO, Stover GL, Bauerlein R, et al. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. Nature cell biology. 2001 Nov;3(11):1014-9. PubMed PMID: 11715023. Epub 2001/11/21. eng.
55. Rommel C, Bodine SC, Clarke BA, Rossman R, Nunez L, Stitt TN, et al. Mediation of IGF-1-induced skeletal myotube hypertrophy by PI(3)K/Akt/mTOR and

PI(3)K/Akt/GSK3 pathways. Nature cell biology. 2001 Nov;3(11):1009-13. PubMed PMID: 11715022. Epub 2001/11/21. eng.
56. Musaro A, McCullagh K, Paul A, Houghton L, Dobrowolny G, Molinaro M, et al. Localized Igf-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. Nature genetics. 2001 Feb;27(2):195-200. PubMed PMID: 11175789. Epub 2001/02/15. eng.
57. Pizon V, Iakovenko A, Van Der Ven PF, Kelly R, Fatu C, Furst DO, et al. Transient association of titin and myosin with microtubules in nascent myofibrils directed by the MURF2 RING-finger protein. Journal of cell science. 2002 Dec 1;115(Pt 23):4469-82. PubMed PMID: 12414993. Epub 2002/11/05. eng.
58. Baehr LM, Furlow JD, Bodine SC. Muscle sparing in muscle RING finger 1 null mice: response to synthetic glucocorticoids. The Journal of physiology. 2011 Oct 1;589(Pt 19):4759-76. PubMed PMID: 21807613. Pubmed Central PMCID: PMC3213422. Epub 2011/08/03. eng.
59. Labeit S, Kohl CH, Witt CC, Labeit D, Jung J, Granzier H. Modulation of muscle atrophy, fatigue and MLC phosphorylation by MuRF1 as indicated by hindlimb suspension studies on MuRF1-KO mice. Journal of biomedicine & biotechnology. 2010;2010:693741-. PubMed PMID: 20625437. Epub 06/24.
60. Bodine SC, Baehr LM. Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1. American Journal of Physiology-Endocrinology and Metabolism. 2014;307(6):E469-E84. PubMed PMID: 25096180.
61. Sun LJ, Sun YN, Chen SJ, Liu S, Jiang GR. Resveratrol attenuates skeletal muscle atrophy induced by chronic kidney disease via MuRF1 signaling pathway. Biochemical and biophysical research communications. 2017 May 20;487(1):83-9. PubMed PMID: 28392400. Epub 2017/04/11. eng.
62. Hughes SM, Taylor JM, Tapscott SJ, Gurley CM, Carter WJ, Peterson CA. Selective accumulation of MyoD and myogenin mRNAs in fast and slow adult skeletal muscle is controlled by innervation and hormones. Development (Cambridge, England). 1993 Aug;118(4):1137-47. PubMed PMID: 8269844. Epub 1993/08/01. eng.
63. Alapat DV, Chaudhry T, Ardakany-Taghavi R, Kohtz DS. Fiber-types of sarcomeric proteins expressed in cultured myogenic cells are modulated by the dose of myogenin activity. Cellular signalling. 2009 Jan;21(1):128-35. PubMed PMID: 18955131. Epub 2008/10/29. eng.
64. Flynn JM, Meadows E, Fiorotto M, Klein WH. Myogenin regulates exercise capacity and skeletal muscle metabolism in the adult mouse. PloS one. 2010 Oct 22;5(10):e13535. PubMed PMID: 21042574. Pubmed Central PMCID: PMC2962629. Epub 2010/11/03. eng.
65. Kim S, Jin Y, Choi Y, Park T. Resveratrol exerts anti-obesity effects via mechanisms involving down-regulation of adipogenic and inflammatory processes in mice2011. 1343-51 p.
66. Jeon BT, Jeong EA, Shin HJ, Lee Y, Lee DH, Kim HJ, et al. Resveratrol attenuates obesity-associated peripheral and central inflammation and improves memory deficit in mice fed a high-fat diet. Diabetes. 2012 Jun;61(6):1444-54. PubMed PMID: 22362175. Pubmed Central PMCID: PMC3357272. Epub 2012/03/01. eng.
67. Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, et al. Resveratrol improves health and survival of mice on a high-calorie diet. Nature. 2006 Nov 16;444(7117):337-42. PubMed PMID: 17086191. Pubmed Central PMCID: PMC4990206. Epub 2006/11/07. eng.
68. Konings E, Timmers S, Boekschoten MV, Goossens GH, Jocken JW, Afman LA, et al. The effects of 30 days resveratrol supplementation on adipose tissue

morphology and gene expression patterns in obese men. International journal of obesity (2005). 2014 Mar;38(3):470-3. PubMed PMID: 23958793. Epub 2013/08/21. eng.
69. Rayalam S, Yang JY, Ambati S, Della-Fera MA, Baile CA. Resveratrol induces apoptosis and inhibits adipogenesis in 3T3-L1 adipocytes. Phytotherapy research : PTR. 2008 Oct;22(10):1367-71. PubMed PMID: 18688788. Epub 2008/08/09. eng.
70. Zou T, Chen D, Yang Q, Wang B, Zhu MJ, Nathanielsz PW, et al. Resveratrol supplementation of high-fat diet-fed pregnant mice promotes brown and beige adipocyte development and prevents obesity in male offspring. The Journal of physiology. 2017 Mar 1;595(5):1547-62. PubMed PMID: 27891610. Pubmed Central PMCID: PMC5330906. Epub 2016/11/29. eng.
71. Chen S, Zhou N, Zhang Z, Li W, Zhu W. Resveratrol induces cell apoptosis in adipocytes via AMPK activation. Biochemical and biophysical research communications. 2015 Feb 20;457(4):608-13. PubMed PMID: 25603053. Epub 2015/01/21. eng.
72. Lane MD, Lin FT, MacDougald OA, Vasseur-Cognet M. Control of adipocyte differentiation by CCAAT/enhancer binding protein alpha (C/EBP alpha). International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 1996 Mar;20 Suppl 3:S91-6. PubMed PMID: 8680485. Epub 1996/03/01. eng.
73. Hamm JK, el Jack AK, Pilch PF, Farmer SR. Role of PPAR gamma in regulating adipocyte differentiation and insulin-responsive glucose uptake. Annals of the New York Academy of Sciences. 1999 Nov 18;892:134-45. PubMed PMID: 10842659. Epub 2000/06/08. eng.
74. Farmer SR. Transcriptional control of adipocyte formation. Cell metabolism. 2006 Oct;4(4):263-73. PubMed PMID: 17011499. Pubmed Central PMCID: PMC1958996. Epub 2006/10/03. eng.
75. Deng Q, Li X, Fu S, Yin L, Zhang Y, Wang T, et al. SREBP-1c gene silencing can decrease lipid deposits in bovine hepatocytes cultured in vitro. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology. 2014;33(5):1568-78. PubMed PMID: 24854845. Epub 2014/05/24. eng.
76. Yang XR, Wat E, Wang YP, Ko CH, Koon CM, Siu WS, et al. Effect of Dietary Cocoa Tea (Camellia ptilophylla) Supplementation on High-Fat Diet-Induced Obesity, Hepatic Steatosis, and Hyperlipidemia in Mice. Evidence-based complementary and alternative medicine : eCAM. 2013;2013:783860. PubMed PMID: 23935682. Pubmed Central PMCID: PMC3723092. Epub 2013/08/13. eng.
77. Carreras A, Zhang SX, Peris E, Qiao Z, Wang Y, Almendros I, et al. Effect of resveratrol on visceral white adipose tissue inflammation and insulin sensitivity in a mouse model of sleep apnea. International journal of obesity (2005). 2015 Mar;39(3):418-23. PubMed PMID: 25298275. Epub 2014/10/10. eng.
78. Liu Z, Gan L, Liu G, Chen Y, Wu T, Feng F, et al. Sirt1 decreased adipose inflammation by interacting with Akt2 and inhibiting mTOR/S6K1 pathway in mice. Journal of lipid research. 2016 Aug;57(8):1373-81. PubMed PMID: 27317762. Pubmed Central PMCID: PMC4959854. Epub 2016/06/19. eng.
79. Iskandar HN, Ciorba MA. Biomarkers in inflammatory bowel disease: current practices and recent advances. Translational research : the journal of laboratory and clinical medicine. 2012 Apr;159(4):313-25. PubMed PMID: 22424434. Pubmed Central PMCID: PMC3308116. Epub 2012/03/20. eng.

80. Don BR, Kaysen G. Serum albumin: relationship to inflammation and nutrition. Seminars in dialysis. 2004 Nov-Dec;17(6):432-7. PubMed PMID: 15660573. Epub 2005/01/22. eng.
81. Kinoshita A, Onoda H, Imai N, Iwaku A, Oishi M, Tanaka K, et al. The C-reactive protein/albumin ratio, a novel inflammation-based prognostic score, predicts outcomes in patients with hepatocellular carcinoma. Annals of surgical oncology. 2015 Mar;22(3):803-10. PubMed PMID: 25190127. Epub 2014/09/06. eng.
82. Xu XL, Yu HQ, Hu W, Song Q, Mao WM. A Novel Inflammation-Based Prognostic Score, the C-Reactive Protein/Albumin Ratio Predicts the Prognosis of Patients with Operable Esophageal Squamous Cell Carcinoma. PloS one. 2015;10(9):e0138657. PubMed PMID: 26390126. Pubmed Central PMCID: PMC4577080. Epub 2015/09/22. eng.
83. Zhou T, Zhan J, Hong S, Hu Z, Fang W, Qin T, et al. Ratio of C-Reactive Protein/Albumin is An Inflammatory Prognostic Score for Predicting Overall Survival of Patients with Small-cell Lung Cancer. Scientific reports. 2015 Jun 18;5:10481. PubMed PMID: 26084991. Pubmed Central PMCID: PMC4471724. Epub 2015/06/19. eng.
84. Xu X-L, Yu H-Q, Hu W, Song Q, Mao W-M. A Novel Inflammation-Based Prognostic Score, the C-Reactive Protein/Albumin Ratio Predicts the Prognosis of Patients with Operable Esophageal Squamous Cell Carcinoma. PloS one. 2015;10(9):e0138657.

Legends of figures

Figure 1. Effect of Resv on body weight of the control and C57BL/6 mice tumor model. Weight body (g) measurements were daily performed (A). Macroscopic appearances of control and experimental mice on day 12 (B). On the first and seventh day of follow-up, the body weight from experimental mice were estimated discounting the tumor weight. The tumor weight was estimated using a linear regression equation according to formula: $y = 0.0006x + 0.0769$, which x is tumor volumeand and y represents tumor mass, , with value of $R^2 = 0.9848$ (positive co-operation). All animals were sacrificed at twelfth day after CRC diagnosis. After sacrifice, carcass and tumor weights were distinctly measured. Measurements of the mice body weight (carcass) on day 12 (C). Significant differences for body weight measurements were noted between groups at day 1: $Control_{tumor}$ *vs.* $Control_{non\text{-}tumor}$ ($p < 0.01$), $Control_{tumor}$ *vs.* $Resv200_{tumor}$ ($p < 0.05$), $Control_{tumor}$ *vs.* $Control_{non\text{-}tumor/Resv200}$ ($p < 0.05$); at day 7: $Control_{tumor}$ *vs.* $Control_{non\text{-}tumor}$ ($p < 0.01$), $Control_{non\text{-}tumor}$ *vs.* $Resv200_{tumor}$ ($p < 0.01$); and at day 12: $Control_{tumor}$ *vs.* $Resv200_{tumor}$ ($p < 0.05$).

Figure 2. Effect of Resv administration on occurrence of cancer-related cachexia (CRC) and on overall cancer-related survival (CRS) in C57BL/6 mice with syngeneic tumor model. Follow-up of all mice bearing tumor lasted 18 days. CRC diagnosis (in days) was established at weight loss ≥ 10%. Time of CRC diagnosis was individually and daily assessed in each animal. Data from mice bearing tumor groups (n = 10, in each group) were compared using Log-rank test and curves were represented by Kaplan-Meier plots. CRC diagnosis and CRS data were compared between study groups (A) $Control_{tumor}$ *vs* $Resv200_{tumor}$ and $Control_{tumor}$ *vs* $Resv400_{tumor}$ ($p = 0.018$). CRC diagnosis occurred in mice from $Control_{tumor}$ (5.12 ± 1.24 days), $Resv200_{tumor}$ (8 ± 2.77 days), and $Resv400_{tumor}$ (10.75 ± 2.31 days). According to our findings, significant differences between groups were noted for CRC diagnosis occurrence were noted in $Control_{tumor}$ *vs* $Resv200_{tumor}$ ($p = 0.05$) and $Control_{tumor}$ *vs* $Resv400_{tumor}$ ($p = 0.001$) (B). In to concerns to CRS, significant differences were observed between $Control_{tumor}$ *vs* $Resv200_{tumor}$ ($p = 0.038$) (C), and $Control_{tumor}$ *vs* $Resv400_{tumor}$ ($p = 0.040$) (D).

Figure 3. Effect of Resv on right hindlimb skeletal muscle volume (SMV) hindlimbof the C57BL/6 mice control and syngeneic CM cancer model. SMV assessment occurred at 2 (tumor induction), 7, and 11 days. Resv treatments started at baseline. SMV was assessed using an ultrassound device (A). Mice bearing tumors exhibited significant reduction of the SM volume compared to control mice ($p < 0.001$, for all comparisons). Mice from $Resv200_{tumor}$ and $Resv400_{tumor}$ groups exhibited a higher gain of SM volume compared to $Control_{tumor}$ mice ($p < 0.001$ and $p < 0.05$, respectively) (B).

Figure 4. Effect of Resv on right hindlimb skeletal muscle (SM) weight from right hindlimb of the control and C57BL/6 mice bearing tumor. All SM weight measurements occurred on the last day. Macroscopic appearance of the right hindlimb from control and experimental mice soon after sacrifice (A). Student´s t test was performed in order to compare SM weight between groups. Resv200 and Resv400 administration promoted SM weight gain in both control and mice bearing tumor (B). Mice from Resv400 exhibited SM weight gain compared to $Control_{non\text{-}tumor}$ group ($p < 0.05$). In mice bearing tumors, our findings showed that $Resv200_{tumor}$ and $Resv400_{tumor}$ groups exhibited a higher SM weight gain compared to $Control_{tumor}$ mice ($p < 0.05$ and $p < 0.0015$, respectively). Comparisons of the individual weight analysis of the SM which make up hindlimb showed that Mice from $Control_{tumor}$ group exhibited lower gain of the relative SM weight compared to $Resv200_{tumor}$ mice (quadriceps: $p= 0.001$ and tibial: $p < 0.05$) and compared to $Resv400_{tumor}$ mice (gastrocnemius: $p < 0.05$, femoral quadriceps: $p= 0.001$, and tibial: $p = 0.001$) (C-E).

Figure 5. Effect of Resv on skeletal muscle (SM) relative strength from forelimbs of control and C57BL/6 mice bearing tumor. SM strength assessment occurred at fourth, seventh, $Control_{tumor}$ *vs.* Resv200, and $Control_{tumor}$ *vs.* Resv400 ($p < 0.001$ and $p < 0.01$, respectively), and twelfth days $Control_{tumor}$ *vs.* Resv200 ($p < 0.05$) (A). Notably, $Control_{tumor}$ mice exhibited lower SM strength compared to normal ($Control_{non\text{-}tumor}$, Resv200, and Resv400) ($p < 0.05$) and mice bearing tumor treated with ($p < 0.05$).

Figure 6. Effect of Resv on number (unity) and SM fiber area (μm^2) from gastrocnemius muscle in control and C57BL/6 mice bearing tumor. Cytomorphometrical analysis was performed using ImageJ software. Student´s t test was performed in order to compare WAT weight between study groups. Mice from $Control_{tumor}$ showed a lower number of

the SM fibers compared to study groups $Control_{non\text{-}tumor}$, Resv200, Resv400, $Resv200_{tumor}$ ($p < 0.001$, for all associations) and $Resv400_{tumor}$ ($p < 0.01$) (A). Assessment of the SM fiber area (μm²) of the gastrocnemius not showed difference between study groups (B). Representative microscopical tissue images of the gastrocnemius muscle of both control and mice bearing tumor groups treated with Resv (stain: Masson´s trichrome, magnification: x200) (C).

Figure 7. Effect of Resv on number (unity) and adipocytes area (μm^2) from epididymal white adipose tissue (WAT) in control and C57BL/6 mice bearing tumor. Cytomorphometrical analysis was performed using ImageJ software. Student´s t test was performed in order to compare WAT weight between study groups. Notably, control and mice bearing tumor treated with Resv exhibited decrease of the WAT weight. However, significant differences were only showed for $Control_{non\text{-}tumor}$ *vs*. $Resv400_{tumor}$ ($p < 0.05$) and $Control_{non\text{-}tumor}$ *vs*. Resv400 ($p < 0.05$) (A). The number of adipocytes were compared between groups. Mice from $Control_{non\text{-}tumor}$ group exhibited higher adipocyte number compared to Resv200, Resv400 $Resv200_{tumor}$ and $Resv400_{tumor}$ ($p < 0.001$, for all associations). Additionally, mice from $Control_{tumor}$ exhibited lower adipocytes number compared to $Control_{non\text{-}tumor}$, Resv200, and Resv400 mice groups ($p < 0.05$, for all associations) and to $Resv200_{tumor}$ and $Resv400_{tumor}$ mice groups ($p < 0.001$, for all associations). Similarly, both control and mice bearing tumor treated with Resv showed a lower adipocyte area compared to non-treated mice. Comparisons of the adipocyte area between groups showed significant differences between $Control_{non\text{-}tumor}$ *vs* Resv200, Resv400, $Resv200_{tumor,}$ and $Resv400_{tumor}$ ($p < 0.01$, for all associations) while mice from $Control_{tumor}$ exhibited higher adipocyte area compared to $Resv200_{tumor}$ and $Resv400_{tumor}$ mice groups ($p < 0.01$, for both associations). Figure 7D exhibits adipocyte area/unity relationship according to study groups. Representative microscopical tissue images of the gastrocnemius muscle of both control and experimental mice groups (stain: Masson´s trichrome, magnification: 200X).

Figure 8. Effect of Resv on relative gene expression of selected myokines (*MYOG, FXOB32, IGF1, IGF2, TRIM55*, and *TRIM63*) in SM tissue from controls and C57BL/6 mice bearing tumor. *MYOG* expression in SM tissues from $Control_{tumor}$ mice was significantly reduced in comparison to $Resv_{tumor}$ mice ($p < 0.001$) (A). *FBXO32* expression in SM from $Control_{tumor}$ was significantly higher that $Resv_{tumor}$ mice ($p <$

0.01) (B). *IGF1* expression in SM tissues from $Control_{tumor}$ was increased in comparison to $Resv_{tumor}$ mice ($p < 0.5$) (D). *IGF2* expression in SM tissues from $Control_{tumor}$ mice were reduced in comparison to $Contro_{non\text{-}tumor}$, Resv, and $Resv_{tumor}$ mice groups ($p <$ 001, for all associations) (D). TRIM55 expression was reduced in SM from $Control_{tumor}$ compared to Resv and $Resv_{tumor}$ ($p < 0.05$, for both associations) (E). TRIM63 expression was increased in SM from $Control_{tumor}$ mice compared to $Resv_{tumor}$ mice ($p < 0.05$) (F).

Figure 9. Effect of Resv on relative gene expression of selected adipokines *PPARγ* and *SREBF1*) in WAT from controls and C57BL/6 mice bearing tumor. A higher *PPARγ* and *SREBF1* expressions were noted in WAT from $Control_{tumor}$ mice. Mice from $Control_{tumor}$ exhibited higher WAT *PPARγ* expression compared Resv and $Resv_{tumor}$ mice groups ($p < 0.01$, for both associations) (A). WAT from $Control_{tumor}$ mice exhibited a higher *SREBF1* expression compared to $Control_{non\text{-}tumor}$, Resv, and $Resv_{tumor}$ mice groups ($p < 0.001$, for all associations) (B).

Figure 10. Plasma levels of albumin and C-reactive protein (CRP) of control and mice bearing tumor treated with Resv. Mice from $Control_{tumor}$ group exhibited lower albumin levels compared to Resv400 and $Resv200_{tumor}$ ($p < 0.05$, for both associations) (A). In the other hand, mice from $Control_{tumor}$ group exhibited higher CRP levels compared to $Control_{non\text{-}tumor}$, $Resv200_{tumor}$, and $Resv400_{tumor}$ mice groups ($p < 0.01$, for all associations) (B).

Figures

Figure 1.

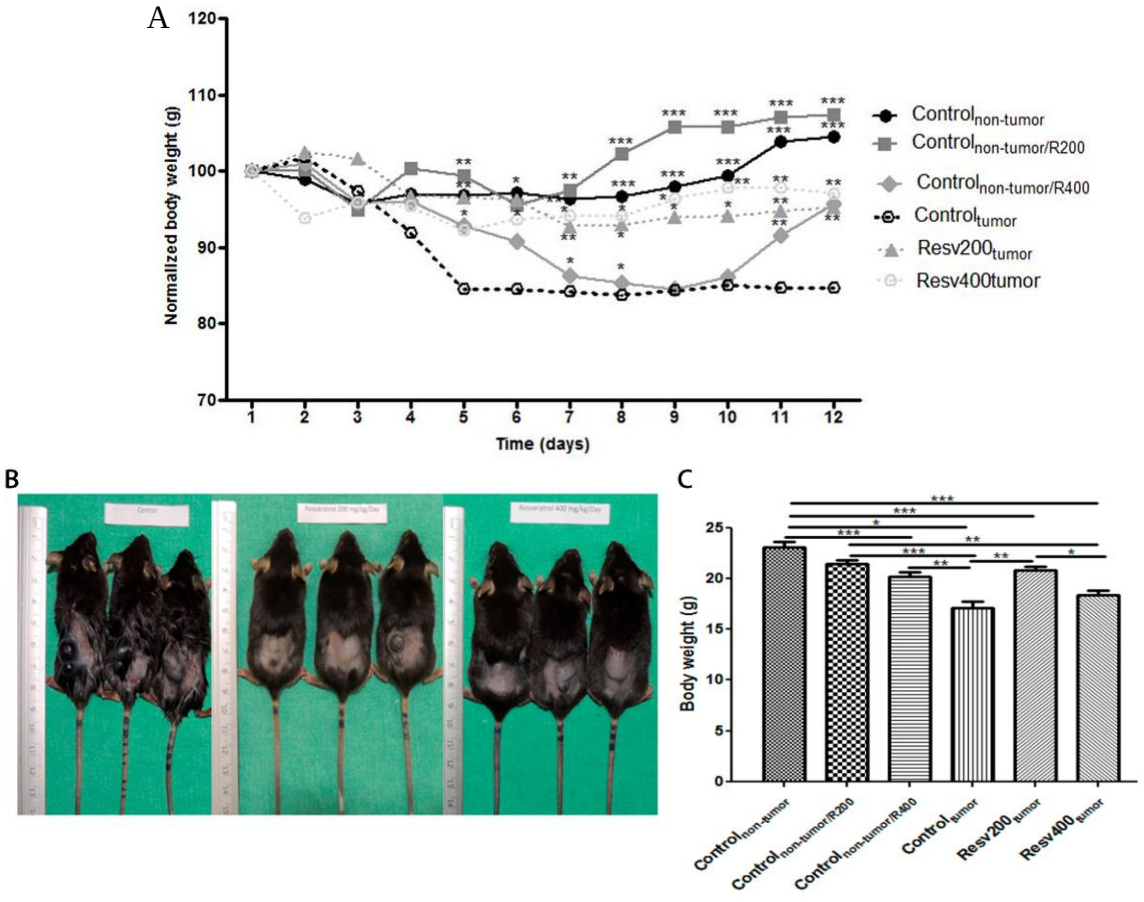


Figure 2.

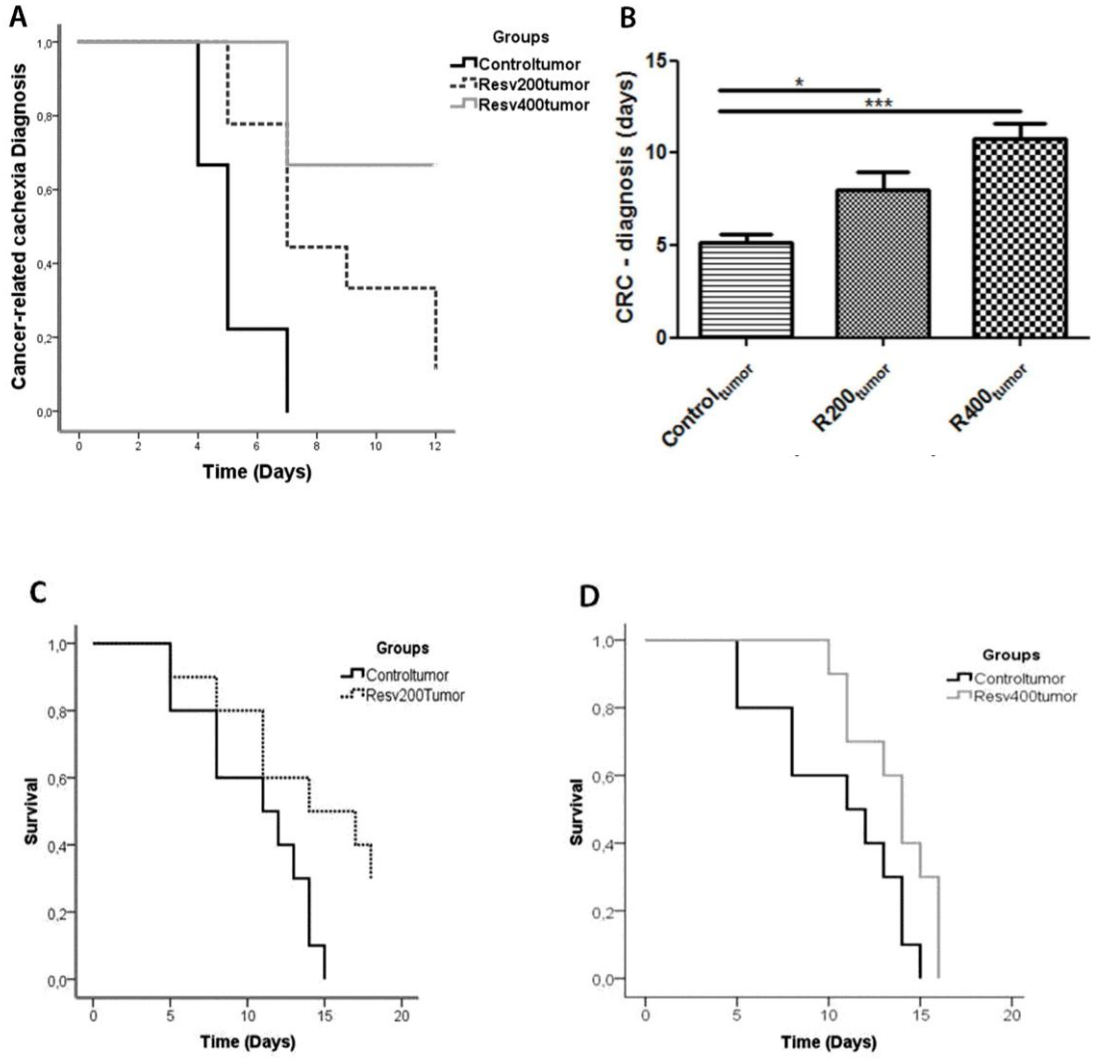

A
Cancer-related cachexia Diagnosis
Time (Days)
Groups
Controltumor
Resv200tumor
Resv400tumor
B
CRC - diagnosis (days)
*
***
Control tumor
R200 tumor
R400 tumor
C
Survival
Time (Days)
Groups
Controltumor
Resv200Tumor
D
Survival
Time (Days)
Groups
Controltumor
Resv400tumor

Figure 3.

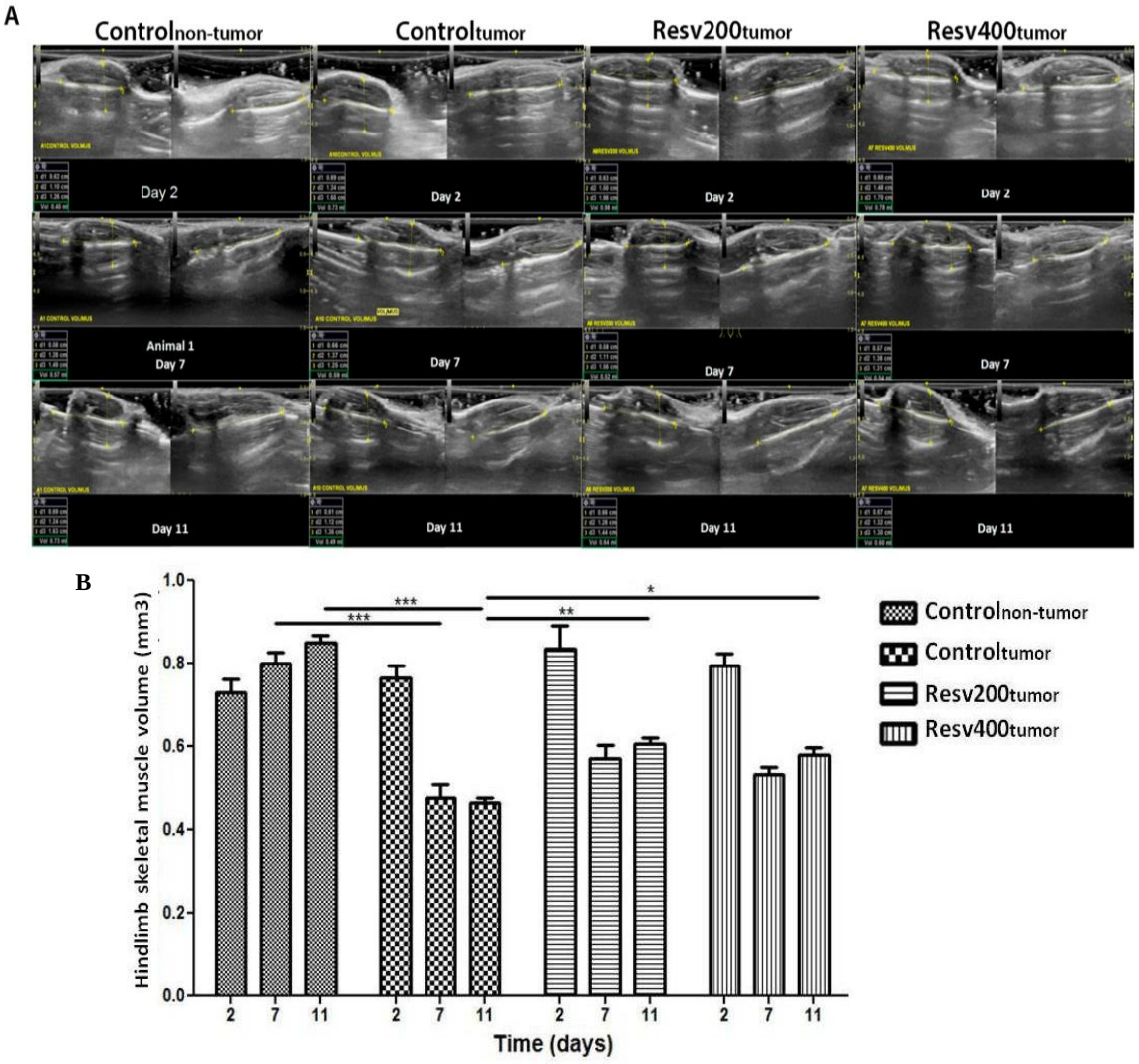

A
Controlnon-tumor
Controltumor
Resv200tumor
Resv400tumor
Day 2
Animal 1
Day 7
Day 11
B
Hindlimb skeletal muscle volume (mm3)
1.0
0.8
0.6
0.4
0.2
0.0
***
***
**
*
2
7
11
Time (days)
Controlnon-tumor
Controltumor
Resv200tumor
Resv400tumor

Figure 4.

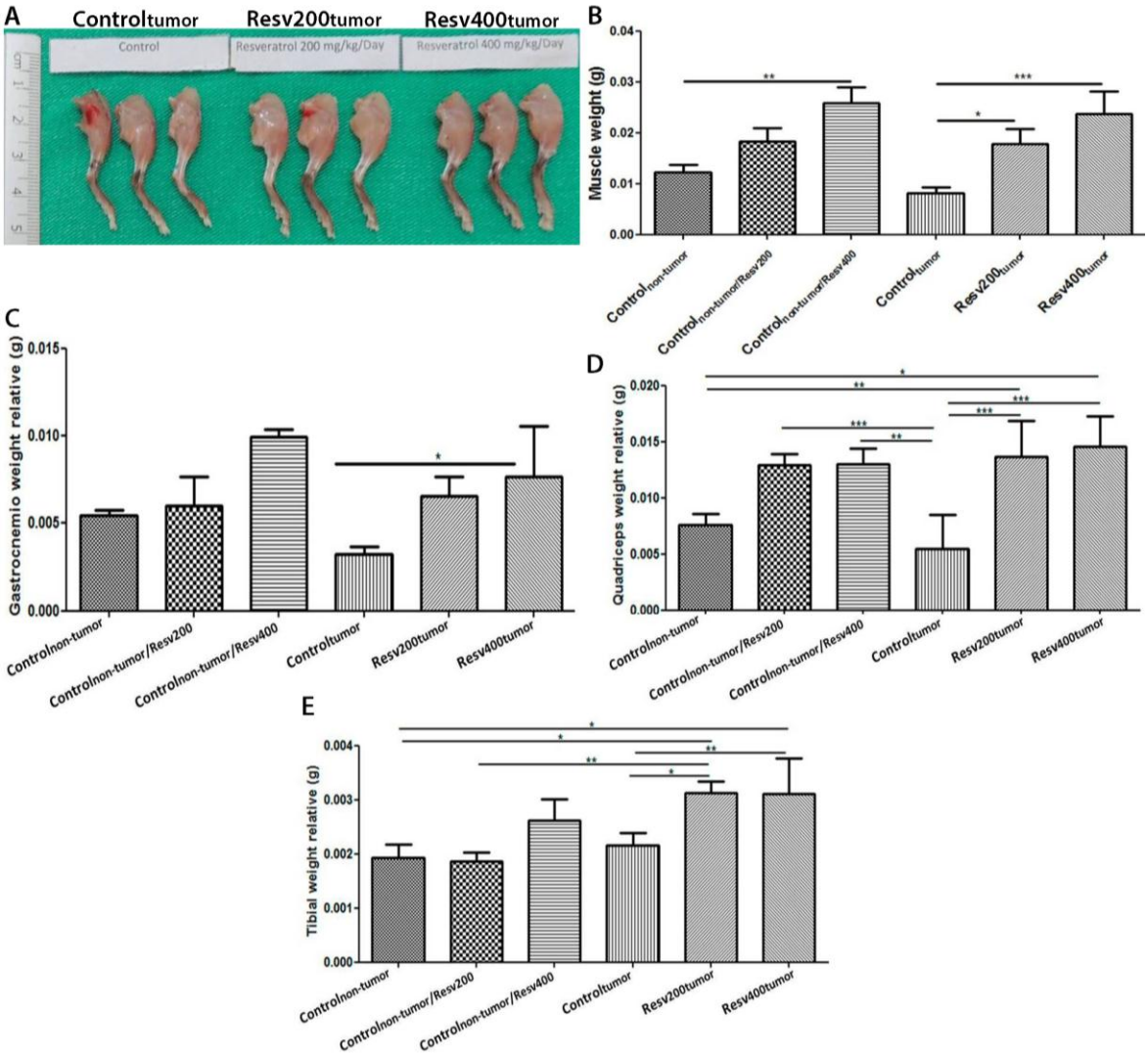

A
Controltumor
Resv200tumor
Resv400tumor
Control
Resveratrol 200 mg/kg/Day
Resveratrol 400 mg/kg/Day
B
Muscle weight (g)
0.04
0.03
0.02
0.01
0.00
Controlnon-tumor
Controlnon-tumor/Resv200
Controlnon-tumor/Resv400
Controltumor
Resv200tumor
Resv400tumor
C
Gastrocnemio weight relative (g)
0.015
0.010
0.005
0.000
Controlnon-tumor
Controlnon-tumor/Resv200
Controlnon-tumor/Resv400
Controltumor
Resv200tumor
Resv400tumor
D
Quadriceps weight relative (g)
0.020
0.015
0.010
0.005
0.000
Controlnon-tumor
Controlnon-tumor/Resv200
Controlnon-tumor/Resv400
Controltumor
Resv200tumor
Resv400tumor
E
Tibial weight relative (g)
0.004
0.003
0.002
0.001
0.000
Controlnon-tumor
Controlnon-tumor/Resv200
Controlnon-tumor/Resv400
Controltumor
Resv200tumor
Resv400tumor

Figure 5

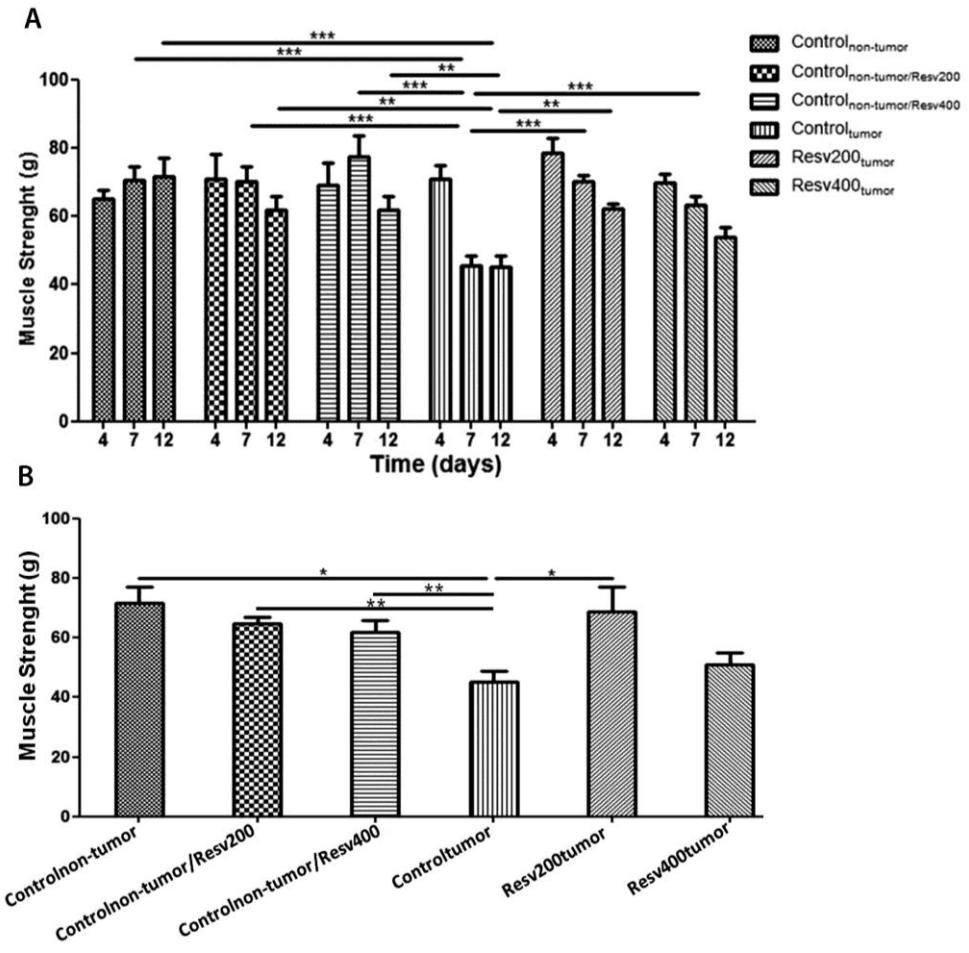

A
Muscle Strenght (g)
Time (days)
Control non-tumor
Control non-tumor/Resv200
Control non-tumor/Resv400
Control tumor
Resv200 tumor
Resv400 tumor
B
Muscle Strenght (g)
Controlnon-tumor
Controlnon-tumor/Resv200
Controlnon-tumor/Resv400
Controltumor
Resv200tumor
Resv400tumor

Figure 6.

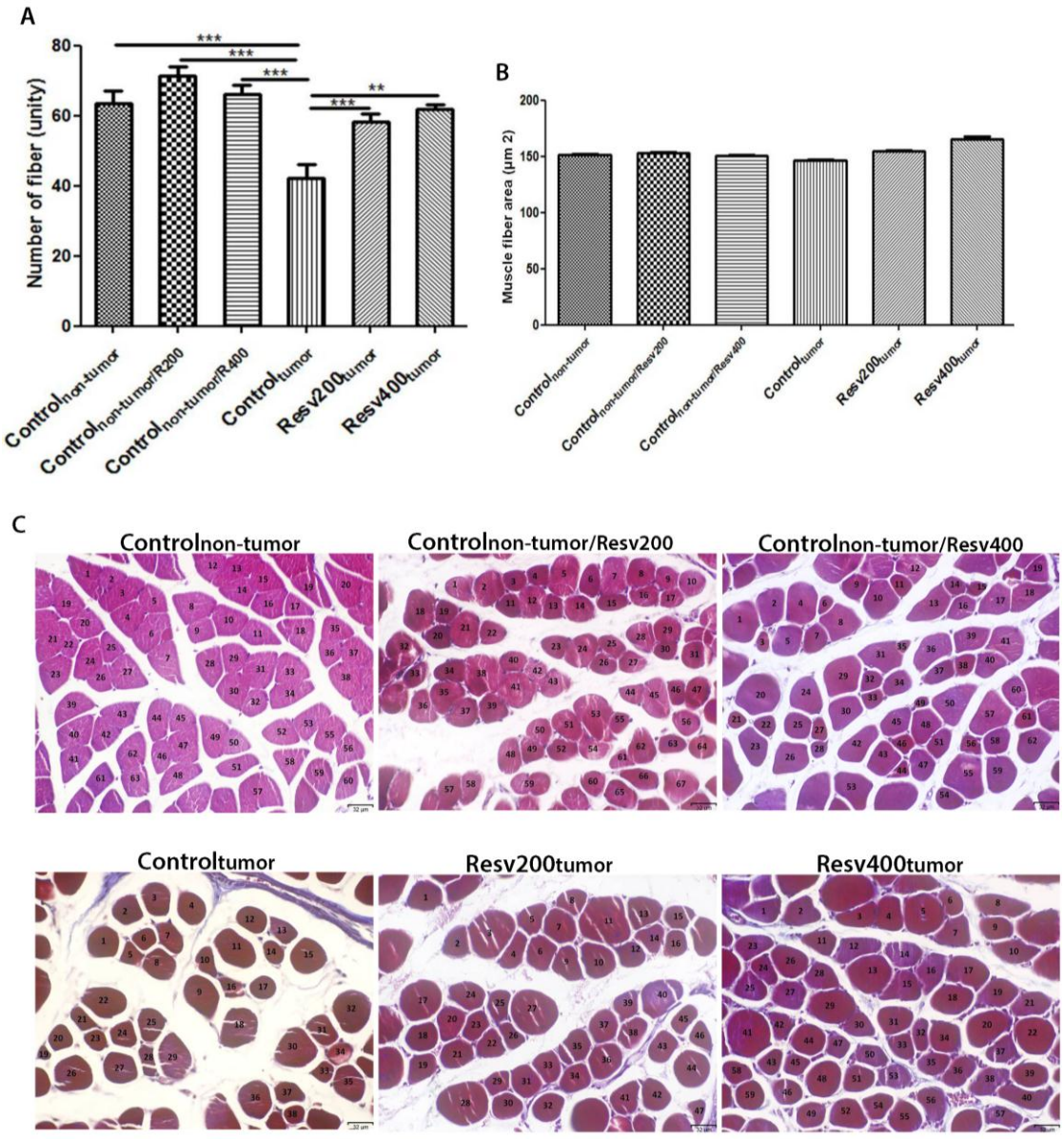

A
Number of fiber (unity)
80
60
40
20
0
***
***
***
***
**
Control non-tumor
Control non-tumor/R200
Control non-tumor/R400
Control tumor
Resv200 tumor
Resv400 tumor
B
Muscle fiber area (μm 2)
200
150
100
50
0
Control non-tumor
Control non-tumor/Resv200
Control non-tumor/Resv400
Control tumor
Resv200 tumor
Resv400 tumor
C
Control non-tumor
Control non-tumor/Resv200
Control non-tumor/Resv400
Control tumor
Resv200 tumor
Resv400 tumor

Figure 7.

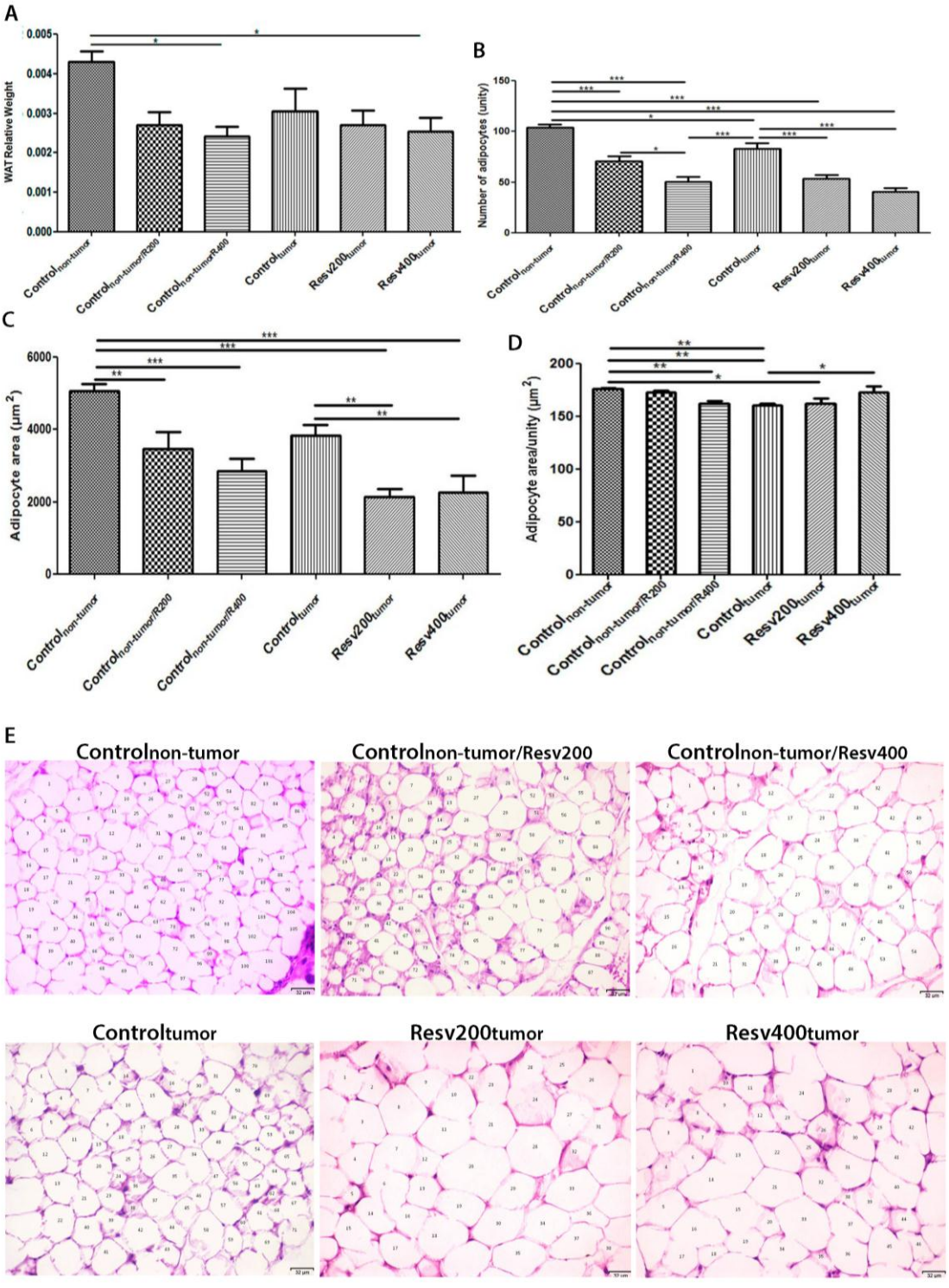

A
WAT Relative Weight
0.005
0.004
0.003
0.002
0.001
0.000
Control non-tumor
Control non-tumor/R200
Control non-tumor/R400
Control tumor
Resv200 tumor
Resv400 tumor
B
Number of adipocytes (unity)
150
100
50
0
C
Adipocyte area (μm 2)
6000
4000
2000
0
D
Adipocyte area/unity (μm 2)
200
150
100
50
0
E
Controlnon-tumor
Controlnon-tumor/Resv200
Controlnon-tumor/Resv400
Controltumor
Resv200tumor
Resv400tumor

Figure 8.

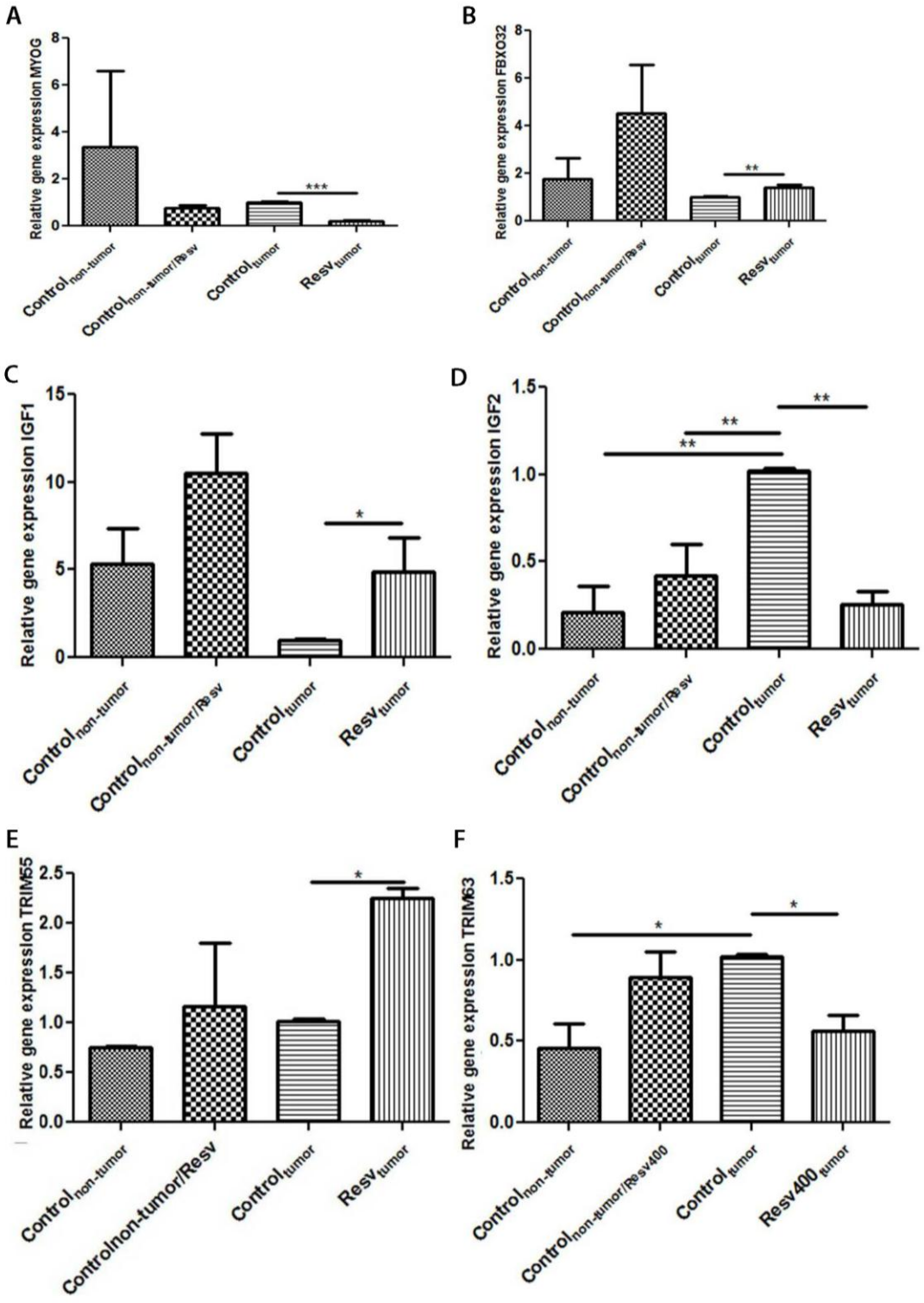

A
Relative gene expression MYOG
8
6
4
2
0
***
Control non-tumor
Control non-tumor/Resv
Control tumor
Resv tumor
B
Relative gene expression FBXO32
8
6
4
2
0
**
Control non-tumor
Control non-tumor/Resv
Control tumor
Resv tumor
C
Relative gene expression IGF1
15
10
5
0
*
Control non-tumor
Control non-tumor/Resv
Control tumor
Resv tumor
D
Relative gene expression IGF2
1.5
1.0
0.5
0.0
**
**
**
Control non-tumor
Control non-tumor/Resv
Control tumor
Resv tumor
E
Relative gene expression TRIM55
2.5
2.0
1.5
1.0
0.5
0.0
*
Control non-tumor
Control non-tumor/Resv
Control tumor
Resv tumor
F
Relative gene expression TRIM63
1.5
1.0
0.5
0.0
*
*
Control non-tumor
Control non-tumor/Resv400
Control tumor
Resv400 tumor

Figure 9.

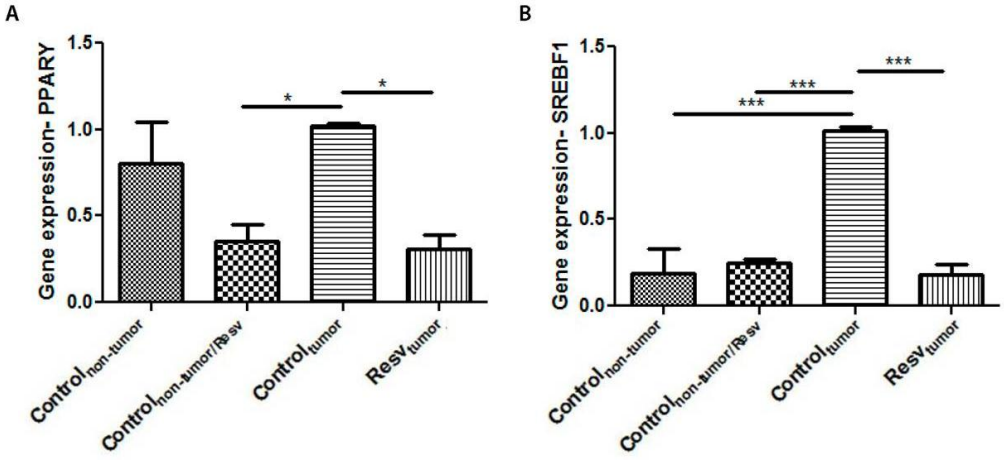
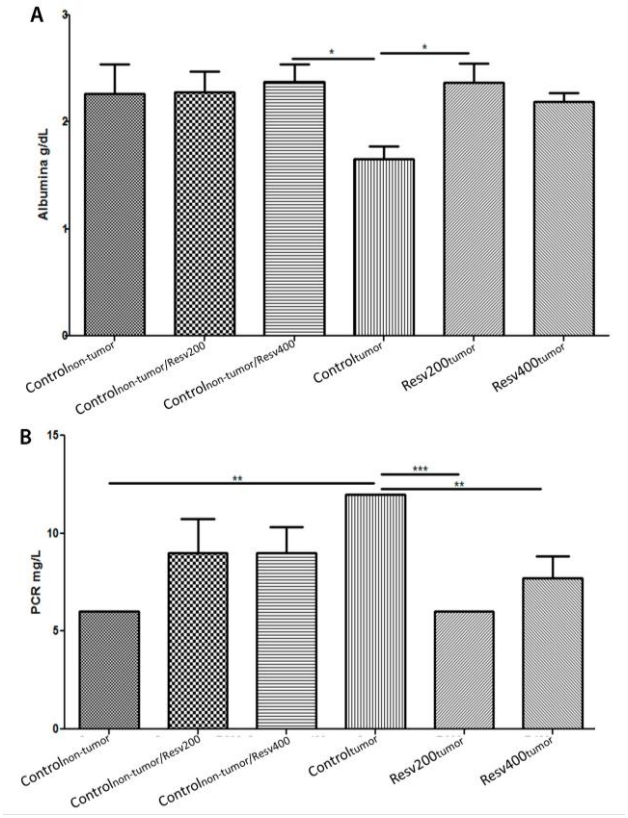

A
Gene expression- PPARY
1.5
1.0
0.5
0.0
*
*
Control non-tumor
Control non-tumor/Resv
Control tumor
Resv tumor
B
Gene expression- SREBF1
1.5
1.0
0.5
0.0
***
***
***
Control non-tumor
Control non-tumor/Resv
Control tumor
Resv tumor

Figure 10


A
Albumina g/dL
3
2
1
0
*
*
Control non-tumor
Control non-tumor/Resv200
Control non-tumor/Resv400
Control tumor
Resv200 tumor
Resv400 tumor
B
PCR mg/L
15
10
5
0
**
***
**
Control non-tumor
Control non-tumor/Resv200
Control non-tumor/Resv400
Control tumor
Resv200 tumor
Resv400 tumor

Legends of Figures

Figure S1: Water (ml) and food (g) consumption measurements were daily performed.

Figure S2: linear equation of the correlation between weight and tumor volume.

Figure S1:

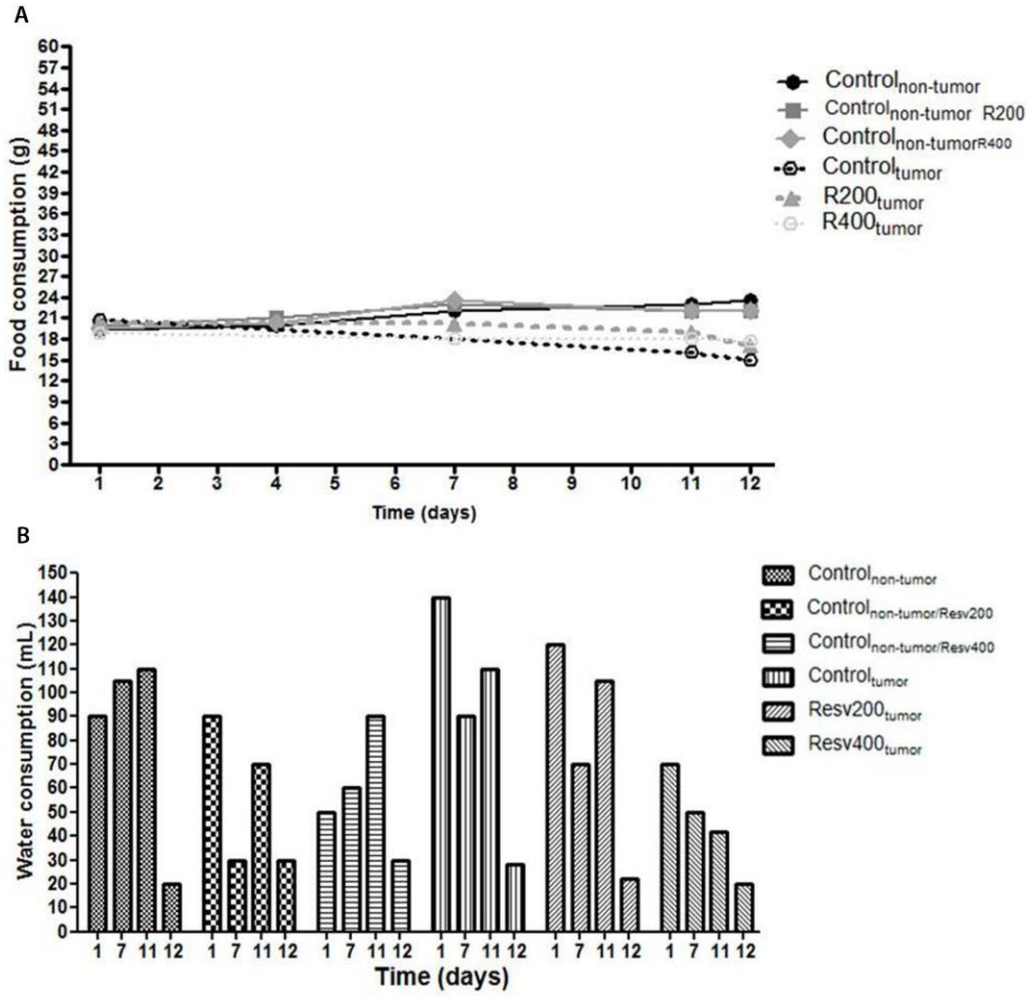
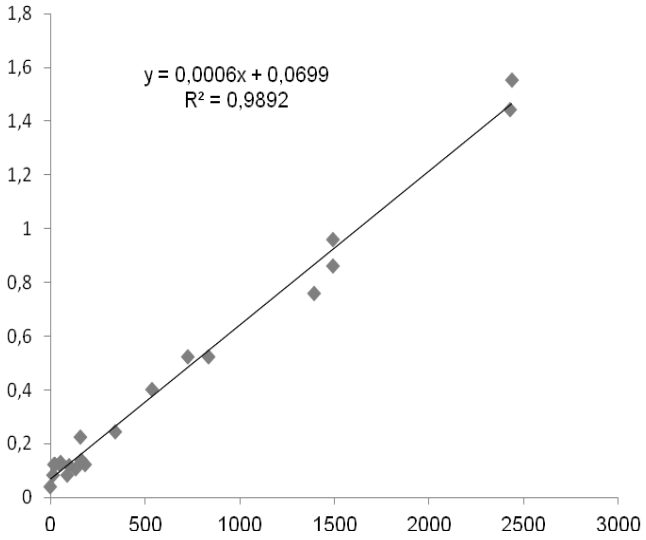


Figure S2:


y = 0,0006x + 0,0699
R² = 0,9892
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
0
500
1000
1500
2000
2500
3000

4 CONCLUSÕES

Os modelos de caquexia relacionados ao melanoma cutâneo são desenvolvidos pela inoculação subcutânea de células B16F10 em camundongos fêmeas C57BL/6. E o diagnostico da caquexia é predominantemente definido pela monitoração do peso corporal dos animais, secundariamente, pelas medidas da massa muscular esquelética e da massa do tecido adiposo do epidídimo. A ação antiinflamátoria dos agentes farmacológicos, intensifica o efeito anticaquético quando ocorre também redução do volume tumoral.

A administração de resveratrol promoveu significativos efeitos anticaquéticos em camundongos C57BL/6 com modelo de caquexia relacionado ao melanoma cutâneo, com resultante melhora da sobrevida dos animais.

REFERÊNCIAS

1. Slingluff CL, Jr., Petroni GR, Chianese-Bullock KA, Smolkin ME, Ross MI, Haas NB, et al. Randomized multicenter trial of the effects of melanoma-associated helper peptides and cyclophosphamide on the immunogenicity of a multipeptide melanoma vaccine. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2011 Jul 20;29(21):2924-32. PubMed PMID: 21690475. Pubmed Central PMCID: PMC3138719. Epub 2011/06/22. eng.
2. Nikolaou V, Stratigos AJ. Emerging trends in the epidemiology of melanoma. The British journal of dermatology. 2014 Jan;170(1):11-9. PubMed PMID: 23815297. Epub 2013/07/03. eng.
3. Naghavi M, Abajobir AA, Abbafati C, Abbas KM, Abd-Allah F, Abera SF, et al. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980–2016: a systematic analysis for the Global Burden of Disease Study 2016. The Lancet. 2017;390(10100):1151-210.
4. Erdmann F, Lortet-Tieulent J, Schuz J, Zeeb H, Greinert R, Breitbart EW, et al. International trends in the incidence of malignant melanoma 1953-2008--are recent generations at higher or lower risk? International journal of cancer. 2013 Jan 15;132(2):385-400. PubMed PMID: 22532371. Epub 2012/04/26. eng.
5. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. International journal of cancer. 2015 Mar 1;136(5):E359-86. PubMed PMID: 25220842. Epub 2014/09/16. eng.
6. Whiteman DC, Green AC, Olsen CM. The Growing Burden of Invasive Melanoma: Projections of Incidence Rates and Numbers of New Cases in Six Susceptible Populations through 2031. The Journal of investigative dermatology. 2016 Jun;136(6):1161-71. PubMed PMID: 26902923. Epub 2016/02/24. eng.
7. Wainstein AJA, Belfort FA. Conduta para o melanoma cutâneo. Revista do Colégio Brasileiro de Cirurgiões. 2004;31:204-14.
8. Whiteman DC, Pavan WJ, Bastian BC. The melanomas: a synthesis of epidemiological, clinical, histopathological, genetic, and biological aspects, supporting distinct subtypes, causal pathways, and cells of origin. Pigment cell & melanoma research. 2011 08/16;24(5):879-97. PubMed PMID: PMC3395885.
9. Tuong W, Cheng LS, Armstrong AW. Melanoma: epidemiology, diagnosis, treatment, and outcomes. Dermatologic clinics. 2012 Jan;30(1):113-24, ix. PubMed PMID: 22117873. Epub 2011/11/29. eng.
10. Garbe C, Peris K, Hauschild A, Saiag P, Middleton M, Bastholt L, et al. Diagnosis and treatment of melanoma. European consensus-based interdisciplinary guideline - Update 2016. European journal of cancer (Oxford, England : 1990). 2016 Aug;63:201-17. PubMed PMID: 27367293. Epub 2016/07/02. eng.
11. Grignol V, Fairchild ET, Zimmerer JM, Lesinski GB, Walker MJ, Magro CM, et al. miR-21 and miR-155 are associated with mitotic activity and lesion depth of borderline melanocytic lesions. British journal of cancer. 2011 Sep 27;105(7):1023-9. PubMed PMID: 21863027. Pubmed Central PMCID: PMC3185929. Epub 2011/08/25. eng.
12. Uong A, Zon LI. Melanocytes in Development and Cancer. Journal of cellular physiology. 2010;222(1):38-41. PubMed PMID: PMC2783760.

13. Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, et al. Distinct sets of genetic alterations in melanoma. The New England journal of medicine. 2005 Nov 17;353(20):2135-47. PubMed PMID: 16291983. Epub 2005/11/18. eng.
14. Eggermont AM, Spatz A, Robert C. Cutaneous melanoma. Lancet (London, England). 2014 Mar 1;383(9919):816-27. PubMed PMID: 24054424. Epub 2013/09/24. eng.
15. Tsao H, Chin L, Garraway LA, Fisher DE. Melanoma: from mutations to medicine. Genes & Development. 2012;26(11):1131-55. PubMed PMID: PMC3371404.
16. Clark WH, Jr., From L, Bernardino EA, Mihm MC. The histogenesis and biologic behavior of primary human malignant melanomas of the skin. Cancer Res. 1969 Mar;29(3):705-27. PubMed PMID: 5773814. Epub 1969/03/01. eng.
17. Moehrle M, Dietz K, Garbe C, Breuninger H. Conventional histology vs. three-dimensional histology in lentigo maligna melanoma. The British journal of dermatology. 2006 Mar;154(3):453-9. PubMed PMID: 16445775. Epub 2006/02/01. eng.
18. Moehrle M, Metzger S, Schippert W, Garbe C, Rassner G, Breuninger H. "Functional" surgery in subungual melanoma. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al]. 2003 Apr;29(4):366-74. PubMed PMID: 12656815. Epub 2003/03/27. eng.
19. Temple CL, Arlette JP. Mohs micrographic surgery in the treatment of lentigo maligna and melanoma. J Surg Oncol. 2006 Sep 15;94(4):287-92. PubMed PMID: 16917877. Epub 2006/08/19. eng.
20. Garbe C, Peris K, Hauschild A, Saiag P, Middleton M, Bastholt L, et al. Diagnosis and treatment of melanoma. European consensus-based interdisciplinary guideline – Update 2016. European Journal of Cancer. 2016;63:201-17.
21. Holmes D. The cancer that rises with the Sun. Nature. 2014 11/19/online;515:S110.
22. Ruiter DJ, Spatz A, van den Oord JJ, Cook MG. Pathologic staging of melanoma. Seminars in oncology. 2002 Aug;29(4):370-81. PubMed PMID: 12170440. Epub 2002/08/10. eng.
23. Saiag P, Bosquet L, Guillot B, Verola O, Avril MF, Bailly C, et al. Management of adult patients with cutaneous melanoma without distant metastasis. 2005 update of the French Standards, Options and Recommendations guidelines. Summary report. European journal of dermatology : EJD. 2007 Jul-Aug;17(4):325-31. PubMed PMID: 17540641. Epub 2007/06/02. eng.
24. Mills JK, White I, Diggs B, Fortino J, Vetto JT. Effect of biopsy type on outcomes in the treatment of primary cutaneous melanoma. American journal of surgery. 2013 May;205(5):585-90; discussion 90. PubMed PMID: 23592167. Epub 2013/04/18. eng.
25. Robert C, Dummer R, Gutzmer R, Lorigan P, Kim KB, Nyakas M, et al. Selumetinib plus dacarbazine versus placebo plus dacarbazine as first-line treatment for BRAF-mutant metastatic melanoma: a phase 2 double-blind randomised study. The Lancet Oncology. 2013 Jul;14(8):733-40. PubMed PMID: 23735514. Epub 2013/06/06. eng.
26. Rubin KM. Management of primary cutaneous and metastatic melanoma. Seminars in oncology nursing. 2013 Aug;29(3):195-205. PubMed PMID: 23958217. Epub 2013/08/21. eng.
27. Kirkwood JM, Manola J, Ibrahim J, Sondak V, Ernstoff MS, Rao U. A pooled analysis of eastern cooperative oncology group and intergroup trials of adjuvant high-dose interferon for melanoma. Clinical cancer research : an official journal of the

American Association for Cancer Research. 2004 Mar 1;10(5):1670-7. PubMed PMID: 15014018. Epub 2004/03/12. eng.
28. Jonasch E, Kumar UN, Linette GP, Hodi FS, Soiffer RJ, Ryan BF, et al. Adjuvant high-dose interferon alfa-2b in patients with high-risk melanoma. Cancer journal (Sudbury, Mass). 2000 May-Jun;6(3):139-45. PubMed PMID: 10882328. Epub 2000/07/06. eng.
29. Delaney G, Barton M, Jacob S. Estimation of an optimal radiotherapy utilization rate for melanoma: a review of the evidence. Cancer. 2004 Mar 15;100(6):1293-301. PubMed PMID: 15022299. Epub 2004/03/17. eng.
30. Kawamura I, Yamamoto N, Sakai F, Yamazaki H, Naoe Y, Inami M, et al. Activation of lipoprotein lipase and inhibition of B16 melanoma-induced cachexia in mice by ponalrestat, an aldose reductase inhibitor. Anticancer research. 1999 Jan-Feb;19(1A):341-8. PubMed PMID: 10226565. Epub 1999/05/05. eng.
31. Das SK, Eder S, Schauer S, Diwoky C, Temmel H, Guertl B, et al. Adipose triglyceride lipase contributes to cancer-associated cachexia. Science (New York, NY). 2011 Jul 8;333(6039):233-8. PubMed PMID: 21680814. Epub 2011/06/18. eng.
32. Voltarelli FA, Frajacomo FT, Padilha CdS, Testa MTJ, Cella PS, Ribeiro DF, et al. Syngeneic B16F10 Melanoma Causes Cachexia and Impaired Skeletal Muscle Strength and Locomotor Activity in Mice. Frontiers in Physiology. 2017 09/29
05/12/received
09/05/accepted;8:715. PubMed PMID: PMC5626871.
33. Fearon K, Strasser F, Anker SD, Bosaeus I, Bruera E, Fainsinger RL, et al. Definition and classification of cancer cachexia: an international consensus. The Lancet Oncology. 2011 May;12(5):489-95. PubMed PMID: 21296615. Epub 2011/02/08. eng.
34. Argilés JM, Fontes-Oliveira CC, Toledo M, López-Soriano FJ, Busquets S. Cachexia: a problem of energetic inefficiency. Journal of Cachexia, Sarcopenia and Muscle. 2014 08/15
01/10/received
06/22/accepted;5(4):279-86. PubMed PMID: PMC4248416.
35. Ebner N, Steinbeck L, Doehner W, Anker SD, von Haehling S. Highlights from the 7th Cachexia Conference: muscle wasting pathophysiological detection and novel treatment strategies. Journal of cachexia, sarcopenia and muscle. 2014;5(1):27-34. PubMed PMID: 24595460. Epub 03/05.
36. Fearon KC, Glass DJ, Guttridge DC. Cancer cachexia: mediators, signaling, and metabolic pathways. Cell metabolism. 2012 Aug 8;16(2):153-66. PubMed PMID: 22795476. Epub 2012/07/17. eng.
37. Soares RJ, Cagnin S, Chemello F, Silvestrin M, Musaro A, De Pitta C, et al. Involvement of MicroRNAs in the Regulation of Muscle Wasting during Catabolic Conditions. Journal of Biological Chemistry. 2014 August 8, 2014;289(32):21909-25.
38. Suzuki H, Asakawa A, Amitani H, Nakamura N, Inui A. Cancer cachexia—pathophysiology and management. Journal of Gastroenterology. 2013 03/20
02/15/received
02/20/accepted;48(5):574-94. PubMed PMID: PMC3698426.
39. Tisdale MJ. Mechanisms of cancer cachexia. Physiological reviews. 2009 Apr;89(2):381-410. PubMed PMID: 19342610. Epub 2009/04/04. eng.
40. Blum D, Omlin A, Fearon K, Baracos V, Radbruch L, Kaasa S, et al. Evolving classification systems for cancer cachexia: ready for clinical practice? Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer. 2010 Mar;18(3):273-9. PubMed PMID: 20076976. Epub 2010/01/16. eng.

41. Mondello P, Mian M, Aloisi C, Fama F, Mondello S, Pitini V. Cancer cachexia syndrome: pathogenesis, diagnosis, and new therapeutic options. Nutrition and cancer. 2015;67(1):12-26. PubMed PMID: 25513730. Epub 2014/12/17. eng.
42. Allen M, Louise Jones J. Jekyll and Hyde: the role of the microenvironment on the progression of cancer. The Journal of pathology. 2011 Jan;223(2):162-76. PubMed PMID: 21125673. Epub 2010/12/03. eng.
43. Chesler L. Paraneoplasia, cancer development and immunity: what are the connections? Nature reviews Cancer. 2014 Jul;14(7):447-8. PubMed PMID: 25093228. Epub 2014/08/06. eng.
44. Diffee GM, Kalfas K, Al-Majid S, McCarthy DO. Altered expression of skeletal muscle myosin isoforms in cancer cachexia. American journal of physiology Cell physiology. 2002 Nov;283(5):C1376-82. PubMed PMID: 12372798. Epub 2002/10/10. eng.
45. Tijerina AJ. The biochemical basis of metabolism in cancer cachexia. Dimensions of critical care nursing : DCCN. 2004 Nov-Dec;23(6):237-43. PubMed PMID: 15586034. Epub 2004/12/09. eng.
46. Dewys WD, Begg C, Lavin PT, Band PR, Bennett JM, Bertino JR, et al. Prognostic effect of weight loss prior to chemotherapy in cancer patients. Eastern Cooperative Oncology Group. The American journal of medicine. 1980 Oct;69(4):491-7. PubMed PMID: 7424938. Epub 1980/10/01. eng.
47. Tan BH, Fearon KC. Cachexia: prevalence and impact in medicine. Current opinion in clinical nutrition and metabolic care. 2008 Jul;11(4):400-7. PubMed PMID: 18541999. Epub 2008/06/11. eng.
48. Tisdale MJ. Cachexia in cancer patients. Nature reviews Cancer. 2002 Nov;2(11):862-71. PubMed PMID: 12415256. Epub 2002/11/05. eng.
49. Skipworth RJ, Stewart GD, Dejong CH, Preston T, Fearon KC. Pathophysiology of cancer cachexia: much more than host-tumour interaction? Clinical nutrition (Edinburgh, Scotland). 2007 Dec;26(6):667-76. PubMed PMID: 17507116. Epub 2007/05/18. eng.
50. Teunissen SC, Wesker W, Kruitwagen C, de Haes HC, Voest EE, de Graeff A. Symptom prevalence in patients with incurable cancer: a systematic review. Journal of pain and symptom management. 2007 Jul;34(1):94-104. PubMed PMID: 17509812. Epub 2007/05/19. eng.
51. Argiles JM. Cancer-associated malnutrition. European journal of oncology nursing : the official journal of European Oncology Nursing Society. 2005;9 Suppl 2:S39-50. PubMed PMID: 16437757. Epub 2006/01/27. eng.
52. Argiles JM, Busquets S, Stemmler B, Lopez-Soriano FJ. Cancer cachexia: understanding the molecular basis. Nature reviews Cancer. 2014 Nov;14(11):754-62. PubMed PMID: 25291291. Epub 2014/10/08. eng.
53. Blum D, Omlin A, Baracos VE, Solheim TS, Tan BH, Stone P, et al. Cancer cachexia: a systematic literature review of items and domains associated with involuntary weight loss in cancer. Critical reviews in oncology/hematology. 2011 Oct;80(1):114-44. PubMed PMID: 21216616. Epub 2011/01/11. eng.
54. Deans DAC, Tan BH, Wigmore SJ, Ross JA, de Beaux AC, Paterson-Brown S, et al. The influence of systemic inflammation, dietary intake and stage of disease on rate of weight loss in patients with gastro-oesophageal cancer. British Journal of Cancer. 2009 01/06
11/05/revised
11/21/accepted;100(1):63-9. PubMed PMID: PMC2634686.

55. Rose Tan C, M Yaffee P, Jamil L, Lo S, Nissen N, J Pandol S, et al. Pancreatic cancer cachexia: A review of mechanisms and therapeutics2014. 88 p.
56. Pepys MB, Hirschfield GM, Tennent GA, Gallimore JR, Kahan MC, Bellotti V, et al. Targeting C-reactive protein for the treatment of cardiovascular disease. Nature. 2006 4/27;440(7088):1217-21.
57. Christensen JF, Jones LW, Andersen JL, Daugaard G, Rorth M, Hojman P. Muscle dysfunction in cancer patients. Annals of oncology : official journal of the European Society for Medical Oncology. 2014 May;25(5):947-58. PubMed PMID: 24401927. Epub 2014/01/10. eng.
58. Soares RJ, Cagnin S, Chemello F, Silvestrin M, Musaro A, De Pitta C, et al. Involvement of microRNAs in the regulation of muscle wasting during catabolic conditions. The Journal of biological chemistry. 2014 Aug 8;289(32):21909-25. PubMed PMID: 24891504. Pubmed Central PMCID: PMC4139209. Epub 2014/06/04. eng.
59. Oliveira MV, Fraga CA, Barros LO, Pereira CS, Santos SH, Basile JR, et al. High expression of S100A4 and endoglin is associated with metastatic disease in head and neck squamous cell carcinoma. Clinical & experimental metastasis. 2014 Aug;31(6):639-49. PubMed PMID: 24798676. Epub 2014/05/07. eng.
60. Fearon KC, Baracos VE. Cachexia in pancreatic cancer: new treatment options and measures of success. HPB : the official journal of the International Hepato Pancreato Biliary Association. 2010 Jun;12(5):323-4. PubMed PMID: 20590907. Pubmed Central PMCID: PMC2951820. Epub 2010/07/02. eng.
61. Vaughan VC, Martin P, Lewandowski PA. Cancer cachexia: impact, mechanisms and emerging treatments. Journal of cachexia, sarcopenia and muscle. 2013 Jun;4(2):95-109. PubMed PMID: 23097000. Pubmed Central PMCID: PMC3684701. Epub 2012/10/26. eng.
62. Aoyagi T, Terracina KP, Raza A, Matsubara H, Takabe K. Cancer cachexia, mechanism and treatment. World Journal of Gastrointestinal Oncology. 2015 04/15
10/21/received
01/17/revised
03/30/accepted;7(4):17-29. PubMed PMID: PMC4398892.
63. Del Fabbro E. More is better: a multimodality approach to cancer cachexia. The oncologist. 2010;15(2):119-21. PubMed PMID: 20133501. Pubmed Central PMCID: PMC3227946. Epub 2010/02/06. eng.
64. Mousa AB. Sorafenib in the Treatment of Advanced Hepatocellular Carcinoma. Saudi Journal of Gastroenterology : Official Journal of the Saudi Gastroenterology Association. 2008 10/17/received
10/22/accepted;14(1):40-2. PubMed PMID: PMC2702892.
65. Wilken R, Veena MS, Wang MB, Srivatsan ES. Curcumin: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma. Molecular Cancer. 2011 02/07
08/04/received
02/07/accepted;10:12-. PubMed PMID: PMC3055228.
66. Kawamura I, Lacey E, Inami M, Nishigaki F, Naoe Y, Tsujimoto S, et al. Ponalrestat, an aldose reductase inhibitor, inhibits cachexia syndrome in nude mice bearing human melanomas G361 and SEKI. Anticancer research. 1999 Sep-Oct;19(5B):4091-7. PubMed PMID: 10628359. Epub 2000/01/11. eng.
67. Rainer L, Heiss CJ. Conjugated linoleic acid: health implications and effects on body composition. Journal of the American Dietetic Association. 2004 Jun;104(6):963-8, quiz 1032. PubMed PMID: 15175596. Epub 2004/06/04. eng.

68. Carter LG, D'Orazio JA, Pearson KJ. Resveratrol and cancer: focus on in vivo evidence. Endocrine-Related Cancer. 2014 02/03/revised
02/05/accepted;21(3):R209-R25. PubMed PMID: PMC4013237.
69. Trela BC, Waterhouse AL. Resveratrol: Isomeric Molar Absorptivities and Stability. Journal of Agricultural and Food Chemistry. 1996 1996/01/01;44(5):1253-7.
70. Diaz-Gerevini GT, Repossi G, Dain A, Tarres MC, Das UN, Eynard AR. Beneficial action of resveratrol: How and why? Nutrition (Burbank, Los Angeles County, Calif). 2016 Feb;32(2):174-8. PubMed PMID: 26706021. Epub 2015/12/27. eng.
71. Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, et al. Resveratrol improves health and survival of mice on a high-calorie diet. Nature. 2006 Nov 16;444(7117):337-42. PubMed PMID: 17086191. Pubmed Central PMCID: PMC4990206. Epub 2006/11/07. eng.
72. Shakibaei M, Harikumar KB, Aggarwal BB. Resveratrol addiction: to die or not to die. Molecular nutrition & food research. 2009 Jan;53(1):115-28. PubMed PMID: 19072742. Epub 2008/12/17. eng.
73. Maier-Salamon A, Bohmdorfer M, Thalhammer T, Szekeres T, Jaeger W. Hepatic glucuronidation of resveratrol: interspecies comparison of enzyme kinetic profiles in human, mouse, rat, and dog. Drug metabolism and pharmacokinetics. 2011;26(4):364-73. PubMed PMID: 21512262. Epub 2011/04/23. eng.
74. Kim YJ, Chung SO, Kim JK, Park SU. Recent studies on resveratrol and its biological and pharmacological activity. EXCLI Journal. 2017 04/27
03/05/received
04/11/accepted;16:602-8. PubMed PMID: PMC5491918.
75. Lin HY, Lansing L, Merillon JM, Davis FB, Tang HY, Shih A, et al. Integrin alphaVbeta3 contains a receptor site for resveratrol. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2006 Aug;20(10):1742-4. PubMed PMID: 16790523. Epub 2006/06/23. eng.
76. Hsieh TC, Wu JM. Differential effects on growth, cell cycle arrest, and induction of apoptosis by resveratrol in human prostate cancer cell lines. Experimental cell research. 1999 May 25;249(1):109-15. PubMed PMID: 10328958. Epub 1999/05/18. eng.
77. Liu FC, Tsai YF, Tsai HI, Yu HP. Anti-Inflammatory and Organ-Protective Effects of Resveratrol in Trauma-Hemorrhagic Injury. Mediators of inflammation. 2015;2015:643763. PubMed PMID: 26273141. Pubmed Central PMCID: PMC4529946. Epub 2015/08/15. eng.
78. Rai G, Mishra S, Suman S, Shukla Y. Resveratrol improves the anticancer effects of doxorubicin in vitro and in vivo models: A mechanistic insight. Phytomedicine : international journal of phytotherapy and phytopharmacology. 2016 Mar 15;23(3):233-42. PubMed PMID: 26969377. Epub 2016/03/13. eng.
79. Kumar A, Dhar S, Rimando AM, Lage JM, Lewin JR, Zhang X, et al. Epigenetic potential of resveratrol and analogs in preclinical models of prostate cancer. Annals of the New York Academy of Sciences. 2015 Aug;1348(1):1-9. PubMed PMID: 26214308. Epub 2015/07/28. eng.
80. Li W, Cao L, Chen X, Lei J, Ma Q. Resveratrol inhibits hypoxia-driven ROS-induced invasive and migratory ability of pancreatic cancer cells via suppression of the Hedgehog signaling pathway. Oncology reports. 2016 Mar;35(3):1718-26. PubMed PMID: 26707376. Epub 2015/12/29. eng.

81. Tan L, Wang W, He G, Kuick RD, Gossner G, Kueck AS, et al. Resveratrol inhibits ovarian tumor growth in an in vivo mouse model. Cancer. 2016 Mar 1;122(5):722-9. PubMed PMID: 26619367. Epub 2015/12/01. eng.
82. Yun H, Park S, Kim MJ, Yang WK, Im DU, Yang KR, et al. AMP-activated protein kinase mediates the antioxidant effects of resveratrol through regulation of the transcription factor FoxO1. The FEBS journal. 2014 Oct;281(19):4421-38. PubMed PMID: 25065674. Epub 2014/07/30. eng.
83. Sin TK, Yung BY, Yip SP, Chan LW, Wong CS, Tam EW, et al. SIRT1-dependent myoprotective effects of resveratrol on muscle injury induced by compression. Front Physiol. 2015;6:293. PubMed PMID: 26557094. Pubmed Central PMCID: PMC4617057. Epub 2015/11/12. eng.
84. Hyatt JP, Nguyen L, Hall AE, Huber AM, Kocan JC, Mattison JA, et al. Muscle-Specific Myosin Heavy Chain Shifts in Response to a Long-Term High Fat/High Sugar Diet and Resveratrol Treatment in Nonhuman Primates. Front Physiol. 2016;7:77. PubMed PMID: 26973542. Pubmed Central PMCID: PMC4773583. Epub 2016/03/15. eng.
85. Polley KR, Jenkins N, O'Connor P, McCully K. Influence of exercise training with resveratrol supplementation on skeletal muscle mitochondrial capacity. Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme. 2016 Jan;41(1):26-32. PubMed PMID: 26638911. Epub 2015/12/08. eng.
86. Chang CC, Lin KY, Peng KY, Day YJ, Hung LM. Resveratrol exerts anti-obesity effects in high-fat diet obese mice and displays differential dosage effects on cytotoxicity, differentiation, and lipolysis in 3T3-L1 cells. Endocrine journal. 2016;63(2):169-78. PubMed PMID: 26698690. Epub 2015/12/25. eng.
87. Faghihzadeh F, Hekmatdoost A, Adibi P. Resveratrol and liver: A systematic review. Journal of Research in Medical Sciences : The Official Journal of Isfahan University of Medical Sciences. 2015 12/01/received
02/27/revised
09/01/accepted;20(8):797-810. PubMed PMID: PMC4652315.
88. Pereira S, Park E, Moore J, Faubert B, Breen DM, Oprescu AI, et al. Resveratrol prevents insulin resistance caused by short-term elevation of free fatty acids in vivo. Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme. 2015 Nov;40(11):1129-36. PubMed PMID: 26455923. Epub 2015/10/13. eng.
89. Raj P, Zieroth S, Netticadan T. An overview of the efficacy of resveratrol in the management of ischemic heart disease. Annals of the New York Academy of Sciences. 2015 Aug;1348(1):55-67. PubMed PMID: 26227659. Epub 2015/08/01. eng.
90. Dong W, Yang R, Yang J, Yang J, Ding J, Wu H, et al. Resveratrol pretreatment protects rat hearts from ischemia/reperfusion injury partly via a NALP3 inflammasome pathway. International journal of clinical and experimental pathology. 2015;8(8):8731. PubMed PMID: 26464617. Pubmed Central PMCID: PMC4583849. Epub 2015/10/16. eng.
91. Kaur G, Verma N. Nature curing cancer – review on structural modification studies with natural active compounds having anti-tumor efficiency. Biotechnology Reports. 2015 6//;6:64-78.
92. Sautter CK, Denardin S, Alves AO, Mallmann CA, Penna NG, Heckttheuer LH. Determinação de resveratrol em sucos de uva no Brasil. Food Science and Technology (Campinas). 2005;25:437-42.
93. Buhrmann C, Shayan P, Kraehe P, Popper B, Goel A, Shakibaei M. Resveratrol induces chemosensitization to 5-fluorouracil through up-regulation of intercellular

junctions, Epithelial-to-mesenchymal transition and apoptosis in colorectal cancer. Biochemical pharmacology. 2015 Nov 1;98(1):51-68.
94. Demoulin B, Hermant M, Castrogiovanni C, Staudt C, Dumont P. Resveratrol induces DNA damage in colon cancer cells by poisoning topoisomerase II and activates the ATM kinase to trigger p53-dependent apoptosis. Toxicology in vitro : an international journal published in association with BIBRA. 2015 Aug;29(5):1156-65. PubMed PMID: 25952326. Epub 2015/05/09. eng.
95. Jeong, K. J. Egfr Mediates Lpa-Induced Proteolytic Enzyme Expression and Ovarian Cancer Invasion: Inhibition by Resveratrol. Molecular Oncology, 2013.
96. Gomez, L. S. et al. Resveratrol Decreases Breast Cancer Cell Viability and Glucose Metabolism by Inhibiting 6-Phosphofructo-1-Kinase. Biochimie, 2013.
97.Kumar B, Iqbal MA, Singh RK, Bamezai RN. Resveratrol inhibits TIGAR to promote ROS induced apoptosis and autophagy. Biochimie. 2015 Nov;118:26-35.
98.Carter LG, D'Orazio JA, Pearson KJ. Resveratrol and cancer: focus on in vivo evidence. Endocrine-related cancer. 2014 Jun;21(3):R209-25. PubMed PMID: 24500760.
99.Jang M, Pezzuto JM. Effects of resveratrol on 12-*O*-tetradecanoylphorbol-13-acetate-induced oxidative events and gene expression in mouse skin. Cancer Lett. 1998;134:81–89. 1998.
100. De Pinho L, Andrade JM, Paraiso A, Filho AB, Feltenberger JD, Guimaraes AL, et al. Diet composition modulates expression of sirtuins and renin-angiotensin system components in adipose tissue. Obesity (Silver Spring, Md). 2013 Sep;21(9):1830-5.
101. Kimura Y, Okuda H. Resveratrol isolated from *Polygonum cuspidatum* root prevents tumor growth and metastasis to lung and tumor-induced neovascularization in Lewis lung carcinoma-bearing mice. J Nutr 2001;131:1844–9.
102. Yann Schneider, Benoit Duranton, Francine Goss, Ren Schleiffer,Nikolaus Seiler & Francis Raul. Resveratrol inibe Intestinal Tumorigênese e modula Host-Defesa-relacionadas Expressão Gênica em um modelo animal de Human polipose adenomatosa familiar Nutrition and Cancer. Volume 39, n1, 2009.102-107.

ANEXOS

ANEXO A – Parecer do Comitê de Ética e Pesquisa

UNIVERSIDADE ESTADUAL DE MONTES CLAROS
COMISSÃO DE ÉTICA EM EXPERIMENTAÇÃO E BEM-ESTAR ANIMAL Unimontes

PARECER CONSUBSTANCIADO

Montes Claros, 20 de fevereiro de 2017.

Processo N.º **131**

Título do Projeto: **EFEITOS DO EXERCÍCIO FÍSICO, DO RESVERATROL NO SISTEMA UBIQUINA-PROTEASSOMA, NO CRESCIMENTO TUMORAL, NA OCORRÊNCIA DE METÁSTASE E NA CAQUEXIA ASSOCIADA AO MODELO SINGÊNICO DE MELANOMA CUTÂNEO EM CAMUNDONGOS C57Bl/6.**

Orientador: **Prof. Dr. Alfredo Maurício Batista de Paula**

Histórico
O melanoma humano representa um tipo de câncer que ocorre a partir de alterações moleculares genéticas e epigenética em melanócitos localizados nos revestimentos mucoso e cutâneo e em órgãos internos. O melanoma cutâneo (MC) apresenta altas taxas de prevalência e de incidência em várias populações em todo o mundo sendo, portanto, um importante problema de saúde pública mundial. Frequentemente, o MC apresenta altas taxas de morbidade e mortalidade devido à sua usual precocidade de disseminação metastática. Uma parcela importante dos pacientes com MC manifesta uma síndrome paraneoplásica conhecida como caquexia, caracterizada como uma progressiva consumação física decorrente principalmente de alterações do metabolismo proteico em fibras musculares esqueléticas. A caquexia promove um impacto significativamente negativo sobre a resposta terapêutica antineoplásica, a qualidade de vida e o tempo de sobrevida de indivíduos com câncer. Embora há muito reconhecida clinicamente, a caquexia associada ao câncer não responde às terapias atuais devido à complexidade dos distúrbios moleculares associadas a essa condição clínica.

Mérito
Avaliar os efeitos do exercício físico, do resveratrol no sistema ubiquinaproteassoma, no crescimento tumoral, na ocorrência de metástase e na caquexia associada ao modelo singênico de melanoma cutâneo em camundongos C57Bl/6.
Baseada nas Informações contidas no projeto e no Protocolo para uso de animais em Pesquisa o Projeto tem Mérito.

Parecer
A Comissão de Ética em Experimentação e Bem-Estar Animal da Unimontes analisou o processo **131** e entende que o mesmo está dentro das normas da Comissão Sendo assim, somos pela **APROVAÇÃO** do projeto de pesquisa.

Prof. Orlando Raphael Lopasso Júnior
Presidente da Comissão de Ética em Experimentação
e Bem-Estar Animal da UNIMONTES

1

ANEXO B – Primeiro parecer do periódico Critical Reviews in Oncology/Hematology em relação ao produto 1

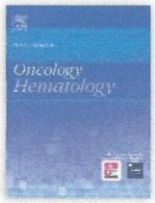
ELSEVIER

OTAVIO Cardoso Filho | Sign out | Help

My Co-authored Submissions

Critical Reviews in Oncology / Hematology

Overview



Review Article | CROH_2018_329

Anticachectic Effects of Pharmacological Agents on Mouse Models of Cutaneous Melanoma-Related Cachexia: a Systematic Review.

alfredo de paula, Amanda Souto Machado, Amanda Rodrigues Santos, Andréia de Souza Brito, Erivelton Pereira Santos, João Lucas Rodrigues dos Santos, Ludmilla Souza, Magda Mendes Vieira, Marcos Vinícius Oliveira, OTAVIO Cardoso Filho, Renato Monteiro Junior

Submitted 19 Sep 2018

Reviewer Invited 10 Nov 2018

View PDF

Alert: keep me informed about the submission status for this manuscript

Abstract +

Decision Letter - RESUBMISSION: 18 September 2018 −

Ref: CROH_2018_329
Title: Anticachectic Effects of Pharmacological Agents on Mouse Models of Cutaneous Melanoma-Related Cachexia: a Systematic Review.
Journal: Critical Reviews in Oncology / Hematology

Dear Professor de paula,