UNIVERSIDADE ESTADUAL DE MONTES CLAROS

Cláudio Marcelo Cardoso

Alterações genéticas e epigenéticas associadas a neoplasias de cabeça e pescoço.

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Orientador: Prof. Dr André Luíz de Sena Guimarães.

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Dedico este trabalho à minha esposa Cristiane, presença eterna em minhas conquistas e amparo nos momentos de angústia, e às minhas filhas Brenda, Júlia e Sofia, por fazerem tudo valer a pela.

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A Deus, por manter sempre viva a minha fé.

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RESUMO

Câncer é a principal causa de morte em países economicamente desenvolvidos e a segunda causa de morte nos países em desenvolvimento sendo as neoplasias malignas de cabeça e pescoço, responsáveis por 5% dos casos novos de câncer no mundo e a sexta mais frequente no Brasil, representados em sua maioria por carcinoma de células escamosas (HNSCC) e em parte por neoplasias de glândulas salivares (SGNs), estas formando um grupo com ampla diversidade morfológica entre os diferentes tipos de neoplasia e, por vezes, até mesmo dentro de uma única massa tumoral. Recentemente, alterações genéticas têm sido intensamente investigadas em HNSCC, dentre elas os polimorfismos. O gene *TP53* está localizado no cromossomo 17 (17p13.1) e codifica uma fosfoproteína de 53 kDa (P53) e está envolvido no controle do ciclo celular e associado a diversos tipos de neoplasia. O polimorfismo de nucleotídio único no codon 72 (72 SNP), localizado no exon 4 do gene *TP53* pode estar relacionado com a gênese e a progressão de alguns tipos de neoplasia. A hipóxia é um importante mecanismo de resistência ao tratamento em cânceres. Alterações epigenéticas têm se mostrado importantes no contexto das neoplasias de cabeça e pescoço. MiR-210 é um gene hipóxia-induzido, regulado pelo fator hipóxia-induzido 1α (HIF-1α) e exerce vários papéis na célula, como inibir a apoptose e aumentar a angiogênese. Estudos têm demonstrado grande potencial como biomarcardor no diagnóstico e prognóstico de diversos tipos de neoplasias. A bioinformática é uma importante ferramenta para avaliação de diversos fenômenos. Os objetivos desta dissertação foram: avaliar a associação do 72 SNP do gene *TP53* com o aumento do risco de desenvolvimento e pior prognóstico de HNSCC, avaliar os níveis de marcadores de hipóxia (HIF-1α e miR-210) em SGNs benignas e malignas e avaliar os principais processos associados à quimioterapia em SGNs, através de bioinformática. A avaliação do 72 SNP do gene *TP53* em indivíduos com HNSCC e no grupo controle foi feita através da Reação em Cadeia da Polimerase (PCR), análise multivariada foi realizada para avaliar o odds ratio do HNSCC e Fuzzy C Means Clustering foi usado para agrupar os indivíduos com HNSCC, para análise de sobrevivência. A avaliação da expressão do HIF-1α e do miR-210 ocorreu através de PCR em 21 amostras, incluindo 7 (33,33%) de SGNs malignas, 7 (33,33%) de SGNs benignas e 7 de tecido salivar não neoplásico. Análise

bioinformática para identificar os principais processos biológicos envolvidos com a aplicação de quimioterapia em SGNs foi realizada. Observou que indivíduos portadores de um alelo arginina em 72 SNP de *TP53* tem risco aumentado para HNSCC, porém, não se observou associação entre o codon 72 SNP de *TP53* e prognóstico em HNSCC. Não houve diferença entre os níveis de HIF1α e miR-210 em neoplasias benignas e malignas de glândulas salivares, em comparação com o grupo controle de glândula salivar normal. Análise bioinformática demonstrou que processos relacionados com o DNA e divisão celular, são os mais importantes para SGNs.

Palavras-chave: *TP53* . Polimorfismo de nucleotídeo único . Carcinoma de Células Escamosas de Cabeça e Pescoço . HIF-1α . miR-210 . neoplasia de glândula salivar .

ABSTRACT

Cancer is the leading cause of death worldwide and head and neck malignant neoplasias account for 5% of all new cancer cases in the world. This cancer subtype is the sixth most common form of cancer in Brazil. Specifically, squamous cell carcinoma (SCC) and salivary gland neoplasias (SGNs) account for the majority of reported cases within this subtype. SGNs exhibit a broad spectrum of phenotypic heterogeneity, both within the same tumor as well as when compared to other types of neoplasias. Recently, genetic alterations, including polymorphisms, have been extensively investigated in SCC. The *TP53* (Tumor Protein P53) gene is localized to chromosome 17 (17p13.1) and encodes a 53kDa phosphoprotein (P53), which is involved in cell cycle control and associated with a number of neoplasias. Interestingly, a 72 codon single-nucleotide-polymorphism (72 SNP), localized to exon 4 of *TP53* is associated with the onset and progression of some types of neoplasia. Hypoxia is an important resistance mechanism for cancer therapy and epigenetic modifications have been shown to be associated with head and neck neoplasias. In fact, miR-210, a miRNA regulated by hypoxia via the hypoxia-inducible factor 1α (HIF-1α), is involved in inhibition of apoptosis and increased angiogenesis. Studies have suggested that miR-210 might be a useful biomarker for both diagnosis and prognosis of various neoplasias. Bioinformatics is a powerful tool for the comprehensive evaluation of differential gene expression in tumours. The aim of this dissertation was to evaluate the relationship between expression of the *TP53* codon 72 SNP and both the risk of developing HNSCC as well as prognosis in patients with HNSCC. Furthermore, the goal was to determine whether the expression of the hypoxia markers, HIF-1α and miR-210, are altered in benign and malignant SGNs. Finally, through the use of bioinformatics, our aim was to investigate the main processes altered following chemotherapeutic treatment in SGNs. Expression of the *TP53* codon 72 SNP in HNSCC and control individuals was evaluated using Polymerase Chain Reaction (PCR), which was followed with a multivariate analysis to determine the odds ratio of HNSCC. Additionally, a Fuzzy C Means Clustering was used to group HSSCC individuals for survival analysis. PCR was also used to evaluate the expression of HIF-1α and miR-210 in 21 samples, including 7

malignant SGNs (33.33%), 7 benign SGNs (33.33%) and 7 control non-neoplastic salivary tissue samples (33.33%). Through the use of bioinformatics, we evaluated the main biological processes regulated by chemotherapy in SGNs. Individuals expressing one arginine of the *TP53* codon72 SNP had a greater risk of HNSCC development. However, no association was observed between *TP53* codon72 SNP expression and worse HNSCC prognosis. Furthermore, there was no difference in HIF-1α and miR-210 expression in either benign or malignant neoplasias of salivary glands, as compared to control, non-neoplastic salivary glands. Finally, bioinformatic analyses revealed the most robust alterations in processes specifically related to DNA and cellular division in SGNs, as compared to control healthy tissue samples.

Key-words – *TP53* . single-nucleotide-polymorphism . head and neck squamous cells carcinoma . HIF-1α . miR-210 . salivary gland neoplasias

SUMÁRIO

1 INTRODUÇÃO

Câncer é a principal causa de morte em países economicamente desenvolvidos e a segunda causa de morte nos países em desenvolvimento. O aumento dos casos de câncer nos países em desenvolvimento é resultado do crescimento e envelhecimento da população, bem como da adoção de estilo de vida associados ao câncer, como tabagismo, etilismo, sedentarismo e hábitos alimentares "ocidentalizados" (1), porém, fatores intrínsecos individuais também exercem importante papel no desenvolvimento e prognóstico do câncer (2).

Carcinoma de células escamosas de cabeça e pescoço (HNSCC) é responsável por 5% dos casos novos de câncer no mundo, apresentando variações em determinadas áreas do globo, como na Índia, onde o câncer de cavidade oral é mais comum, perfazendo um total de 35% de todos os cânceres (3, 4). No Brasil, é o sexto tipo mais frequente de câncer, sendo a cavidade oral o sítio mais comum. Em 2014, foram estimados 15.290 novos casos, com aproximadamente 10% dos casos ocorrendo no estado de Minas Gerais (5). Apesar do avanço tecnológico, do melhor conhecimento da biologia tumoral e do surgimento de novas drogas, a cirurgia continua sendo o tratamento padrão, estando a radioterapia, associada ou não à quimioterapia, indicada nos casos iniciais ou em adjuvância ao tratamento cirúrgico, sendo indicada ainda, nos casos irressecáveis ou em protocolos para preservação de órgão (3, 4, 6, 7). A sobrevida em cinco anos é acima de 80% nos estágios iniciais, caindo para cerca de 40% na presença de metástases linfonodais e 20% na presença de metástase à distância (3) (8).

As neoplasias de glândulas salivares representam um grupo com ampla diversidade morfológica entre os diferentes tipos de neoplasia e, por vezes, até mesmo dentro de uma única massa tumoral. Além disso, a ampla variedade de apresentação clínica, os diferentes padrões de metastatização, a presença de tumores híbridos, desdiferenciação e proprensão para transformação maligna de algumas neoplasias benignas podem dificultar a interpretação histopatológica. Essas características, associadas com a baixa incidência, pode tornar o diagnóstico difícil, apesar da abundância de entidades tumorais classificadas (9).

A incidência das neoplasias de glândulas salivares varia de 0,4 a 6,5 casos por 100.000 pessoas e ocorrem em cerca de 2% a 6,5% de todas as neoplasias de cabeça e pescoço (10).

As neoplasias benignas são cerca de 54% a 79% e as malignas estão entre 21% e 46% dos tumores de glândula salivar, sendo que 64% a 80% de todos os tumores primários epiteliais ocorrem nas parótidas, 7% a 11% nas submandibulares, menos de 1% nas sublinguais e de 9% a 23% nas glândulas salivares menores. Das neoplasias de parótida, de 76% a 80% são benignas, sendo o adenoma pleomórfico a mais frequente, seguido pelo tumor de Warthin, que pode ser bilateral, multicêntrico ou ambos em 10% dos casos (11).

O Carcinoma mucoepidermóide é a neoplasia maligna mais comum (31,7%), seguido pelo Adenocarcinoma sem outras especificações- SOE (14,3%), Carcinoma de células acinares (14,3%) e Carcinoma adenoidecístico (11,9%) (12).

Das neoplasias da glândula submandibular, cerca da metade são benignas, sendo o adenoma pleomórfico a mais comum e a neoplasia maligna mais frequente é o Carcinoma adenoidecístico, seguido do Carcinoma mucoepidermóide.

As neoplasias da glândula sublingual, assim como as neoplasias das glândulas salivares menores são em sua maioria malignos (78% a 82%). A neoplasia benigna mais frequente é o adenoma pleomórfico e a maligno é o Carcinoma adenoide cístico, seguido pelo Carcinoma mucoepidermoide e pelo Adenocarcinoma (13).

O diagnóstico das neoplasias de glândulas salivares baseia-se no exame clínico e na punção aspirativa por agulha fina (PAAF).

A PAAF auxilia na diferenciação entre neoplasias benignas e malignas, e entre tumores salivares e linfonodomegalias. Seu índice de positividade está em torno de 77 a 95%, ajudando na escolha da terapêutica adequada (14). A ultra-sonografia tem sua importância principalmente na diferenciação entre tumores sólidos ou císticos, além de serem usados também para guiar a PAAF (9).

A tomografia computadorizada e a Ressonância Magnética são utilizados para estadiamento da lesão e sua relação com as estruturas adjacentes.(9)

O tratamento cirúrgico é a base do tratamento das neoplasias de glândulas salivares, optando-se, sempre que possível pelo tratamento loco-regional (15).

A radioterapia está reservada para os tumores de comportamento mais agressivo, como os carcinomas de alto grau de malignidade, os estádios III e IV, presença de metástase cervical, paralisia facial e margens exíguas ou comprometidas. A radioterapia exclusiva pode ser indicada para pacientes inoperáveis, mas os resultados não são satisfatórios. Alguns estudos

mostram melhora na sobrevida em pacientes com câncer inoperável de glândulas salivares, com o uso de irradiação com nêutrons (16). Os resultados com o uso de quimioterapia em tumores de glândulas salivares são pobres, embora alguma resposta tem sido descrita com o uso de cisplatina, doxorrubicina, 5FU e ciclofosfamida (12).

O papel do polimorfismo genético na função/nível de proteínas e a predisposição destes pacientes a uma variedade de doenças, tem sido extensivamente demonstrado (2, 17). Por exemplo, o polimorfismo de nucleotídeo único (SNP) pode estar associado ao prognóstico de pacientes com HNSCC, câncer esofágico e câncer de pâncreas (17-19).

O gene *TP53* está localizado no cromossomo 17 (17p13.1) e codifica uma fosfoproteína de 53 kDa (P53) (20), está envolvido no controle do ciclo celular, e consequentemente, associado com diversos tipos de câncer (21). Numerosas variações genéticas do gene *TP53* têm sido descritas (22). Alguns destes estudos têm demonstrado o importante papel do 72 SNP, localizado no exon 4 (rs 1042522) do gene *TP53* (23). Este polimorfismo consiste da mudança de uma única base pareada (CCCG- CGCG), que resulta na mudança do aminoácido prolina para arginina (Arg-Pro) (24) e que podem estar associados com a gênese e progressão de alguns tipos de câncer (23, 25).

Oxigênio (O_2) é essencial para a sobrevivência de todos os organismos aeróbios. O_2 é requerido pelo metabolismo aeróbico que mantém o balanço energético intracelular. A maioria dos mamíferos vivem em uma atmosfera composta por 21% de O_2 , que é considerado um ambiente fisiológico. No entanto, existem algumas circunstâncias em que a célula pode tornar-se hipóxica, que pode ocorrer em condições fisiológicas (desenvolvimento embrionário, adaptação a altas altitudes, cicatrização de feridas) bem como em condições patológicas (doenças isquêmicas e câncer). No caso de doença isquêmica, esta adaptação é benéfica ao paciente pois promove sobrevida das células que estão expostas a baixa pressão de oxigênio. Por outro lado, hipóxia é sinônimo de mau prognóstico no câncer, selecionando células que são capazes de sobrevirer em ambientes extremamente desfavoráveis (26). A principal proteína da via da hipóxia é o fator induzido por hipóxia-1 (HIF-1). HIF-1 é um fator de transcrição que tem como alvo diversos genes que promovem adaptações sob condições hipóxicas. É um hétero-dímero composto por uma subunidade α regulatória (HIF-1α), e uma subunidade β constitutiva (HIF-1β). Sob condições normóxicas, HIF-1β é ativado e HIF-1α é degradado por proteossomas, com a ajuda de prolyl hidroxilases, mais

especificamente HIF-prolyl hidroxilase (PHD) e HIF-asparaginil hidroxilase, também chamada de fator inibidor de HIF (FIH). No microambiente tumoral, a via da degradação O_2 dependente da subunidade HIF-1α está alterada, resultando na elevação dos níveis de HIF-1α. Em tais condições, HIF é capaz de controlar uma série de genes de adaptação associados aos elementos responsivos a hipóxia (HRE) que são sequências específicas de DNA (27-29).

Micro-RNA (miRNA) é uma classe de pequenos RNAs não codificantes, de 19-24 nucleotídeos de comprimento, e que estão envolvidos em numerosos processos fisiológicos de regulação celular incluindo diferenciação, proliferação, apoptose e metabolismo e são também importantes no desenvolvimento do câncer, alguns funcionando como oncogenes e outros com anti-oncogenes (30, 31). São originados de transcritos muito maiores (pri-miRNAs). No núcleo, estes transcritos primários são processados por uma RNA endonuclease Drosha tipo III em pré-miRNAs, que têm 60-70 nucleotídeos de comprimento. O subsequente transporte do pré-miRNA do núcleo para o citoplasma ocorre através da exportina-5. No citoplasma, sofre a ação de outra RNA endonuclease, Dicer tipo III, gerando miRNAs maduros, com cerca de 22 nucleotídeos de comprimento. MiRNA maduro, de cadeia simples forma com proteínas da família Argonauta, um complexo RNA-proteína conhecido como complexo silenciador RNA-induzido (RISC). Este complexo regula a expressão gênica através da inibição da tradução do RNA ou degradação de alvos específicos no RNA mensageiro (mRNA) (32, 33). Muitos estudos tem revelado que a desregulação do miRNA está envolvida no início e progressão em diversos tipos de câncer, em diferentes partes do corpo, inclusive na região de cabeça e pescoço (31, 34-39). Dentre todos os miRNAs, o que aparece mais robustamente expresso em condições hipóxicas é o miR-210.

O miR-210 é um gene hipóxia-induzido, regulado pelo fator HIF-1α e exerce vários papéis na célula incluindo proliferação celular, reparo de DNA, remodelagem de cromatina, metabolismo e migração celular, apoptose e angiogênese (30, 40) Alta expressão de miR-210 está associado a aumento de recidiva loco-regional e diminuição da sobrevida global em diversos tipos de câncer (34, 35, 41), entretanto, outros estudos têm apresentado resultados insignificantes ou contrários (42-44).

Bioinformática tem surgido como uma importante ferramenta para a análise de grandes quantidades de dados gerados através de estudos em diversas doenças (45).

2 OBJETIVOS

2.1 Objetivo Geral

Analisar alterações genéticas e epigenéticas em carcinoma de células escamosas de cabeça e pescoço e em neoplasias de glândulas salivares.

2.2 Objetivos Específicos

- Analisar a possível associação do polimorfismo de nucleotídeo único (SNP) 72 do gene *TP53* com o risco de desenvolvimento de carcinoma de células escamosas de cabeça e pescoço.

- Analisar a possível associação do polimorfismo de nucleotídeo único (SNP) 72 do gene *TP53* com o pior prognóstico em indivíduos com carcinoma de células escamosas de cabeça e pescoço.

- Investigar os níveis de marcadores de hipóxia (HIF-1α e miR-210) em neoplasias benignas e malignas de glândulas salivares.

- Investigar os principais processos associados à quimioterapia em neoplasias de glândulas salivares através de bioinformática.

3 PRODUTOS

- Artigo científico, formatado segundo as normas para publicação do periódico *Tumor Biology*, publicado em dezembro de 2015 com o título "Fuzzy clustering demonstrates that codon 72 SNP rs1042522 of TP53 gene associated with HNSCC but not with prognoses."

- Artigo científico, formatado segundo as normas para publicação do periódico *Journal of Oral Pathology & Medicine*, submetido em agosto de 2016 com o título "Investigation of Hypoxia Markers in Benign and Malignant Salivary Neoplasms."

3.1 ARTIGO 1

Fuzzy clustering demonstrates that codon 72 SNP rs1042522 of *TP53* gene associated with HNSCC but not with prognoses

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Abstract It is estimated that 7.6 million people will die as a consequence of head and neck squamous cell carcinoma (HNSCC). Genetic predisposition has emerged as an important risk factor in the development and prognosis of HNSCC. Considering this, the aim of the current study is to assess whether codon 72 SNP of the *TP53* gene (rs1042522) is associated with an increased odds ratio of developing HNSCC or with a worse prognosis in patients with HNSCC. Analysis of the rs1042522 in HNSCC patients and in control individuals. Differences between the case and control groups were determined using chi-squared tests. Multivariate analysis was performed to evaluate the odds ratio of HNSCC. Fussy C Means Clustering was to cluster HNSCC patients for survival analyses. Time of survival was calculated using the Kaplan- Meier estimator and comparing this to the log rank test. Statistical significance was set at $p<0.05$. A total of 71.4 % of the Arg/Arg genotype were from HNSCC patients, while only 28.6 % of Arg/Arg genotype were found in the control group. Logistic regression demonstrated that the Arg/Arg genotype, smoking, and alcohol consumption increase the odds ratio of HNSCC. No association between *TP53* codon 72 polymorphism and P53 expression. No association between rs1042522 and survival or prognoses was observed. This study identified that individuals carrying the arginine allele at rs1042522 have an increased odds ratio of HNSCC. However, no association between codon 72 SNP of the *TP53* gene and HNSCC prognosis or P53 expression was observed.

Keywords Tumor suppressor gene · Survival · Oral cancer · Survival · p53

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Introduction

Globally, about 12.7 million cancer cases and 7.6 million cancer deaths are estimated to have occurred in 2008, with 56 % of the cases and 64 % of the deaths occurring in economically developing countries [1]. In Brazil, head and neck squamous cell carcinoma (HNSCC) is the sixth most prevalent type of cancer [2], and HNSCC patients have poor prognoses [3]. In 2014, 15,290 new cases of cancer in the oral cavity were estimated, with approximately 10 % of these cases occurring in the state of Minas Gerais [2].

Considering its etiology, HNSCC has been associated with chemical and physical agents [4, 5]. Generally, exposure to carcinogenic agents is a consequences of smoking tobacco and drinking alcohol [4]. However, it is important to note that individual intrinsic factors also have an important role in HNSCC development and prognosis [6–14]. Specifically, genetic variations affect the ways that proteins are expressed and how they function [15, 16]. For example, there is a great deal of evidence that single-nucleotide polymorphisms (SNPs) may be associated to HNSCC prognosis [6, 9–11, 14, 17, 18].

The *TP53* gene is located on chromosome 17 (17p13.1) and encodes the nuclear phosphoprotein 53 kDa (P53) [19]. The *TP53* gene is involved in cell cycle control, and consequently, this gene is associated with many types of cancer [20]. Numerous genetic variations of the *TP53* gene have been described [21]. Some such studies have demonstrated the important role of codon 72 SNP, located on exon 4 of the *TP53* gene [22]. The polymorphism consists of a single base pair change that results in an amino acid change of proline (Pro, CCCG) to arginine (Arg, CGCG) [23]. Studies related to functional changes caused by codon 72 SNP, located on exon 4 (rs1042522), are contradictory [22, 24]. For example, a positive association between the production of arginine due to codon 72 SNP of the *TP53* gene was associated with a reduction of apoptotic tumor cell death in HNSCC cells [24]. In addition,

mutant alleles containing arginine are preferentially selected during tumor genesis [25]. On the other hand, it was suggested that Pro/Pro at codon 72 SNP codon of the *TP53* gene was associated with increases in the susceptibility to bladder cancer in Asians [22]. Based on these data, the aim of this study is to assess whether codon 72 SNP of the *TP53* gene is associated with an increased odds ratio of HNSCC and prognoses of the disease.

Methods

Patients

The present analysis was based on a retrospective study de- sign. Active patients from 1997 through 2011 were recruited from a head and neck surgery service database. The study group included 62 patients with HNSCC. As part of the inclusion criteria, only samples with sufficient material available for DNA extraction were included in the study. These cases underwent surgical resection with postoperative radiotherapy. Treatment of the patients consisted of standard radiotherapy using the conventional two-dimensional technique delivered with megavoltage (cobalt-60 or 6 MV linear accelerator). Total doses ranged from 50 to 70 Gy in daily fractions of 2 Gy, 5 times a week. Patients, who had undergone preoperative treatment or had a diagnosis of carcinoma in situ or multiple head and neck carcinomas, as well as those with other histological types of cancer, were excluded. A control group of 60 randomly chosen individuals without HNSCC were also enrolled in the study. Sample size calculation of the control group was performed to estimate the number of people, considering the size of the local population (confidence level of 95 %, standard error of <12 %, and event prevalence of 50.0 %). Both case and control groups came from the same geographical region. The control group was age-matched.

Clinical data

The mean age was 62.24 years (SD 13.92 years; range, 40–92 years) for HNSCC patients. Physical description of skin color was not used because, in Brazil, it is a poor predictor of genomic ancestry [49, 50]. The study was approved by the Institutional Review Board (CAAE 04337412.8.0000.5146). Information on age, sex, tobacco smoking, alcohol drinking, medical history, family cancer history, tumor site, TNM clinical staging, and survival was obtained from medical charts. Individuals who were not capable for the evaluation of tobacco smoking or alcohol drinking were considered as undetermined.

All patients were staged according to the UICC TNM Classification of Malignant Tumors (1997)(51). Lesions of HNSCC were classified according to the primary site as de- scribed in the International Classification of Diseases (ICD-10) for Oncology. The anatomical sites reviewed in this study included the following: (1) 45 (72 %) mouth and perioral region (C00, C01, C02, C04,

C05, C06.0, C06.2); 6 (10 %) oropharynx (C09–C10) of the patients; and hypo pharynx–larynx 11 (18 %) (C11, C12, C13, C32).

DNA isolation and genotyping

DNA was isolated from HNSCC specimens as described in a previous study [18]. Codon 72 polymorphism of the *TP53* gene was assessed by RFLP. Polymerase chain reaction for codon 72 SNP of the *TP53* gene was performed in a total volume of 25 μL containing approximately 800 ng genomic DNA as template, 0.5 μL of the primers 5′ATCTACAGTCCC CCTTGCCG-3′ and 5′-GCAACTGACCGTGCAAGTCA-3′ (20 pmol/μL), 2.5 μL dNTP-mix (25 mM of each, AMRE SCO, Ohio, CA), 2.5-μL 10× PCR buffer, 1.25 μL magnesium chloride (50 mM), 2.5 U of Platinum Taq DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA, USA). The 296-bp PCR product from codon 72 SNP of the *TP53* gene was digested with BstUI restriction endonuclease (New England BioLabs, Inc., Beverly, MA). The Arg allele has a BstU I restriction site, resulting in two bands (169 and 127 bp), and the Pro allele lacks the BstU I restriction site and therefore produces a single 296-bp band. For this analysis, 10 μL of amplified DNA was digested with 2.5 U of 52 Bln1 for 16 h at 37 °C. PCR and restriction reactions were performed in a thermocycler (Eppendorf AG, Hamburg, GE). The PCR products for polymorphism and digested fragments were verified on 10 % polyacrylamide gel electrophoresis as described in a previous study [26].

Immunohistochemistry

After the genetic analysis, cases which had enough tissue (n=18) were chosen for the immunohistochemistry. The primary antibody against P53 protein (Mouse monoclonal anti- P53, clone DO7 from Novocastra Laboratories in Newcastle, UK) was detected by a LSAB kit (Dako, Carpinteria, California, USA). Negative controls were performed by replacing the primary antibody with universal negative control mouse (Dako, Carpinteria, California, USA). A biopsy sample of HNSCC, which was previously shown to be strongly P53- positive, was used as a positive control in every set of experiments. Only cells that presented a nuclear brown-colored staining in neoplastic cells were considered positive, regard- less of the staining intensity. Positive P53-expression was de- fined when there were more than 10 % of positively stained cells, similarly to a previous study [3].

Statistical analysis

Statistical significance of the differences between case and control group distributions for alleles and genotypes was determined using Fisher or chi-square tests. In addition, a multivariate analysis using binary logistic regression was per- formed to build a model of variables to evaluate the odds ratio of HNSCC.

Time survival was calculated from date of diagnosis to the date of last follow-up visit or to the date of death. Time survival was displayed by means of the Kaplan-Meier method for the relevant variables.

The results of Kaplan-Meier were com- pared to the log-rank test. All analyses were assessed using SPSS 18.0 (SPSS Inc., Chicago), and statistical significance was set at $p<0.05$.

Clustering of numerical data forms the basis of many classification and system modeling algorithms. The purpose of clustering is to identify natural groupings of data from a large data set to produce a concise representation of a system s behavior. Fuzzy Logic tools allow you to find clusters in input–output training data. You can use the cluster information to generate a Sugeno-type fuzzy inference system that best models the data behavior using a minimum number of rules. The rules partition themselves according to the fuzzy qualities associated with each of the data clusters. Fuzzy C-Means Clustering is a data clustering technique wherein each data point belongs to a cluster to some degree that is specified by a membership grade. This technique was originally introduced by Jim Bezdek in 1981 [27] as an improvement on earlier clustering methods. It provides a method that shows how to group data points that populate some multidimensional space into a specific number of different clusters. After multivariate analyses, Fuzzy C-Means Clustering was used to select the cases that were higher pertinence (more than 70 %) to evaluate the survival and compare to all HNSCC group.

Results

Association of rs1042522 and HNSCC patients

To determine whether rs1042522 is associated with HNSCC in the study population, data from the HNSCC group were compared with 60 controls (Table 1). A total of 71.4 % of HNSCC patients presented Arg/Arg genotypes, while only 29.6 % of Arg/Arg genotype was observed in the control group. On the other hand, 76.5 % of the control group presented at least one Proline allele, and only 23.1 % of HNSCC patients presented at least one Proline allele. Independent of Fuzzy C-Means Clustering, an association was observed between Arg/Arg genotypes and HNSCC patients ($p>0.001$ in Table 1). In addition, when compared to data from the dbSNP database (ss342443172 and ss491729176), an association was observed between Arg/Arg genotypes and HNSCC patients ($p>0.001$, Supplementary Table 5). The association between Arg/Arg genotype and HNSCC was also observed in multivariate analyses (Table 2). In addition, binary logistic regression also indicated that smoking consumption increases the odds ratio of HNSCC (Table 2).

Table 1 Genotype frequencies of the *P53 72 Arg/Pro* polymorphisms in HNSCC and the control group

Gene Variant/genotype	Controls n (%)	HNSCC n (%)	*p* value
Without Fuzzy C-Means Clustering			
TP53 72 Genotype			
Arg/Arg	21(29,6)	50(71,4)	
Arg/Pro + Pro/Pro	39(76,5)	12(23,1)	<0,001
With Fuzzy C-Means Clustering			
TP53 72 Genotype			
Arg/Arg	7(21,2)	26(78,8)	
Arg/Pro + Pro/Pro	13(72,2)	5(27,8)	<0,001

All values were calculated using X^2 test. In bold significant differences

Table 2 Multivariate analyses of the odds ratio to HNSCC evaluated by binary logistic regression

Variables	p value	OR	95% IC for OR	
			Lower	Upper
Genotype				
Pro/Pro + Arg/Pro	Referent			
Arg/Arg	<0,00	12,17	3,15	47,09
Ethylism				
Absent	Referent			
Present	<0,31	1,84	0,56	5,99
Tabagism				
Absent	Referent			
Present	<0,00	3,81	58,52	127,52

In bol, significant p value <0,05
OR odds ratio
The term referent is associated with categorical variables with a lower risk of developing the disease according the literature

Association of rs1042522 and clinical pathological parameters of HNSCC patients

rs1042522 and their association with and clinicopathological features in HNSCC patients are summarized in Table 3. No significant association between codon 72 SNP of the *TP53* gene and clinic pathological data was identified. The Arg\Arg genotype was not associated with alcohol consumption (p=0.329), family history of cancer (p=0.369), smoking status (p=0.397), TNM clinical staging (p=0.462), or loco regional metastasis (p=0.426) (Table 3). No association was observed between P53 immunohistochemistry and *TP53* co- don 72 variables, 50 % of Arg/Pro or Pro/Pro genotypes and 75 % of Arg/Arg were positive for P53 staining (p=0.49, data not shown).

Table 3 TP53 codon 72 genotype and their association with and clinicopathological features in HNSCC patients

Variables	Arg/Pro + Pro/Pro n (%)	Arg/Arg n (%)	p value
Etilism			
Never	6 (50,0)	19 (38,0)	0,329
Ever	6 (50,0)	31 (62,0)	
Family history of any cancer			
Absent	8 (66,7)	28 (56,0)	0,369
Present	4 (33,3)	22 (44,0)	
Smoking status			

Never	2 (16,7)	13 (26,0)	0,397
Ever	10 (83,3)	37 (64,0)	
TNM clinical staging			
I/II	3 (25,0)	16 (32,0)	0,462
III/IV	9 (75,0)	34 (68,0)	
Locoregional metastasi			
Absent	6 (50,0)	29 (58,0)	0,426
Present	6 (50,0)	21 (42,0)	

In bold, significant p value <0,05

*Only samples with sufficient tissue were included. Analyzed by X^2 test

Clustering control group and HNSCC patients

For clustering samples, genotyping, alcohol consumption, and smoking status were used. It was observed that 30 (50 %) of the HNSCC patients could not be clustered as case group. On the other hand, on control group, only 20 (33.3 %) samples might be clustered in the control group (Supplementary Table 4). Figure 1 shows graphical distributions of case and control groups according to Fuzzy C Means clustering.

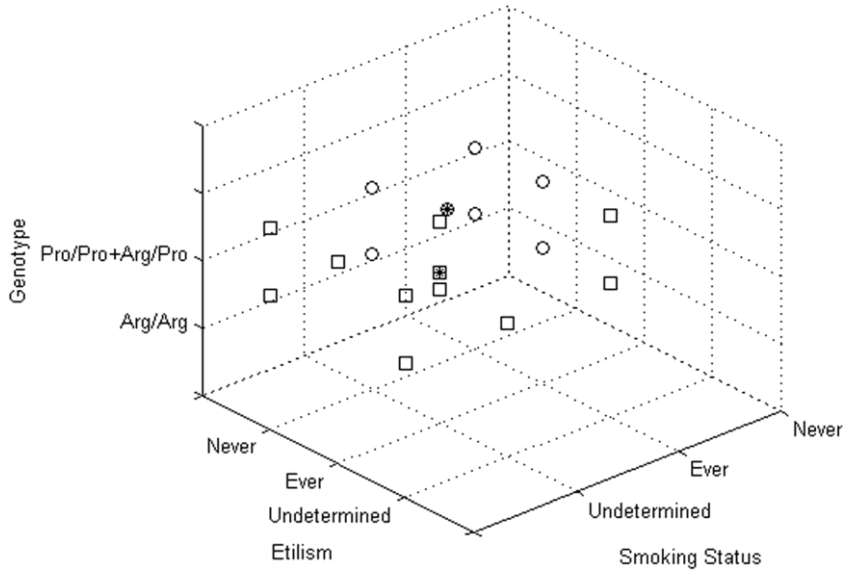

Fig. 1 Graphical distributions of case and control groups according to Fuzzy C Means Clustering. Distribution of samples according to genotype, smoking and ethylism. Unfilled circles and squares indicate individuals from control and HNSCC groups, with ○ indicate individuals control and □ indicating HNSCC individuals. Filled circles and squares represent the control and HNSCC groups, with ❁ indicating the control group and ▣ indicating the HNSCC group.

Association of codon 72 SNP of the TP53 gene and survival

Taken together, the mean survival of HSNCC patients was 1827.70 days after initial diagnosis. HNSCC patients who presented more than 70 % of pertinence of case group according to Fussy C Means had the mean survival of 1226.18 days. Independent of Fuzzy C Means clustering, co- don 72 SNP of the *TP53* gene did not impact on survival (Fig. 2). On the other hand, HNSCC patients with presence of advanced stage TNM presented a higher risk of death (Supplementary Fig. 3)

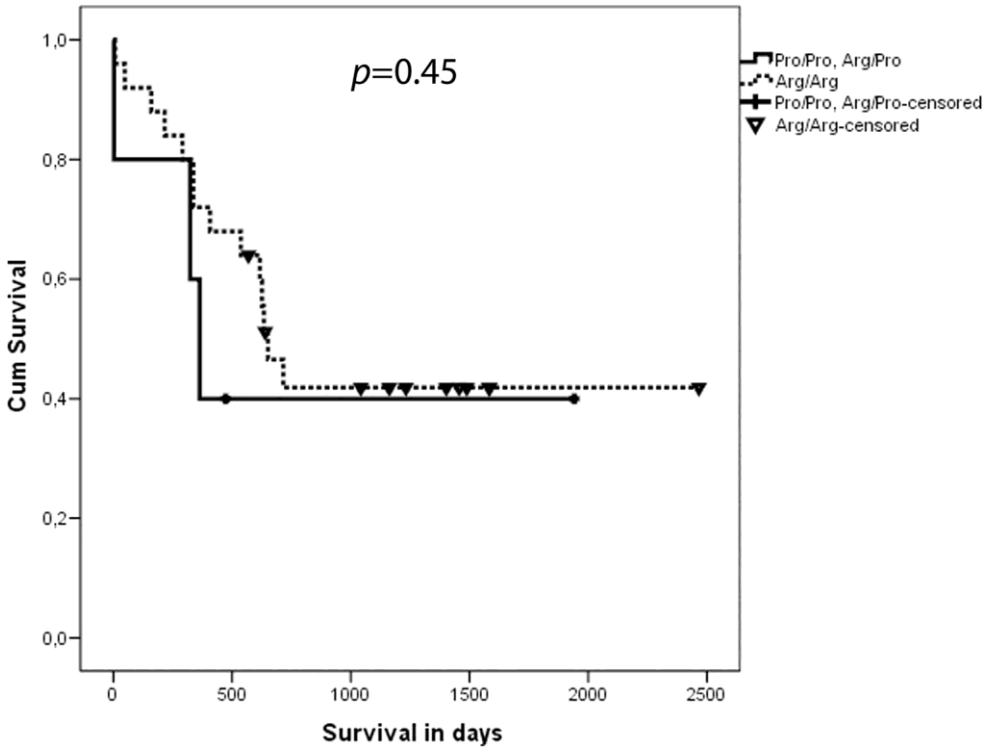

Fig.2 Survival of HNSCC patients according to genotypes. The results of the Kaplan-Meier estimator were compared to the log rank test. A without Fuzzy C Means Clustering (p=0,07) and B was with Fuzzy C Means Clustering (p=0,49)

Discussion

The P53 protein is activated in response to several forms of cellular stress and exerts multiple, anti-proliferative functions [28]. Consequently, disruption of P53 protein expression or function is implicated in many cancers, including HNSCC [29]. Despite of several studies that investigate the role of the TP 53 gene, its classification as a tumor suppressor gene re- mains in debate [20]. With regard to molecular changes related to HNSCC pathogenesis, it is important to highlight the role of SNP [6–12, 16–18, 22, 23, 30]. Some SNPs present divergent results in different populations [29, 31–33]. Considering the rs1042522, a plethora of information is found in the literature [20, 28, 34]. In the current study, it was observed that the arginine allele was associated with HNSCC. Our data are in agreement with some studies that

demonstrated more susceptibility of the arginine allele to mutations that could inactivate the P53 protein [24] and, consequently, lead to the development of cancer. Moreover, the arginine allele was associated with HNSCC [34], lung cancer [33], and cervical carcinoma in Brazil [35] and India [36]. On the other hand, some studies did not observe association of rs1042522 and cancer [31, 32] or even observed an association with the proline allele [22, 37, 38]. The current study was the first to adjust HNSCC patients using Fussy C Means clustering to confirm the absence of association between rs1042522 and survival. These contradictions in results could be attributed to the fact that not only SNP but also epigenetics [18] or posttranslational modification [39] might alter protein function or expression. The DO-7 anti-P53 clone, which was used in the current study, detects wild-type and mutant-type P53 protein and accumulation in human neoplasia [40]. It is important to highlight that due to the complexity of the immunohistochemistry of P53, which is dependent on antibody and tissue preservation, conflicting results regarding *P53* expression in HNSCC are ob- served in the literature [3, 41, 42]. In the current study, no association was observed between *P53* expression and rs1042522. The effect of rs1042522 on P53 protein expression seems to be complex [22, 34, 38, 43] and might explain the absence of an association between rs1042522 and HNSCC survival as observed in the current study. No association of rs1042522 and prognosis was observed. There are some studies that suggest that the proline allele of rs1042522 could be associated with higher risk of HPV infection [43]. As some studies suggest that HPV positive HNSCC tumors present better prognoses [44], the rationale could be that the proline allele might be associated with better prognosis as well [45]. However, our data are very similar to a study by Brant et al. [34], who did not observe an association between rs1042522 and survival. Similarly, both the study by Brant et al. [34] and the present study did not find any differences between codon rs1042522 gene and age. Interestingly, in the current study, classical parameter advanced stage TNM was associated with increased risk of death even after Fussy C Means. In this way, the current study might contribute an important piece to solving the TP 53 puzzle.

In conclusion, this study identified that individuals carrying the arginine allele at rs1042522 have an increased odds ratio of HNSCC. But, no association was observed between rs1042522 and HNSCC survival or P53 expression. These findings may contribute to enhancing the assessment of risk for HNSCC.

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4.2 ARTIGO 2

Investigation of Hypoxia Markers in Benign and Malignant Salivary Neoplasms

Hypoxia Markers in Salivary Neoplasms

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Abstract

Background

Salivary gland neoplasia (SGN) presents at low frequencies but is an aggressive form of head and neck neoplasia. SGNs exhibit a broad spectrum of phenotypic heterogeneity. Hypoxia is an important concurrent phenomenon that may be associated with SGN prognosis and valuable to determine to the choice of treatment regimen.

Objective

There is significant controversy in the literature regarding the relationship between hypoxia and SGNs. As such, the purpose of the current study is to investigate mRNA levels of hypoxia markers in both benign and malignant salivary neoplasms. Additionally, the present study aims to investigate, using bioinformatics, the main biological processes that are altered following chemotherapeutic treatment of SGNs.

Patients and Methods

Ethical approval and signed, informed consent was obtained from all patients. The current study sample is comprised of a total of 21 samples, including malignant neoplasms (n=7, 33.33%), benign neoplasms (n=7, 33.33%), and samples from normal salivary glands (n=7, 33.33%). miR-210 expression and HIF-1α mRNA levels were evaluated using qRT-PCR. Bioinformatics analyses were also performed to identify the main biological processes altered in SGNs following chemotherapy.

Results

There was no difference in miR-210 expression between case and control groups. Similarly, HIF-1α mRNA levels were similar between benign and malignant SGNs. The ontological analyses suggested that post-replication repair, error-prone translesion synthesis, translesion synthesis and error-free translesion synthesis were the most important biological processes related to chemotherapy in these patients. Additionally, the main biological processes altered with chemotherapy were related to the DNA metabolic process and strand elongation.

Conclusion

The angiogenic markers, mir-210 and HIF-1α, do not appear to distinguish malignancy in salivary glands. Bioinformatic analyses demonstrated that biological processes related to DNA and cell division were the most important for SGNs. The current study suggests that salivary gland neoplasms do not exhibit increased expression of hypoxia markers.

Introduction

Salivary gland neoplasias (SGNs) are less common than other head and neck cancers, including oral squamous cell carcinoma (45-49). SGNs exhibit a broad spectrum of phenotypic heterogeneity and are divided into five categories, according to World Heath Organization (WHO) (49). Prognosis of SGNs is also related to the anatomical location of the neoplasia (50, 51).

Recently, a large number of studies have attempted to evaluate the role of hypoxia in cancer development and prognosis (52-55). Hypoxia-inducible factor 1-alpha (HIF-1α) is a marker of hypoxia and is activated under hypoxic conditions (56, 57). HIF-1α has been shown to regulate the expression of several miRNAs, including miR-210. miRNAs are a class of single-stranded noncoding RNAs 21–22 nucleotides in length (58) which regulate gene expression through the inhibition of RNA translation or degradation of target messenger RNA (mRNA) (59). Recent evidence has suggested that miR-210 plays a crucial role in the cellular response to hypoxia (60). HIF-1α can promote isoform-specific stabilization of miR-210 by binding to the Hypoxia Responsive Element (HRE) present in the proximal promoter of miR-210 (61). Similar to HIF-1α, hypoxia induces miR-210 expression, which regulates cellular proliferation, DNA stability, mitochondrial metabolism, apoptosis and angiogenesis (60, 62). Furthermore, miR-210 expression is also significantly upregulated in other types of cancer, including non-small cell lung cancer (63).

Although first choice treatment for malignant SGNs is surgery (64), adjuvant therapy has historically been determined based on data from studies focused on squamous cell carcinomas of the upper aerodigestive tract (51). Importantly, there are conflicting reports with regards to the relationship between hypoxia and clinical behavior or treatment responsiveness in patients diagnosed with SGNs (65, 66). Recent studies have demonstrated that hypoxia might promote an increase in radioresistance (67-69), specifically via miRNA-mediated modulation of the hypoxic response (70). These data suggest that the use of chemoradiation might be a valuable alternative treatment option for patients presenting with radioresistant neoplasias (51). Considering the controversial literature regarding the relationship between hypoxia and SGNs, the current study aimed to investigate mRNA levels of hypoxia markers in both benign and malignant salivary neoplasms. Additionally, in the present study, we took advantage of bioinformatics to investigate the main biological processes associated with chemotherapy of SGNs.

Methods

Patients

Ethical approval for this study was obtained from the relevant Institutional Review Board (process number CAAE 52767316.6.0000.5146) and signed, informed consent was obtained from all patients. The current study population consisted of 21 total patient samples, including 7 with malignant neoplasms (33.33%), 7 with benign neoplasms (33.33%), and 7 with normal salivary glands (33.33%).

RNA isolation and real-time PCR

RNA was isolated using Trizol (Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's protocol. Total RNA was treated with DNase I, Amplification Grade (Invitrogen, cat number 18068015, Carlsbad, CA, USA) and 1.5 µg of RNA was reversely transcribed with the SuperScript® First-Strand Synthesis System for qRT-PCR (Invitrogen, cat number 11904018, Carlsbad, CA, USA). Each reaction for SYBR green-based qRT-PCR (total volume 20 µl) contained 10 µl of SYBR Green master mix, 0.25 µl of both forward and reverse primers, 1 µl of cDNA (66 ng/reaction) and 8.5 µl H_2O. Non-Template Control (NTC) was included for each assay. The thermal cycling conditions were as follows: 95°C for 10 min, followed by 40 cycles of the following steps: 95°C for 15 sec and 60°C for 1 minute. The specific primers/probes (Life Technologies, Carlsbad, CA, USA) were described previously (52). Specifically, the following primers were used for HIF1α: 5′-TCTGCAACATGGAAGGTATTGC-3′ and 5′-CTGAGGTTGGTTACTGTTGGTATCA-3. Beta-Actin was used to normalize HIF1α gene expression and was amplified using the following primers: 5′-TGCCGACAGGATGCAGAAG-3′ and 5′-CTCAGGAGGAGCAATGATCTTGA-3′. qPCR was performed on a StepOne Real-Time PCR System (Life Technologies, Carlsbad, CA, USA).

For mir-210 (ID: Hs04231470_s1, Life Technologies, Carlsbad, CA, USA), a TaqMan assay was performed according to the manufacturer's protocol. RNU44 was used as an endogenous control for mi-210 analysis (ID: 001094, Life Technologies, Carlsbad, CA, USA), All reactions were done in triplicate. Samples of M were used as calibrator. The following reaction steps were used for amplification: 95°C for 10 min, 95°C for 15s and 60°C for 1 min. The Ct values of endogenous controls were subtracted from the Ct values of the respective targets to calculate the ΔCt. The ΔCt values from each experimental group were averaged and converted to log base 2 using the equation 2^-ΔΔCt in order to compare expression among different samples.

Bioinformatics and interaction network analysis

The bioinformatics approach utilized here has been described previously (52, 71). Briefly, 5FU, bevacizumab, carboplatin, cisplatin, CPT11, cyclophosphamide, doxorubicin, gemcitabine, hydroxyurea, paclitaxel, pirarubicin, trastuzumab, and Vp16 were used as chemicals, as suggested previously (51). All chemotherapy agents were taken together to build a

biological network in the open-source software, STITCH (version 4.0) (72). Only experimental studies with a high degree of confidence (0.9-0.99) were considered. The initial gene list was then expanded and STITCH was used to score each interaction in order to build an interaction map among the identified genes. To evaluate differences among classes with regards to the weighted number of links (WNL), the Markov Cluster Algorithm (MCL) were used. Topological analysis was carried out with Cytoscape (73), while ontological analysis was performed with BinGO (74). In order to confirm the STITCH results, STRING analyses were performed as previously described (52, 71). Briefly, the main targets of chemotherapeutic agents used to treat SGNs were used to build the network. P53, GAAD45, MDM2, ABL1, BAX, CASP3, CASP9, MLH1, MSH2, TOP2A, ERCC1, ERCC2, RRM1, TUBB2A, TOP2B, TOP2A and ERBB2 were included in STRING (version 10.0) (75). Only experimental studies with a high degree of confidence (0.9-0.99) were considered. The initial gene list was then expanded using STRING (version 10.0) (75), which was used to score each interaction and build an interaction map among the identified genes. In order to evaluate differences regarding a weighted number of links (WNL) among classes, the Markov Cluster Algorithm (MCL) were used. The topological analysis was carried out with Cytoscape (73), while ontological analysis was performed with BinGO (74).

Statistical analysis

Analyses were performed using GraphPad Prism software (Version 5.0, GraphPad Software Inc., San Diego, CA, USA). The Kolmogorov-Smirnov and Shapiro-Wilk Tests were carried out to evaluate data distribution. These analyses revealed that the data were non-parametrically distributed; therefore, the Mann-Whitney and Kruskal-Wallis tests were performed. All data are given as means ± S.D. $p < 0.05$ was considered statistically significant. .

Results

The individuals in the control group were 28.3 years of age, on average. All control samples were obtained from oral mucoceles located in the inferior lip. As shown in Table 1, the majority of the samples were from male subjects (57.1%). All benign tumors were pleomorphic adenomas and the patients had an average age of 36.4 years. With regards to the location of the tumor, one (14.3%) was in the minor salivary glands (hard palate) while six (85.7%) were located in the parotid gland. In the patient population with malignant neoplasms, one patient presented with acinar cell carcinoma (14.3%), two with adenoid cystic carcinoma (28.6%) and two with myoepithelial carcinoma (n=2, 28.56%). The average age of patients with malignant neoplasias was 52.3 years.

No difference in miR-210 expression was observed between case and control groups (Fig 1A). Similarly, miR-210 levels were similar between malignant and benign SGNs (Fig 1A). Additionally, HIF-1α mRNA levels did not differ between the case and control groups or between benign and malignant SGNs (Fig 1B).

A bioinformatics approach was utilized to evaluate the main pathways affected by chemotherapic agents. Bevacizumab and trastuzumab treatment did not appear to be related in STITCH and 16 proteins appeared in the network (Fig 2A and Supplementary material). Correlation and R-squared values were 0.767 and 0.630, respectively. Ontological analyses suggested that postreplication repair, error-prone translesion synthesis, translesion synthesis and error-free translesion synthesis were the biological processes most affected by chemotherapeutic drugs (Fig 2B). A second bioinformatics analysis was performed to evaluate STITCH results. STRING analyses revealed a total of 18 main targets (Supplementary material). After expansion, 66 proteins were used to built the network (Fig 2C). 357 interactions were observed (average of 10.8) and the clustering coefficient was 0.611. Correlation and R-squared values were 0.781 and 0.581, respectively. DNA metabolic process, DNA strand elongation, DNA strand elongation involved in DNA replication, leading strand elongation, DNA replication, DNA-dependent replication, mismatch repair, cellular response to stress and cellular response to a stimulus were the main biological processes observed to correlate with chemotherapy. The results reported in the current study demonstrate that salivary gland neoplasms do not increase markers of hypoxia.

Discussion

Previous reports have suggested that hypoxia is an important mechanism related to radioresistance in specific cancers (76). Due to the fact that the indirect actions of radiation depend on oxygen levels, hypoxia is believed to be the main mechanism leading to radioresistance (51, 67-70, 76, 77). Furthermore, it is important to highlight that vascular damage, which triggers an immune response, is a consequence of radiation-induced tumor hypoxia (78). However, fractionated radiation can induce subsequent tumor revascularization via recruitment of bone marrow-derived cells (BMDCs) in a HIF1α-dependent or –independent manner (79, 80).

Traditionally, SGNs were considered radioresistant (51). However, the main biological mechanism that contribute to the radioresistance observed in SGNs is still not well established. As such, recommendations for the management and treatment of malignant SGNs have changed in recent years (81, 82). The shift in the treatment of SGNs is due to an improvement in imaging technology, as well as the development of various treatment options, including external beam radiation, neutron beam therapy, and chemotherapy (81, 82). It has been shown that miR-210 expression is induced by both HIF-1α (83, 84) as well as hypoxia (84). The current study was the first to investigate miR-210 expression in SGNs. This report suggests that there are not significant alterations in miR-210 expression in this specific tumor subtype. Specifically, no differences in miR-210 levels were observed between SGNs and normal, healthy salivary glands. In support of the miR-210 findings reported here, our results regarding HIF-1α demonstrate that salivary gland neoplasms are not hypoxic. Rather, an elegant study has demonstrated that SGNs are well oxygenated, and it is unlikely that hypoxia is a relevant factor in the clinical progression and treatment responsiveness of these tumors (65). Moreover, repair of sublethal damage during the interval between fractionated radiotherapy doses is minimal in neutron therapy but plays a significant role in external-beam radiotherapy (85), thus suggesting that radioresistance of SGNs is independent of HIF1α. This notion is supported by the fact that, even through the use of extensive bioinformatics, hypoxia processes were not observed to be significantly altered in SGNs. In fact, almost all chemotherapeutic agents currently target DNA damage to induce cell death (51).

Hypoxia is associated with both radioresistance and tumor progression (53, 57, 62, 67, 86). However, the importance of the hypoxic response appears to be specific to the tumor subtype. Specifically, a substantial positive correlation has been observed between adenoid cystic carcinomas and hypoxia (86). However, HIF-1α levels do not appear to be altered in pleomorphic adenomas, when compared with control samples (86).

It is important to note that there are limitations to the current study, including a small sample size, which precluded our ability to perform comprehensive analyses on different specimens and histological types. Irrespective of these limitations, the current study is the first to evaluate RNA levels of hypoxia

markers in SGNs. Furthermore, the present study validates previously published data as we observed no differences in hypoxia markers between control, benign and malignant neoplasias.

In conclusion our data suggest that salivary gland neoplasms do not display increased levels of hypoxia markers. Specifically, the angiogenic markers, mir210 and HIF-α, do not seem to correlate with malignancy of salivary glands. Rather, bioinformatic analyses suggests that biological processes related to DNA repair and cell division are the most important in SGNs.

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Legends:

Figure 1: miR-210 or HIF-1α mRNA levels in control, benign and malignant SGNs. (A) No difference in expression of miR-210 is observed between groups. (B) HIF-1α expression is not altered in SGNs, compared with control samples.

Figure 2: Bioinformatics Analyses of the main chemotherapeutic agents used for the treatment of salivary gland neoplasms. (A) STITCH confidence view of chemotherapeutic agents. Stronger associations are represented by thicker lines. Protein-protein interactions are shown in blue, chemical-protein interactions in green and interactions between chemicals in red. Links between chemicals are not used to extend the network results. (B) Ontological analyses of chemotherapy agents. The most important processes are shown in yellow. (C) STRING confidence view of main targets of chemotherapeutic agents used in SGNs. Thicker lines represent stronger protein-protein interactions and are shown in blue. (D) Ontological analyses of chemotherapeutic targets. The most important processes are shown in yellow.

Table 1: Descriptive data of the patient population:

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5 CONCLUSÕES

Houve associação entre genótipo Arg/Arg no 72 SNP do gene *TP53* (rs 1042522) e HNSCC, independente do Fuzzy C-means Clustering (p<00,1), também observado em análise multivariada.

Não houve associação entre 72 SNP do gene *TP53* (rs 1042522) e impacto na sobrevida de indivíduos com HNSCC, independente do Fuzzy C-means Clustering.

Não houve diferenças estatisticamente significantes entre os níveis de miR-210 e HIF-1α em neoplasias benignas e malignas de glândula salivar, tampouco com o grupo controle de tecido salivar normal.

Análise bioinformática demonstrou que processos relacionados com o DNA e divisão celular, são os alvos quimioterápicos mais importantes em SGNs.

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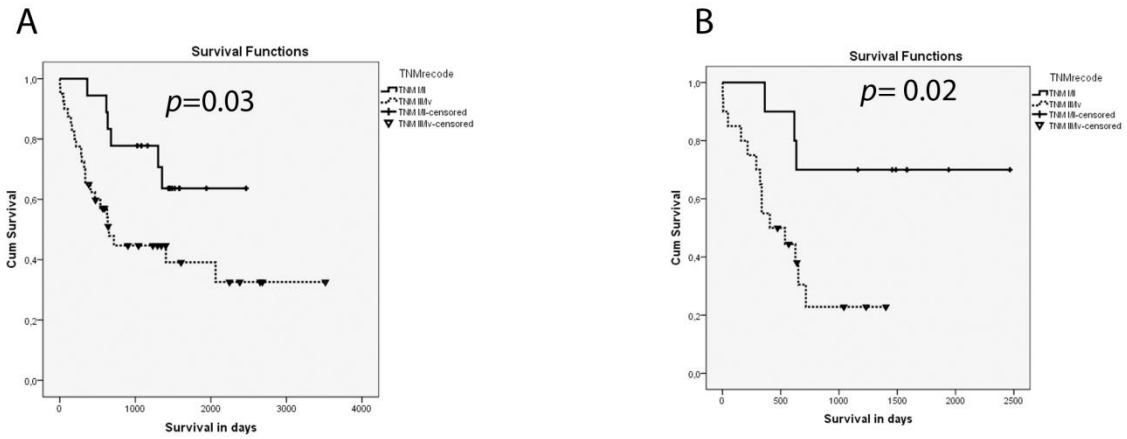
APÊNDICES

APÊNDICE A- Supplementary Fig. 3 do artigo 1



APÊNDICE B- Supplementary Table 4 do artigo 1

Code	Group	*Fuzzy C_means*		Code	Group	*Fuzzy C_means*	
		μ_{Case}	$\mu_{Control}$			μ_{Case}	μ_{Case}
1	Case	**0.896954**	0.103046	32	Case	0.425107	0.574893
2	Case	**0.896954**	0.103046	33	Case	0.425107	0.574893
3	Case	**0.896954**	0.103046	34	Case	0.425107	0.574893
4	Case	**0.896954**	0.103046	35	Case	0.425107	0.574893
5	Case	**0.896954**	0.103046	36	Case	0.425107	0.574893
6	Case	**0.896954**	0.103046	37	Case	0.395895	0.604105
7	Case	**0.896954**	0.103046	38	Case	0.306587	0.693413
8	Case	**0.896954**	0.103046	39	Case	0.306587	0.693413
9	Case	**0.896954**	0.103046	40	Case	0.306587	0.693413
10	Case	**0.896954**	0.103046	41	Case	0.306587	0.693413
11	Case	**0.896954**	0.103046	42	Case	0.306587	0.693413
12	Case	**0.896954**	0.103046	43	Case	0.306587	0.693413
13	Case	**0.896954**	0.103046	44	Case	0.306587	0.693413
14	Case	**0.896954**	0.103046	45	Case	0.306587	0.693413
15	Case	**0.896954**	0.103046	46	Case	0.306587	0.693413
16	Case	**0.896954**	0.103046	47	Case	0.306587	0.693413
17	Case	**0.896954**	0.103046	48	Case	0.306587	0.693413
18	Case	**0.896954**	0.103046	49	Case	0.296088	0.703912
19	Case	**0.896954**	0.103046	50	Case	0.296088	0.703912
20	Case	**0.896954**	0.103046	51	Case	0.296088	0.703912
21	Case	**0.896954**	0.103046	52	Case	0.296088	0.703912
22	Case	**0.896954**	0.103046	53	Case	0.296088	0.703912
23	Case	**0.896954**	0.103046	54	Case	0.162531	0.837469
24	Case	**0.896954**	0.103046	55	Case	0.148876	0.851124
25	Case	**0.896954**	0.103046	56	Case	0.148876	0.851124
26	Case	**0.896954**	0.103046	57	Case	0.148876	0.851124
27	Case	**0.723809**	0.276191	58	Case	0.148876	0.851124
28	Case	**0.723809**	0.276191	59	Case	0.148876	0.851124
29	Case	**0.723809**	0.276191	60	Case	0.148876	0.851124
30	Case	**0.723809**	0.276191	61	Case	0.148876	0.851124
31	Case	**0.723809**	0.276191	62	Case	0.148876	0.851124

Code	Group	*Fuzzy C_means*		Code	Group	*Fuzzy C_means*	
		μ_{Case}	$\mu_{Control}$			μ_{Case}	μ_{Case}
1	Control	0.148876	**0.851124**	31	Control	0.531757	0.468243
2	Control	0.148876	**0.851124**	32	Control	0.531757	0.468243
3	Control	0.148876	**0.851124**	33	Control	0.557397	0.442603
4	Control	0.148876	**0.851124**	34	Control	0.557397	0.442603
5	Control	0.148876	**0.851124**	35	Control	0.557397	0.442603
6	Control	0.148876	**0.851124**	36	Control	0.596998	0.403002
7	Control	0.148876	**0.851124**	37	Control	0.596998	0.403002
8	Control	0.162531	**0.837469**	38	Control	0.596998	0.403002
9	Control	0.162531	**0.837469**	39	Control	0.596998	0.403002
10	Control	0.162531	**0.837469**	40	Control	0.596998	0.403002
11	Control	0.162531	**0.837469**	41	Control	0.596998	0.403002
12	Control	0.162531	**0.837469**	42	Control	0.625107	0.374893
13	Control	0.162531	**0.837469**	43	Control	0.625107	0.374893
14	Control	0.162531	**0.837469**	44	Control	0.72014	0.27986
15	Control	0.162531	**0.837469**	45	Control	0.72014	0.27986
16	Control	0.162531	**0.837469**	46	Control	0.72014	0.27986
17	Control	0.162531	**0.837469**	47	Control	0.723809	0.276191
18	Control	0.296088	**0.703912**	48	Control	0.723809	0.276191
19	Control	0.296088	**0.703912**	49	Control	0.723809	0.276191
20	Control	0.296088	**0.703912**	50	Control	0.736601	0.263399
21	Control	0.306587	0.693413	51	Control	0.736601	0.263399
22	Control	0.395895	0.604105	52	Control	0.736601	0.263399
23	Control	0.395895	0.604105	53	Control	0.736601	0.263399
24	Control	0.395895	0.604105	54	Control	0.736601	0.263399
25	Control	0.425107	0.574893	55	Control	0.736601	0.263399
26	Control	0.425107	0.574893	56	Control	0.765585	0.234415
27	Control	0.425107	0.574893	57	Control	0.765585	0.234415
28	Control	0.531757	0.468243	58	Control	0.765585	0.234415
29	Control	0.531757	0.468243	59	Control	0.797014	0.202986
30	Control	0.531757	0.468243	60	Control	0.797014	0.202986

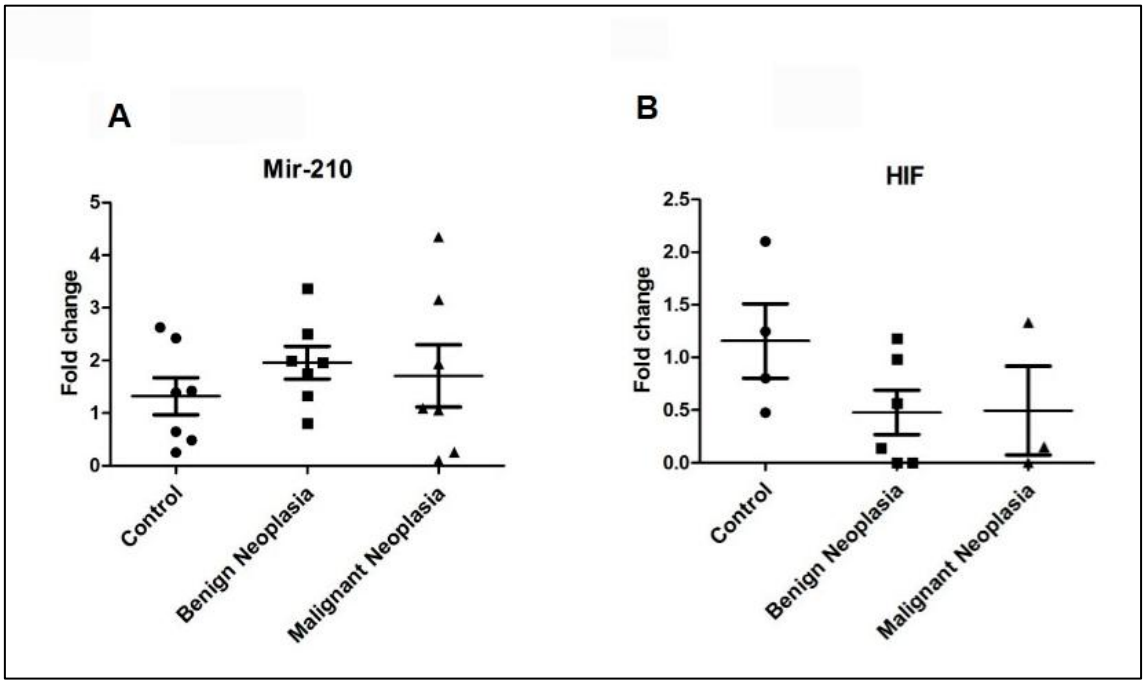
APÊNDICE C- Supplementary Table 5 do artigo 1

Supplementary table 5: Comparison between genotype of HNSCC patients and data from dbSNP.

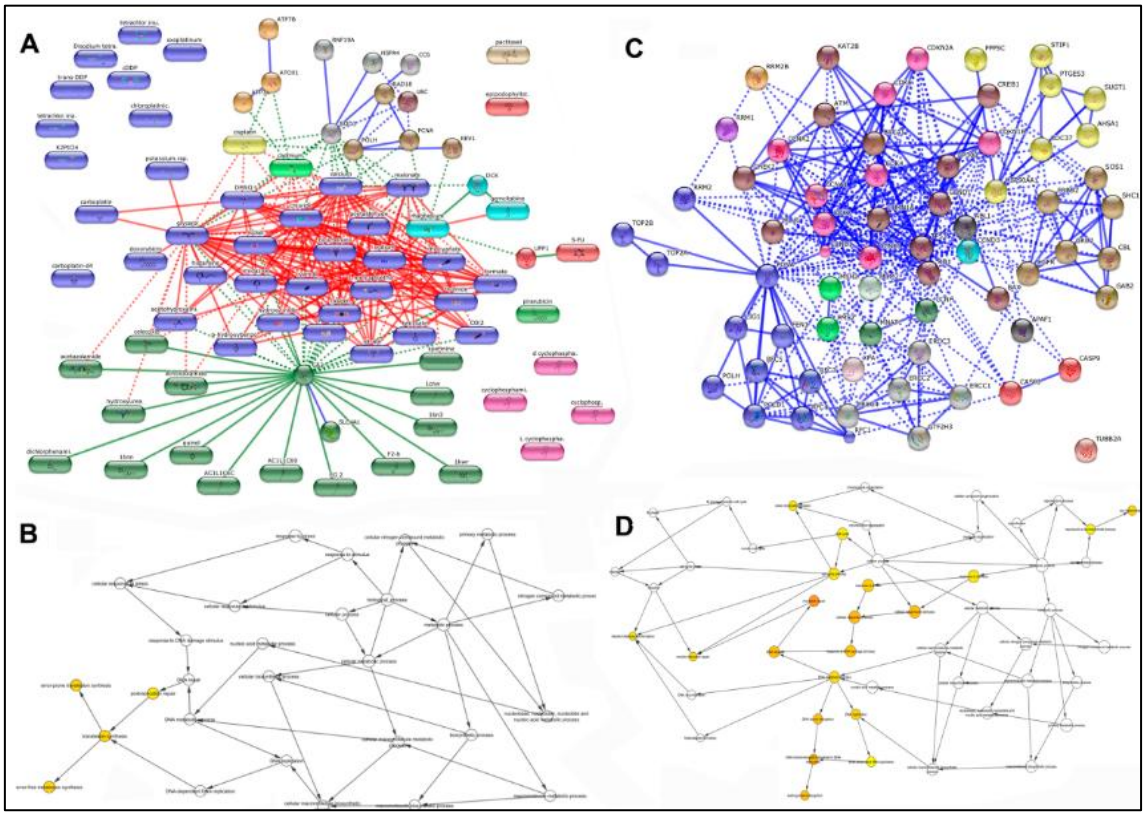
ID	N	Arg/Arg	Pro/Pro + Pro/Arg	p Value
Case Goup Current Study	72	50	22	
ss342443172	3340	1283	2057	**<0.0001**
ss491729176	810	420	390	**0.0028**

All values were calculated using the χ^2 test. **In bold** significant differences.

APÊNDICE D: Figura 1 do artigo 2



APÊNDICE E- Figura 2 do artigo 2



APÊNDICE F- Supplementary material artigo 2

Supplementary Table 1:

Number	Drug	Main Target	Effect
1	5FU	P53, GAAD45, MDM2, ABL1, BAX, CASP3, CASP9	DNA Damage
2	Bevacizumab	VEGF	Angiogenesis
3	Carboplatin	P53, GAAD45, MDM2, ABL1, BAX, CASP3, CASP9	DNA Damage
4	Cisplatin	P53, GAAD45, MDM2, ABL1, BAX, CASP3, CASP9	DNA Damage
5	CPT11	P53, GAAD45, MDM2, ABL1, BAX, CASP3, CASP9	DNA Damage
6	Cyclophosphamide	P53, GAAD45, MDM2, ABL1, BAX, CASP3, CASP9	DNA Damage
7	doxorubicin	P53, GAAD45, MDM2, ABL1, BAX, CASP3, CASP9, MLH1, MSH2, TOP2A, ERCC1, ERCC2	DNA Damage
8	Gemcitabine	P53, GAAD45, MDM2, ABL1, BAX, CASP3, CASP9	DNA Damage
9	Hydroxyurea	RRM1	DNA replication
10	Paclitaxel	TUBB2A	Cell replication
11	Pirarubicin	TOP2B, TOP2A,	DNA replication
12	Trastuzumab	ERBB2	Cell replication
13	Vp16	TOP2B, TOP2A,	DNA replication

Main chemotherapy agents used to treat SGNs according Cerda T, Sun XS, Vignot S, Marcy PY, Baujat B, Baglin AC, et al. A rationale for chemoradiation (vs radiotherapy) in salivary gland cancers? On behalf of the REFCOR (French rare head and neck cancer network). Critical reviews in oncology/hematology. 2014;91(2):142-58.

Supplementary Table 2 STITCH results

#node1	node2	combined_score
Imidazole	sodium	0.900
DMSO	nickel	0.900
CA2	nickel	0.900
REV1	magnesium	0.900
Thiocyanate	2-mercaptoetha.	0.900
Cisplatin	chloroplatinic.	0.026
Chloride	nickel	0.900
Benzoate	bicarbonate	0.900
CO(2	chloride	0.900
CA2	histamine	0.900
Acetaldehyde	oxygen	0.900
Acetaldehyde	sodium	0.900
Calcium	cisplatin	0.900
Glycerol	oxygen	0.900
ATP7A	cadmium	0.900
Nitrate	2-mercaptoetha.	0.900
CA2	imidazole	0.900
CA2	celecoxib	0.900
Bromide	glycerol	0.900
cDDP	tetrachlor.ina.	0.026
Cisplatin	oxoplatinum	0.026
CA2	sodium	0.900
DCK	gemcitabine	0.900
Bicarbonate	nitrate	0.900
Bicarbonate	imidazole	0.900
Thiocyanate	nickel	0.900
CA2	sulfur	0.900
Bicarbonate	sodium	0.900
Chloride	2-mercaptoetha.	0.900
POLH	cisplatin	0.900
CA2	1bnn	0.900
Sodium	nickel	0.900
SOD1	acetaldehyde	0.900
Chloride	DMSO	0.900
potassium.rop.	chloroplatinic.	0.026
Bromide	sodium	0.900
ATOX1	cisplatin	0.900
DMSO	2-mercaptoetha.	0.900
CA2	oxygen	0.900
CA2	1kwr	0.900
Benzoate	magnesium	0.900
Cisplatin	tetrachlor.ina.	0.026

cDDP	Disodium tetra.	0.026
Benzoate	glycerol	0.900
Bromide	calcium	0.900
cyclophosphami.	cyclophosp.de-.	0.026
Calcium	chloride	0.900
Benzoate	malonate	0.900
DMSO	cadmium	0.900
PCNA	POLH	0.998
Acetaldehyde	magnesium	0.900
Benzoate	sodium	0.900
Formate	nickel	0.900
potassium.rop.	K2PtCl4	0.026
p-hydroxybenzo.	bromide	0.900
Formate	nitrate	0.900
SOD1	DMSO	0.900
Acetaldehyde	2-mercaptoetha.	0.900
tetrachlor.inu.	trans-DDP	0.026
Cyanide	thiocyanate	0.900
Sulfur	magnesium	0.900
Malonate	nickel	0.900
Bicarbonate	2-mercaptoetha.	0.900
Magnesium	nitrate	0.900
Cyanide	sodium	0.900
Glycerol	magnesium	0.900
d cyclophospha.	L cyclophospha.	0.026
CO(2	bicarbonate	0.900
trans-DDP	oxoplatinum	0.026
Disodium tetra.	K2PtCl4	0.026
Calcium	cyanide	0.900
Bromide	cyanide	0.900
tetrachlor.inu.	potassium.rop.	0.026
Magnesium	gemcitabine	0.900
Magnesium	nickel	0.900
CA2	AC1L1C6C	0.900
tetrachlor.inu.	tetrachlor.ina.	0.026
RNF19A	SOD1	0.937
Chloride	cadmium	0.900
Glycerol	malonate	0.900
Bromide	chloride	0.900
CA2	spermine	0.900
CA2	SLC4A1	0.974
hydroxyl radic.	oxygen	0.900
CO(2	glycerol	0.900
Sodium	cadmium	0.900
cDDP	tetrachlor.inu.	0.026

Oxoplatinum	Disodium tetra.	0.026
Chloride	oxygen	0.900
Glycerol	histamine	0.900
Chloride	cyanide	0.900
UPP1	5-FU	0.900
tetrachlor.inu.	K2PtCl4	0.026
Bromide	thiocyanate	0.900
CA2	acetazolamide	0.900
Nickel	nitrate	0.900
Thiocyanate	oxygen	0.900
Nickel	2-mercaptoetha.	0.900
SOD1	chloride	0.900
DMSO	magnesium	0.900
Cisplatin	cDDP	0.026
Magnesium	cadmium	0.900
SOD1	cadmium	0.900
DMSO	histamine	0.900
cyclophosphami.	d cyclophospha.	0.026
Formate	magnesium	0.900
Formate	glycerol	0.900
Formate	imidazole	0.900
Benzoate	chloride	0.900
Bicarbonate	nickel	0.900
Bicarbonate	cadmium	0.900
CA2	hydroxyurea	0.900
Bromide	DMSO	0.900
DMSO	cisplatin	0.900
trans-DDP	Disodium tetra.	0.026
Acetaldehyde	cyanide	0.900
Calcium	hydroxyl radic.	0.900
Calcium	formate	0.900
Calcium	CO(2	0.900
DMSO	glycerol	0.900
Carboplatin	carboplatin-d4	0.026
UBC	HSPA4	0.978
Glycerol	thiocyanate	0.900
Sulfur	glycerol	0.900
cDDP	potassium.rop.	0.026
Chloride	imidazole	0.900
Calcium	oxygen	0.900
Glycerol	sodium	0.900
Formate	thiocyanate	0.900
Formate	malonate	0.900
Imidazole	magnesium	0.900
cDDP	chloroplatinic.	0.026

SOD1	calcium	0.900
POLH	REV1	0.994
trans-DDP	tetrachlor.ina.	0.026
Calcium	DMSO	0.900
Chloride	glycerol	0.900
Bicarbonate	celecoxib	0.900
Glycerol	imidazole	0.900
Calcium	nitrate	0.900
tetrachlor.ina.	K2PtCl4	0.026
Sodium	hydroxyl radic.	0.900
Bromide	malonate	0.900
DMSO	bicarbonate	0.900
POLH	magnesium	0.900
chloroplatinic.	tetrachlor.ina.	0.026
Bromide	bicarbonate	0.900
Cisplatin	potassium.rop.	0.026
ATP7A	ATOX1	0.992
trans-DDP	K2PtCl4	0.026
Thiocyanate	sodium	0.900
POLH	sodium	0.900
Magnesium	sodium	0.900
Glycerol	celecoxib	0.900
Magnesium	2-mercaptoetha.	0.900
Cyanide	magnesium	0.900
Glycerol	nickel	0.900
Calcium	histamine	0.900
Chloride	malonate	0.900
Acetaldehyde	calcium	0.900
potassium.rop.	Disodium tetra.	0.026
Sodium	2-mercaptoetha.	0.900
CA2	quinol	0.900
HSPA4	SOD1	0.937
Nickel	oxygen	0.900
Formate	hydroxyl radic.	0.900
CA2	ethoxzolamide	0.900
CA2	hydroxyl radic.	0.900
p-hydroxybenzo.	sodium	0.900
CA2	DMSO	0.900
Bromide	magnesium	0.900
Sulfur	bicarbonate	0.900
CA2	1bn3	0.900
Glycerol	acetohydroxami.	0.900
hydroxyl radic.	cadmium	0.900
Sulfur	nickel	0.900
SOD1	cisplatin	0.900

CA2	EG 2	0.900
Formate	2-mercaptoetha.	0.900
tetrachlor.ina.	oxoplatinum	0.026
CA2	acetaldehyde	0.900
DMSO	imidazole	0.900
Glycerol	acetazolamide	0.900
CO(2	sodium	0.900
Acetaldehyde	formate	0.900
Magnesium	oxygen	0.900
cyclophosp.de-.	d cyclophospha.	0.026
Bicarbonate	magnesium	0.900
cDDP	trans-DDP	0.026
CA2	formate	0.900
Cyanide	cadmium	0.900
DCK	magnesium	0.900
Bromide	nickel	0.900
SOD1	malonate	0.900
Acetaldehyde	chloride	0.900
potassium.rop.	trans-DDP	0.026
UPP1	magnesium	0.900
UBC	SOD1	0.998
Calcium	sodium	0.900
Glycerol	bicarbonate	0.900
potassium.rop.	oxoplatinum	0.026
tetrachlor.inu.	chloroplatinic.	0.026
Glycerol	doxorubicin	0.900
POLH	calcium	0.900
Chloride	sodium	0.900
Glycerol	2-mercaptoetha.	0.900
CA2	bromide	0.900
Chloride	cisplatin	0.900
cDDP	oxoplatinum	0.026
CA2	chloride	0.900
Magnesium	hydroxyl radic.	0.900
CA2	F2-b	0.900
Benzoate	bromide	0.900
chloroplatinic.	K2PtCl4	0.026
Cyanide	nickel	0.900
UBC	RAD18	0.988
cyclophosphami.	L cyclophospha.	0.026
Cisplatin	Disodium tetra.	0.026
PCNA	RAD18	0.998
Calcium	2-mercaptoetha.	0.900
Acetaldehyde	sulfur	0.900
Nickel	cadmium	0.900

CA2	AC1L1C69	0.900
Acetaldehyde	nickel	0.900
p-hydroxybenzo.	2-mercaptoetha.	0.900
potassium.rop.	tetrachlor.ina.	0.026
CO(2	magnesium	0.900
DCK	calcium	0.900
CA2	2-mercaptoetha.	0.900
Glycerol	cisplatin	0.900
Formate	sodium	0.900
CA2	acetohydroxami.	0.900
Calcium	malonate	0.900
p-hydroxybenzo.	glycerol	0.900
malonate	sodium	0.900
thiocyanate	imidazole	0.900
thiocyanate	nitrate	0.900
chloride	ethoxzolamide	0.900
CA2	thiocyanate	0.900
chloride	magnesium	0.900
CA2	CO(2	0.900
SOD1	glycerol	0.900
cisplatin	K2PtCl4	0.026
calcium	magnesium	0.900
acetaldehyde	DMSO	0.900
tetrachlor.inu.	Disodium tetra.	0.026
imidazole	oxygen	0.900
magnesium	spermine	0.900
ATOX1	cadmium	0.900
calcium	glycerol	0.900
CA2	glycerol	0.900
calcium	thiocyanate	0.900
PCNA	magnesium	0.900
calcium	nickel	0.900
acetaldehyde	cadmium	0.900
acetaldehyde	nitrate	0.900
acetaldehyde	bromide	0.900
calcium	bicarbonate	0.900
chloroplatinic.	Disodium tetra.	0.026
SOD1	sodium	0.900
DMSO	carboplatin	0.900
sodium	oxygen	0.900
PCNA	chloride	0.900
SOD1	bicarbonate	0.900
formate	chloride	0.900
DMSO	malonate	0.900
CA2	cyanide	0.900

glycerol	cadmium	0.900
CA2	bicarbonate	0.900
cisplatin	trans-DDP	0.026
glycerol	cyanide	0.900
POLH	RAD18	0.974
sodium	acetohydroxami.	0.900
DMSO	thiocyanate	0.900
chloride	sulfur	0.900
oxoplatinum	K2PtCl4	0.026
chloride	nitrate	0.900
chloroplatinic.	oxoplatinum	0.026
acetaldehyde	malonate	0.900
ATOX1	ATP7B	0.937
acetaldehyde	glycerol	0.900
p-hydroxybenzo.	calcium	0.900
chloride	bicarbonate	0.900
PCNA	UBC	0.998
thiocyanate	magnesium	0.900
glycerol	potassium.rop.	0.900
calcium	imidazole	0.900
CA2	benzoate	0.900
acetaldehyde	thiocyanate	0.900
malonate	2-mercaptoetha.	0.900
cisplatin	tetrachlor.inu.	0.026
glycerol	carboplatin	0.900
CA2	nitrate	0.900
calcium	acetohydroxami.	0.900
chloride	thiocyanate	0.900
CCS	SOD1	0.974
POLH	UBC	0.994
CA2	1cnw	0.900
glycerol	hydroxyurea	0.900
SOD1	thiocyanate	0.900
glycerol	hydroxyl radic.	0.900
bromide	formate	0.900
chloride	hydroxyl radic.	0.900
tetrachlor.ina.	Disodium tetra.	0.026
calcium	cadmium	0.900
cyclophosp.de-.	L cyclophospha.	0.026
imidazole	nickel	0.900
CA2	p-hydroxybenzo.	0.900
glycerol	ethoxzolamide	0.900
DMSO	sodium	0.900
CA2	dichlorphenami.	0.900
bicarbonate	thiocyanate	0.900

sodium	nitrate	0.900
imidazole	2-mercaptoetha.	0.900
POLH	glycerol	0.900
formate	DMSO	0.900
chloroplatinic.	trans-DDP	0.026
glycerol	nitrate	0.900
malonate	magnesium	0.900
cDDP	K2PtCl4	0.026
tetrachlor.inu.	oxoplatinum	0.026
nickel	acetohydroxami.	0.900

Supplementary Table 3 STRING results

#node1	node2	combined_score
RFC3	PCNA	0.999
MDM2	CHEK2	0.999
ERCC2	MNAT1	0.999
TP53	RRM2B	0.999
CCND3	CDKN1A	0.999
RB1	CDK2	0.999
CDK6	CDKN1B	0.999
ERCC2	ERCC3	0.999
ERCC2	CCNH	0.999
POLD1	PCNA	0.999
RFC3	RFC4	0.999
CDK2	CDKN1A	0.999
CCNA2	CDKN1A	0.999
MDM2	TP53	0.999
APAF1	CASP9	0.999
RB1	CDK6	0.999
TP53	MSH2	0.999
CCNA1	CDKN1A	0.999
XPA	ERCC4	0.999
MNAT1	CCNH	0.999
BAX	TP53	0.999
CDK2	CCNB1	0.999
CDKN2A	CCND1	0.999
HSP90AA1	EGFR	0.999
RRM2	RRM1	0.999
CDK6	CCND1	0.999
TP53	CDK2	0.999
CCNB1	CDKN1A	0.999
RFC1	RFC3	0.999
GTF2H3	ERCC3	0.999
HSP90AA1	TP53	0.999
MDM2	CDKN2A	0.999
GTF2H3	ERCC2	0.999
CREB1	EP300	0.999
CCNA2	RB1	0.999
CCNA2	CDKN1B	0.999
SUGT1	HSP90AA1	0.999
GRB2	ERBB2	0.999
SHC1	CBL	0.999
SOS1	EGFR	0.999
CDK4	CDC37	0.999
PCNA	LIG1	0.999

PCNA	POLH	0.999
ABL1	RB1	0.999
MDM2	CDKN1A	0.999
SHC1	SOS1	0.999
RFC5	RFC4	0.999
RRM1	RRM2B	0.999
BRCA1	TP53	0.999
TP53	CDKN1A	0.999
CCND3	CDKN1B	0.999
RFC1	PCNA	0.999
PCNA	MSH2	0.999
MSH2	ERCC1	0.999
HSP90AA1	ERBB2	0.999
HSP90AA1	PTGES3	0.999
PCNA	RFC4	0.999
CDKN2A	CDK4	0.999
PCNA	CDK4	0.999
CCND3	CDK2	0.999
CCNE1	CDKN1A	0.999
CDK2	CCNH	0.999
CDK4	CCND1	0.999
CHEK2	ATM	0.999
PCNA	CDKN1A	0.999
CDKN2A	CDKN1A	0.999
RFC1	RFC4	0.999
EGFR	CBL	0.999
XPA	ERCC3	0.999
KAT2B	EP300	0.999
CDK4	CDKN1A	0.999
RFC5	RFC3	0.999
GRB2	CBL	0.999
ERCC3	MNAT1	0.999
RFC5	PCNA	0.999
CCNA2	CDK2	0.999
PCNA	CCND1	0.999
CCND3	CDK6	0.999
SHC1	GRB2	0.999
GTF2H3	MNAT1	0.999
SHC1	ERBB2	0.999
CASP9	CASP3	0.999
RB1	CCNE1	0.999
ABL1	GRB2	0.999
CDK4	CDKN1B	0.999
TP53	KAT2B	0.999
CDKN1A	CCND1	0.999

CCNE1	CDKN1B	0.999
SHC1	EGFR	0.999
CDK2	CCND1	0.999
HSP90AA1	PPP5C	0.999
CHEK2	TP53	0.999
MDM2	EP300	0.999
HSP90AA1	CDC37	0.999
GRB2	EGFR	0.999
BRCA1	CHEK2	0.999
ATM	TP53	0.999
ERCC4	ERCC1	0.999
CDK6	CDKN1A	0.999
BRCA1	ATM	0.999
CDKN1B	CCND1	0.999
EGFR	ERBB2	0.999
CDKN2A	CDK6	0.999
RFC5	RFC1	0.999
MSH2	MLH1	0.999
HSP90AA1	STIP1	0.999
CDK2	CDKN1B	0.999
SHC1	GAB2	0.999
RB1	CDK4	0.999
ABL1	CBL	0.999
GAB2	GRB2	0.999
TP53	EP300	0.999
ERCC3	CCNH	0.999
CDK2	CCNA1	0.999
ABL1	ATM	0.999
PMS2	MLH1	0.999
CCND3	CDK4	0.999
SOS1	GRB2	0.999
RB1	CCND1	0.999
CDKN2A	TP53	0.999
PCNA	CDK2	0.999
PCNA	FEN1	0.999
HSP90AA1	AHSA1	0.999
MDM2	RB1	0.999
XPA	ERCC1	0.999
CDK2	CCNE1	0.999
PMS2	MSH2	0.999
ERCC4	MSH2	0.998
CREB1	TP53	0.998
PCNA	TP53	0.998
TOP2A	TOP2B	0.998
MDM2	ATM	0.998

CCND3	RB1	0.998
TP53	RB1	0.998
CDK4	CCNH	0.997
HSP90AA1	CDK4	0.997
EGFR	TP53	0.997
APAF1	CASP3	0.997
GTF2H3	CCNH	0.997
CCNB1	CCNA1	0.997
CCNA1	CDKN1B	0.997
TP53	CCNB1	0.996
STIP1	AHSA1	0.996
CDKN2A	RB1	0.996
EP300	CDKN1A	0.996
BRCA1	CDK2	0.996
APAF1	TP53	0.996
PCNA	MLH1	0.996
ERCC2	XPA	0.995
CCNE1	CDK4	0.995
TP53	MLH1	0.995
KAT2B	CCND1	0.995
ERCC2	ERCC1	0.995
ABL1	HSP90AA1	0.995
BRCA1	CDK4	0.995
MDM2	CASP3	0.994
MDM2	KAT2B	0.994
MDM2	ABL1	0.994
CDKN1A	CDKN1B	0.993
ERCC2	ERCC4	0.993
CCND3	CCNH	0.993
CCNA2	CCNB1	0.993
POLD1	ERCC4	0.993
CREB1	CCND1	0.992
ERCC3	TP53	0.992
CDKN2A	CDKN1B	0.992
RB1	CCNA1	0.992
TOP2A	PCNA	0.992
ERCC4	ERCC3	0.992
RB1	CCNB1	0.992
CDK6	CCNE1	0.992
CDK4	CCNB1	0.992
CASP3	CDKN1A	0.992
EP300	CCND1	0.992
BRCA1	EP300	0.992
CDK2	CDK6	0.992
CREB1	ATM	0.991

APAF1	HSP90AA1	0.991
CDKN2A	ABL1	0.990
RB1	EP300	0.990
ERCC3	ERCC1	0.990
ABL1	CCND1	0.990
CDK6	CCNB1	0.990
XPA	POLH	0.989
CCNA2	CDK4	0.989
ABL1	TP53	0.988
TP53	CDK4	0.988
PCNA	RRM2	0.987
RB1	CDKN1A	0.986
POLD1	ERCC1	0.986
ERCC2	TP53	0.985
STIP1	PTGES3	0.985
CHEK2	MSH2	0.985
CDKN2A	CDK2	0.985
CDK6	CDK4	0.984
PCNA	CDK6	0.984
CASP3	RB1	0.984
PCNA	CCND3	0.984
SHC1	ABL1	0.983
ERBB2	CCND1	0.983
ATM	CDK2	0.983
CDK6	CDC37	0.983
EGFR	CDC37	0.983
BRCA1	MSH2	0.982
APAF1	BAX	0.981
PCNA	EP300	0.981
TP53	CCND1	0.979
SUGT1	PTGES3	0.978
ATM	MLH1	0.978
PCNA	CCNB1	0.976
CCNA2	TP53	0.976
GRB2	CDKN1B	0.976
SOS1	CBL	0.976
CCNH	CCNB1	0.975
SOS1	ABL1	0.975
ERBB2	TP53	0.974
ATM	CDKN1A	0.974
BRCA1	CCNE1	0.974
POLD1	ERCC2	0.973
SOS1	ERBB2	0.973
PCNA	PMS2	0.972
ATM	CDK4	0.971

POLD1	FEN1	0.971
CCNA2	CDK6	0.971
CCNH	CCND1	0.970
PCNA	XPA	0.969
EP300	CDK4	0.969
PCNA	CCNA1	0.969
CDKN2A	CCND3	0.968
POLD1	POLH	0.968
BRCA1	RB1	0.968
RB1	CDKN1B	0.967
ERBB2	CBL	0.964
BRCA1	PCNA	0.964
EGFR	CCND1	0.963
CDK2	EP300	0.962
CCNH	CCNA1	0.962
PCNA	CCNA2	0.961
RFC5	POLD1	0.961
PCNA	RRM1	0.961
POLD1	RFC4	0.960
XPA	MSH2	0.960
CDK2	MNAT1	0.960
PCNA	RB1	0.960
CCNA2	CCNE1	0.960
RB1	MNAT1	0.960
POLD1	RFC3	0.959
POLD1	MSH2	0.959
CCNE1	CCNA1	0.959
RFC1	CASP3	0.958
FEN1	LIG1	0.958
FEN1	ERCC3	0.957
CHEK2	KAT2B	0.957
TP53	PMS2	0.956
PCNA	CCNE1	0.955
HSP90AA1	ERCC3	0.955
MDM2	CDK2	0.954
SUGT1	AHSA1	0.953
CREB1	CDKN1A	0.952
CDK2	LIG1	0.951
BRCA1	MLH1	0.951
CCNE1	CCNH	0.951
XPA	CCNH	0.950
SHC1	HSP90AA1	0.950
MNAT1	ERCC1	0.950
SHC1	CREB1	0.950
TP53	CCNH	0.950

CREB1	GRB2	0.950
CCNE1	CCNB1	0.949
MDM2	HSP90AA1	0.949
BRCA1	CDKN1B	0.948
CCNH	CDKN1A	0.948
CHEK2	RB1	0.948
ERCC4	CCNH	0.948
PCNA	ABL1	0.947
GTF2H3	XPA	0.947
POLD1	LIG1	0.947
ERBB2	CDC37	0.947
POLD1	RFC1	0.945
ABL1	CDKN1B	0.945
XPA	MNAT1	0.945
RRM2	TP53	0.944
CCNH	CDKN1B	0.944
CCNH	ERCC1	0.943
CREB1	EGFR	0.943
GTF2H3	ERCC1	0.941
CDK4	MLH1	0.940
GTF2H3	ERCC4	0.939
RRM2	CDK2	0.939
CCNB1	CCND1	0.939
RB1	CCNH	0.939
SOS1	HSP90AA1	0.939
RRM2	CCNE1	0.937
CHEK2	CDKN1A	0.937
ABL1	CDK6	0.935
CDK4	CCNA1	0.934
CCNA1	CCND1	0.934
BAX	EP300	0.933
CCND3	CCNB1	0.933
CCND3	EP300	0.932
STIP1	CDC37	0.931
CDK2	MLH1	0.931
RRM2	CCNA1	0.931
BRCA1	CCND1	0.930
CDK2	RRM2B	0.930
CDK6	CCNA1	0.929
CCNE1	CCND1	0.928
ERCC4	PMS2	0.928
CCND3	CCNA1	0.928
MNAT1	CDK4	0.928
SOS1	GAB2	0.927
POLD1	XPA	0.927

HSP90AA1	RB1	0.927
ABL1	CCNA2	0.926
CCND3	CCNE1	0.925
ABL1	CDK4	0.924
ERCC2	MSH2	0.924
CREB1	SOS1	0.924
MDM2	PCNA	0.924
ABL1	CDK2	0.923
CCND3	GRB2	0.923
MNAT1	CCNB1	0.923
GAB2	EGFR	0.922
SUGT1	CDC37	0.921
ERCC4	MNAT1	0.921
MDM2	CCNA2	0.921
SHC1	CCND3	0.920
TP53	MNAT1	0.919
ATM	CCND1	0.919
CDK2	KAT2B	0.918
ATM	RRM2B	0.918
CDK2	CDK4	0.917
ABL1	CCNE1	0.916
ABL1	CASP9	0.914
ERBB2	CDKN1A	0.914
CASP3	TP53	0.914
ERCC4	FEN1	0.914
MDM2	RFC4	0.912
EGFR	ERCC1	0.912
BRCA1	CCNB1	0.912
CHEK2	CCNB1	0.910
SOS1	CDC37	0.907
CCND3	GAB2	0.907
TOP2B	PCNA	0.906
MNAT1	CCND1	0.905
CCND3	CCND1	0.904
BRCA1	ABL1	0.903
MNAT1	CDKN1A	0.903
SOS1	CCND3	0.901
SHC1	CDC37	0.901
CCNE1	MNAT1	0.900
CHEK2	PCNA	0.900
SHC1	TP53	0.900
MNAT1	CDKN1B	0.900
MDM2	RFC5	0.900
MDM2	RFC3	0.900
PTGES3	CDKN1B	0.900

APÊNDICE G- Justificativa para utilização de material biológico proveniente de biobanco do Institucional de Materiais biológicos humano/Universidade Estadual de Montes Claros - MG (Registro CONEP: B-013)

Eu, André Luiz Sena Guimarães, coordenador do projeto de pesquisa "Alterações genéticas e epigenéticas associadas a neoplasias de cabeça e pescoço", vinculado à Universidade Estadual de Montes Claros - UNIMONTES, venho, através deste documento, justificar a necessidade de requisitar ao Biobanco Institucional desta universidade, amostras biológicas e dados clínicos de indivíduos acometidos por lesões de neoplasia de glândula salivar e carcinoma de células escamosas de cabeça e pescoço, além de amostras não neoplásicas originárias de mucoceles de glândulas salivar menor, sendo estes indivíduos assistidos nas redes públicas e privadas da cidade de Montes Claros-MG. Convém ressaltar que esta requisição é somente para aqueles indivíduos portadores de tal condição patológica que optaram no TCLE do referido biobanco institucional por "NÃO ser consultado da utilização dos meus dados ou material biológico em outra pesquisa, desde que a nova pesquisa seja aprovada pelo Comitê de Ética em Pesquisa".

O biobanco institucional da UNIMONTES encontra-se formalmente regulamentado pela Comissão Nacional de Ética em Pesquisa (registro CONEP: B-013), obedecendo normas e diretrizes estabelecidas pelo Conselho Nacional de Saúde.

A coleta de dados clínicos e materiais biológicos, realizada por este biobanco obedecem, criteriosamente, os preceitos éticos e normas de acondicionamento das amostras, além de já possuir, em seu arquivo, um número considerável de amostras dos grupos considerados nesse projeto. Assim, a utilização de tais amostras já acondicionadas no biobanco em questão irá favorecer o desenvolvimento do projeto de pesquisa por mim coordenado, especialmente pelo rigor utilizado para a coleta do material e número de amostras coletadas disponíveis.

Profª. Dr. André Luiz Sena Guimarães

Docente do Programa de Pós-Graduação em Ciências da Saúde/UNIMONTES

ANEXOS

ANEXO A- Classificação Histopatológica dos tumores de glândula salivar segundo a OMS

Malignant epithelial tumours	
Acinic cell carcinoma	8550/3
Mucoepidermoid carcinoma	8430/3
Adenoid cystic carcinoma	8200/3
Polymorphous low-grade adenocarcinoma	8525/3
Epithelial-myoepithelial carcinoma	8562/3
Clear cell carcinoma, not otherwise specified	8310/3
Basal cell adenocarcinoma	8147/3
Sebaceous carcinoma	8410/3
Sebaceous lymphadenocarcinoma	8410/3
Cystadenocarcinoma	8440/3
Low-grade cribriform cystadenocarcinoma	
Mucinous adenocarcinoma	8480/3
Oncocytic carcinoma	8290/3
Salivary duct carcinoma	8500/3
Adenocarcinoma, not otherwise specified	8140/3
Myoepithelial carcinoma	8982/3
Carcinoma ex pleomorphic adenoma	8941/3
Carcinosarcoma	8980/3
Metastasizing pleomorphic adenoma	8940/1
Squamous cell carcinoma	8070/3
Small cell carcinoma	8041/3
Large cell carcinoma	8012/3
Lymphoepithelial carcinoma	8082/3
Sialoblastoma	8974/1
Benign epithelial tumours	
Pleomorphic adenoma	8940/0
Myoepithelioma	8982/0
Basal cell adenoma	8147/0
Warthin tumour	8561/0
Oncocytoma	8290/0
Canalicular adenoma	8149/0
Sebaceous adenoma	8410/0
Lymphadenoma	
Sebaceous	8410/0
Non-sebaceous	8410/0
Ductal papillomas	
Inverted ductal papilloma	8503/0
Intraductal papilloma	8503/0
Sialadenoma papilliferum	8406/0
Cystadenoma	8440/0
Soft tissue tumours	
Haemangioma	9120/0
Haematolymphoid tumours	
Hodgkin lymphoma	
Diffuse large B-cell lymphoma	9680/3
Extranodal marginal zone B-cell lymphoma	9699/3
Secondary tumours	

[1] Morphology code of the International Classification of Diseases for Oncology (ICD-O) {821} and the Systematized Nomenclature of Medicine (http://snomed.org). Behaviour is coded /0 for benign tumours, /3 for malignant tumours, and /1 for borderline or uncertain behaviour.

ANEXO B- Classificação TNM dos tumores de boca e glândula salivar

TNM classification [1,2]

T – Primary tumour

TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
T1	Tumour 2 cm or less in greatest dimension without extraparenchymal extension*
T2	Tumour more than 2 cm but not more than 4 cm in greatest dimension without extraparenchymal extension*
T3	Tumour more than 4 cm and/or tumour with extraparenchymal extension*
T4a	Tumour invades skin, mandible, ear canal, or facial nerve
T4b	Tumour invades base of skull, pterygoid plates, or encases carotid artery

Note: *Extraparenchymal extension is clinical or macroscopic evidence of invasion of soft tissues or nerve, except those listed under T4a and 4b. Microscopic evidence alone does not constitute extraparenchymal extension for classification purposes.

N – Regional lymph nodes##

NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension
N2	Metastasis as specified in N2a, 2b, 2c below
N2a	Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension
N2b	Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension
N2c	Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
N3	Metastasis in a lymph node more than 6 cm in greatest dimension

Note: Midline nodes are considered ipsilateral nodes.

M – Distant metastasis

MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

Stage Grouping

Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1, T2, T3	N1	M0
Stage IV A	T1, T2, T3	N2	M0
	T4a	N0, N1, N2	M0
Stage IV B	T4b	Any N	M0
	Any T	N3	M0
Stage IV C	Any T	Any N	M1

The regional lymph nodes are the cervical nodes.

[1] {947,2418}.
[2] A help desk for specific questions about the TNM classification is available at http://www.uicc.org/index.php?id=508.

ANEXO C- Parecer CONEP - Biobanco Institucional de Materiais biológicos humano/Universidade Estadual de Montes Claros - MG

CONSELHO NACIONAL DE SAÚDE
COMISSÃO NACIONAL DE ÉTICA EM PESQUISA

PARECER CONEP – BIOBANCO nº. 008/2013

Registro CONEP B-013 (Este nº deve ser citado nas correspondências referentes a este biobanco)

Processo nº 25000.001815/2013-60
Instituição responsável: Universidade Estadual de Montes Claros
Instituição participante: Universidade Estadual de Montes Claros.
Responsável(is) institucional: Dr. Andre Luiz Sena Guimarães.
CEP de origem: Comitê de Ética da Universidade Estadual de Montes Claros.

Sumário geral do protocolo

Tipo de material biológico: amostras de material biológico criopreservadas e/ou emblocadas em parafina, amostras de RNA, DNA, proteína e cultivo celular de materiais biológicos humano, coletados de diferentes sítios anatômicos.

Documentos encaminhados para análise:

1- Protocolo de Desenvolvimento.

2- Regimento interno do Biobanco.

3- Declaração de gerenciamento institucional.

4- TCLE.

Apresentação do protocolo

Trata-se de solicitação de registro de Biobanco ligado à Universidade Estadual de Montes Claros. Destina-se à coleta, armazenamento e distribuição para pesquisa de amostras biológicas humanas obtidas de pacientes/sujeitos de pesquisa da região norte do Estado de Minas Gerais, para o estudo de neoplasias ou outras condições patológicas.

Protocolo de Desenvolvimento:

está estrutudado da seguinte forma:
1 INTRODUÇÃO; 2 JUSTIFICATIVA; 3 OBJETIVOS; 4 PRINCÍPIOS DE ESTRUTURAÇÃO, FUNCIONAMENTO E MANUTENÇÃO E DO BIOBANCO; 5 REGIMENTO INTERNO DO BIOBANCO; 6 DECLARAÇÃO DE GERENCIAMENTO E RESPONSABILIDADE INSTITUCIONAL DO BIOBANCO; REFERÊNCIAS; APÊNDICES.

Em síntese, são abordados de forma detalhada a justificativa e objetivos do biobanco, os cenários previstos para entrada das amostras biológicas humanas, assim como as políticas e procedimentos operacionais padrão para coleta, armazenamento, processamento e distribuição das mesmas, assim como de informações associadas. É descrita a infra-estrutura e de pessoal atualmente disponível, assim como o financiamento da mesma, a cargo da Universidade Estadual de Montes Claros. Os Procedimentos Operacionais Padrão (POPs) enviados para análise estão de acordo com documentos de

melhores práticas internacionais que abordam a coleta, processamento, armazenamento e distribuição de material biológico humano com finalidade de pesquisa.

Um "Termo de concordância da instituição para o acesso à coleta de material biológico e informações associadas" prevê os critérios e condições pelos quais material biológico humano poderá ser coletado de sujeitos de pesquisa de instituições de saúde parceiras do Biobanco, mediante assinatura do Termo de Consentimento Livre e Esclarecido utilizado como modelo.

Um "TERMO DE TRANSFERÊNCIA DE MATERIAL BIOLÓGICO REFERENTE À REMESSA DE AMOSTRA DE COMPONENTE DO PATRIMÔNIO GENÉTICO PARA FINS DE PESQUISA CIENTÍFICA SEM POTENCIAL ECONÔMICO", modelo do Anexo da RESOLUÇÃO CGEN Nº 20, DE 29 DE JUNHO DE 2006, detalha os critérios pelos quais o material armazenado pelo Biobanco será transferido a terceiros para utilização em pesquisa, notadamente a outras Instituições.

Com relação à garantia dos direitos dos sujeitos de pesquisa, os documentos apresentados foram adequados às normativas vigentes. Ao sujeito de pesquisa será fornecida, além de uma via do TCLE, um cartão de participação com formas de contato com o Biobanco. O Termo de Consentimento Livre e Esclarecido, além de informar as formas de contato com o Biobanco e o Comitê de Ética em Pesquisa Institucional, garante:

a) A autonomia do sujeito de pesquisa quanto à necessidade de novo consentimento a cada nova pesquisa que venha a fazer uso de suas amostras biológicas, assim como da dispensa de tal necessidade.
b) A autonomia do sujeito de pesquisa quanto à nomeação de terceiros para decisão quanto à utilização ou descarte do seu material biológico armazenado, em caso de óbito ou condição incapacitante.
c) O acesso, a qualquer momento, aos resultados obtidos a partir do seu material biológico armazenado e às orientações quanto às suas implicações, incluindo o aconselhamento genético, quando aplicável.
d) O direito de desistir da participação em qualquer momento, sem qualquer prejuízo.
e) A garantia ao anonimato.

Comentários/Considerações

1. Este parecer tem como base o disposto na Resolução CNS 441/11 e na Portaria MS 2201/11. Pelo que se depreende do exposto na documentação apresentada, trata-se do estabelecimento de um Biobanco institucional, dedicado à coleta, armazenamento e distribuição de amostras biológicas humanas com finalidade de pesquisa, com um responsável pelo gerenciamento apontado pela Instituição, assim como um comitê gestor, cujas funções adequadamente descritas.

2. No exame da documentação anexada ao Protocolo, inclusive o Termo de Consentimento Livre e Esclarecido, utiliza-se inúmeras vezes as palavras "doador", "doação" e correlatas. Deve-se substituir tais termos (por exemplo, pelo termo "cessão"), retirando-os da documentação apresentada em concordância com a legislação nacional relativa a Biobanco e adicionando o termo "concessão" para substituir "cessão" e o verbo

Cont. Parecer CONEP nº.

"consentir" para subistituir "cedir" (Res. CNS 441 de 2011 e Portaria MS 2201 de 2011). De acordo com as mesmas, as amostras biológicas armazenadas em biobanco são do sujeito de pesquisa (ou cedente), cabendo à Instituição a sua guarda. Solicita-se adequação.

Diante do exposto, a Comissão Nacional de Ética em Pesquisa – CONEP, de acordo com as atribuições definidas na Resolução CNS 196/96, manifesta-se pela aprovação do projeto de pesquisa proposto, devendo o CEP verificar o cumprimento das questões acima e encaminhar à CONEP as recomendações cumpridas antes do início do estudo.

Situação: **Protocolo aprovado com recomendação.**

Brasília, 25 de Abril de 2013

Gyselle Saddi Tannous
Coordenadora da CONEP/CNS/MS

ANEXO D- Termo de Consentimento Livre e Esclarecido utilizado para coleta de materiais biológicos pelo Biobanco de Materiais Biológicos do Norte de Minas Gerais

UNIVERSIDADE ESTADUAL DE MONTES CLAROS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

Código: _____

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Esclarecimentos

Este é um convite para a doação de material biológico excedente, proveniente de sua cirurgia ou procedimento hospitalar que será realizado devido à necessidade já definida pelo médico para o diagnóstico de alguma doença ou tratamento.

A partir da sua autorização, o material será doado e arquivado no banco de materiais biológicos da Universidade Estadual de Montes Claros, destinado à realização de pesquisas científicas, sem fins lucrativos. Garantimos o sigilo dos seus dados. As pesquisas realizadas utilizando o material doado têm como objetivo o melhor entendimento das causas e mecanismos das doenças que nos acomete. Podem, ainda, ajudar na busca de novas formas de tratamento das doenças.

A coleta dos materiais biológicos doados obedece a documentos legais do Conselho Nacional de Saúde e suas complementares. O biobanco onde será arquivado o seu material doado está sob a responsabilidade da UNIMONTES e encontra-se localizado no Hospital Universitário Clemente de Faria.

Sua participação é voluntária, o que significa que você poderá desistir a qualquer momento, retirando seu consentimento, sem que isso lhe traga nenhum prejuízo ou penalidade.

Caso decida aceitar doar o excedente de material biológico proveniente do procedimento médico, gostaríamos de esclarecer você mais alguns pontos importantes:

- O material coletado será o excedente do procedimento cirúrgico realizado pela equipe médica

- Os riscos quanto à doação do material estão relacionados ao tipo de procedimento realizado pela equipe médica, necessário para diagnóstico e tratamento que deverão ser claramente esclarecidos para você pela equipe. Sendo que a coleta de material para o Biobanco envolve apenas o excedente material biológico proveniente desse procedimento médico.

- Através da doação, você estará contribuindo cedendo o material biológico necessário para a realização de pesquisas para melhor entender as causas e mecanismos das doenças.

- Quanto aos benefícios, a doação do material poderá favorecer a realização de pesquisas que buscam um melhor entendimento e possibilidade de controle e tratamento das doenças.

- Você terá direito ao acesso, a qualquer momento, aos resultados obtidos a partir do seu material biológico armazenado e às orientações quanto às suas implicações, incluindo o aconselhamento genético, quando aplicável. Para isso, você deverá entrar em contato com a equipe do biobanco através do endereço ou telefone de contato abaixo, informando o código de cadastro que você recebeu no ato da doação e, também, está escrito no seu cartão de cadastro, que ficou guardado com você:

Biobanco de Materiais Biológicos do norte de Minas Gerais

Laboratório de Pesquisa em Saúde - Hospital Universitário Clemente Faria - UNIMONTES

Av. Cula Mangabeira, 562 - Bairro Santo Expedito, Montes Claros, MG - CEP: 39401-001

Telefone: (38) 3224-8327

- Todas as informações obtidas serão sigilosas e seu nome não será identificado em nenhum momento. Os dados serão guardados em local seguro e a divulgação dos resultados será feita de forma a não identificar os voluntários.

- Se você tiver algum gasto que seja devido à sua participação na pesquisa, você será ressarcido.

Em qualquer momento, se você sofrer algum dano comprovadamente decorrente desta pesquisa, você terá direito a indenização.

Você ficará com uma cópia deste Termo e toda a dúvida que você tiver a respeito desta pesquisa, poderá perguntar diretamente a um dos membros do biobanco através do endereço ou telefone apresentado acima.

Se houver dúvidas sobre a ética da pesquisa entre em contato com o Comitê de Ética em Pesquisa da UNIMONTES através do contato abaixo:

Comitê de Ética em Pesquisa

Campus Universitário Professor Darcy Ribeiro

Vila Mauricéia - Montes Claros – MG, Caixa Postal 126 - CEP 39401-089

Fone: (38) 3229-8000

Consentimento Livre e Esclarecido

Estou de acordo com a participação e doação de material biológico excedente, proveniente de minha cirurgia ou procedimento hospitalar que será realizado devido à necessidade já definida pelo médico para o diagnóstico de alguma doença ou tratamento.

Fui devidamente esclarecido quanto aos objetivos do biobanco, aos procedimentos aos quais serei submetido e dos possíveis riscos que possam advir de tal participação. Foram a mim garantidos esclarecimentos que venha a solicitar durante o curso da pesquisa e o direito de desistir da participação em qualquer momento, sem que minha desistência implique em qualquer prejuízo a minha pessoa ou de minha família. A minha participação na pesquisa não implicará em custos ou prejuízos adicionais, sejam esses custos ou prejuízos de caráter econômico, social, psicológico ou moral. Autorizo assim a publicação dos dados da pesquisa a qual me garante o anonimato e o sigilo dos dados referentes à minha identificação.

Reutilização dos dados ou material biológico:

O material biológico doado ficará armazenado no Banco de Materiais Biológicos Humano do Norte de Minas Gerais, localizado nas dependências do Hospital Universitário Clemente de Faria na cidade de Montes Claros/MG. Em relação à utilização deste material para a realização de pesquisas científicas:

( ) SIM, autorizo a utilização de dados ou material biológico _____(especificar o material) em todas as pesquisas vinculadas ao Biobanco da UNIMONTES.

( ) NÃO autorizo a utilização de dados ou material biológico_____(especificar o material) em mais de uma pesquisa. Então este material será descartado. (especificar a forma de descarte): _____

Para utilização dos meus dados e material coletado_____(especificar o material) em mais de uma pesquisa:

( ) NÃO preciso ser consultado da utilização dos meus dados ou material biológico em outra pesquisa, desde que a nova pesquisa seja aprovada pelo Comitê de Ética em Pesquisa,

( ) SIM, quero ser consultado da utilização dos meus dados ou material biológico em outra pesquisa.

Em caso de óbito ou alguma outra condição em que eu não possa dar o consentimento para utilização dos meus dados em outro estudo, autorizo_____(especificar nome da pessoa)a consentirem a utilização ou descarte de meu material biológico armazenado.

Doador de material biológico ao Biobanco ou responsável legal:

Assinatura do doador ou responsável

Coordenador Geral do Biobanco:

Dr. André Luiz Sena Guimarães

Laboratório de Pesquisa em Saúde - Hospital Universitário Clemente Faria - UNIMONTES

Av. Cula Mangabeira , 562 - Bairro Santo Expedito, Montes Claros, MG - CEP: 39401-001

Telefone: (38) 3224-8327

Responsável técnico pela coleta e transporte do material coletado ao biobanco:

Data: _____

UNIVERSIDADE ESTADUAL DE MONTES CLAROS

BIOBANCO INSTITUCIONAL DE MATERIAIS

BIOLÓGICO HUMANO

O doador de material biológico humano e informações clínicas, através do Termo de Consentimento Livre e Esclarecido, consentiu a doação legal de material biológico humano para a realização de pesquisas científicas, sem fins lucrativos, vinculadas ao Biobanco de Materiais Biológicos Humano da Universidade Estadual de Montes Claros.

Você tem direito a todas as informações de resultados de pesquisas realizadas com seu material doado. Caso tenha interesse em tais resultados, comunique no seguinte endereço ou telefone de contado, informando o seu código de doador apresentado abaixo:

Código do doador: _____

Data: _____

ANEXO E- Declaração de aceite do Biobanco de Materiais Biológicos do Norte de Minas Gerais para a concessão das amostras e dados clínicos para a execução do projeto

Eu, Alfredo Maurício Batista De Paula, Coordenador do Banco de Materiais Humano do Norte do Estado de Minas Gerais (Registro CONEP: B-013: Parecer nº 008/2013), declaro, em nome do Comitê Gestor do biobanco institucional/UNIMONTES, a aceitação da cessão de materiais biológicos e dados clínicos para o projeto de pesquisa " Alterações genéticas e epigenéticas associadas a neoplasias de cabeça e pescoço" coordenado pelo professor Dr. André Luiz Sena Guimarães.

A solicitação inclui amostras teciduais e dados clínicos de indivíduos, independente da faixa etária , com diagnóstico histopatológico confirmado de neoplasia de glândula salivar e carcinoma de células escamosas de cabeça e pescoço, além de amostras não neoplásicas originárias de mucoceles de glândulas salivar menor. Conforme solicitado pelo pesquisador , as amostras biológicas cedidas, deverão ser, somente, daqueles indivíduos que optaram, no TCLE do biobanco institucional, por "NÃO ser consultado da utilização dos meus dados ou material biológico em outra pesquisa, desde que a nova pesquisa seja aprovada pelo Comitê de Ética em Pesquisa".

Declaro ainda que a cessão de tais amostras está vinculada a entrega formal da aprovação do projeto do Comitê de Ética em Pesquisa.

Nessa ocasião, o coordenador do projeto deverá efetuar o aceite legal de cumprimento das normas do Regimento interno do Biobanco, através da assinatura de um " Termo de Compromisso para o Uso de Material Biológico e Informações Associadas". Nesse documento, o mesmo responsabilizar-se-á pelo uso e preservação dos materiais biológicos e informações associadas, resguardando os direitos dos sujeitos, especialmente, no que se refere à confidencialidade, ao sigilo, ao acesso a resultados das pesquisas e à proteção intelectual contra utilização comercial e qualquer forma de patenteamento com todo e qualquer material proveniente do biobanco.

Montes Claros, 1º de Setembro de 2015

_____

Alfredo Maurício Batista De Paula

Coordenador do Banco de Materiais Biológicos Humano do Norte de Minas Gerais