UNIVERSIDADE FEDERAL DE MINAS GERAIS
FACULDADE DE MEDICINA
DEPTO. DE ANATOMIA PATOLÓGICA E MEDICINA LEGAL

Tumores Mistos e Carcinomas Metaplásicos de Glândulas Mamárias Caninas: Aspectos Comparativos com Tumores de Glândulas Salivares da Espécie Humana

Marisa Cristina Genelhu Leite Santos

Belo Horizonte – Minas Gerais
2005

MARISA CRISTINA GENELHU LEITE SANTOS

Tumores Mistos e Carcinomas Metaplásicos de Glândulas Mamárias Caninas: Aspectos Comparativos com Tumores de Glândulas Salivares da Espécie Humana

Tese apresentada ao Programa de Pós-graduação em Patologia da Faculdade de Medicina da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do título de Doutor em Patologia.
Área de concentração: Patologia Geral

Orientador: Prof. Dr. Geovanni Dantas Cassali
Co-orientador: Prof.ª Dr.ª Helenice Gobbi
Universidade Federal de Minas Gerais

Belo Horizonte – Minas Gerais
Faculdade de Medicina da UFMG
2005

Ó meu Deus,

Quisestes nutrir-me com Tua divina substância, a mim, pobre criatura, que ao nada retornaria se Teu Divino olhar não me outorgasse a vida a cada instante...

Vós haveis ido mais longe de minha previsão e eu quero cantar Vossas misericórdias.

Para Vós, unicamente, seja qualquer vitória que me fizerdes adquirir...

Santa Teresa de Lisieux

Aos meus filhos Bernardo, Lucas e Gabriel e ao meu marido Heber. Como uma vez disse Vinícius de Moraes, vocês são "indispensáveis ao meu equilíbrio vital, porque fazem parte do mundo que eu, tremulamente, construí e se tornaram alicerces do meu encanto pela vida".

Aos meus pais João e Zélia e meus irmãos Giovana e João Luis.

AGRADECIMENTOS

Ao Prof. Dr. Geovanni Dantas Cassali, pela orientação neste trabalho, admiração e respeito.

À Profa Dr[a]. Helenice Gobbi, por ter acreditado nos meus sonhos e incentivado de todas as maneiras possíveis para que ele se tornasse realidade.

Ao Pe. Geraldo Hogervorst e Pe. Edson José do Sacramento, verdadeiros instrumentos dóceis nas mãos do Pai, pelo conforto espiritual concedido a cada encontro.

Ao Prof. Giovanni Gazzinelli e ao Dr. Rodrigo Corrêa de Oliveira do Centro de Pesquisas René Rachou CPqRR-FIOCRUZ, por terem observado em mim a vocação para a pesquisa e incentivado a sua realização.

À Dr[a]. Lucia Alves de Oliveira Fraga e ao Dr. Luiz Cosme Cotta Malaquias do Núcleo de Pesquisa em Imunologia da Universidade Vale do Rio Doce (UNIVALE), por serem aqueles amigos e professores que se tornam inesquecíveis e imortais através da continuidade de sua obra por meio de seus discípulos e companheiros.

À Maria José Rocha, anjo colocado por Deus no meu caminho para que eu suportasse as dificuldades e compartilhasse as alegrias deste trabalho.

A todos os colegas do Laboratório de Patologia Comparada do Instituto de Ciências Biológicas e da Faculdade de Medicina da UFMG, pela acolhida e prazerosa convivência.

A todos os colegas da UNIVALE, em especial às professoras e amigas Beatriz Brasileiro e Sílvia Perim e às fiéis colaboradoras, Fátima, Marlucy, Lília, Ivanete, Nelma, Meire e Elaine: saber que vocês me queriam de volta, era um bálsamo nas horas difíceis...

Aos meus amigos Luciene Tafuri, Terezinha Marques, Antônio Hugo Campos, Paôlla Perdigão, Paula Vidigal, Renato Baldan, Sérgio Cardoso e Diele Carine Barreto Arantes e aos professores Maria Cássia Aguiar, Carlos Alberto Ribeiro, Lúcia Porto e Mônica Demas Cabral: a colaboração e o apoio de vocês foram imprescindíveis.

À Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG), à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e à Comissão Institucional de Capacitação Docente e Técnica (CICDT) da UNIVALE.

E a todos que direta ou indiretamente contribuíram para a realização deste trabalho.

SUMÁRIO

TRADUÇÕES ÚTEIS PARA A COMPREENSÃO DA TESE

cluster	**grupo**
de novo	**de origem primária**
in situ	**não invasivo**
follow-up	**seguimento, proservação**
multi-steps	**múltiplos passos**
insights	**novas percepções, novos conceitos**
status	**estado, condição**
pool	**associação**
stem cell	**célula indiferenciada, célula-tronco**
splincing	**clivagem e união de fragmentos de RNA**

LISTA DE ABREVIATURAS E SIGLAS

μm	**micrômetro**
ΔN	**Isoforma ΔN (sem a porção amino-terminal) da proteína p63**
α-SMA	**α-actina de músculo liso (*α-smooth muscle actin*)**
A1	**Glândula mamária canina abdominal-cranial**
A2	**Glândula mamária canina abdominal-cranial**
AE1/AE3	**Anticorpo anti-pan citoqueratinas humanas (1-8, 10, 14-16 e 19)**
AgNORs	**Regiões organizadoras de nucléolo coradas pela prata (*silver-stained nucleolar organizer regions*)**
AP	**Adenoma pleomórfico**
APC	**Gene da polipose adenomatosa do cólon (*adenomatosis polyposis coli*)**
BMP	**Proteínas morfogenéticos ósseas (*bone morphogenetics proteins)***
***BRCA*1**	**Gene do câncer de mama 1 (*breast cancer gene 1*)**
Ca ex-AP	**Carcinoma ex-adenoma pleomórfico**
CD10	**Grupo de moléculas de diferenciação de linfócitos 10 (*cluster of differentiation* 10)**

CD-RAP	**Proteína derivada de cartilagem ácido retinóico específica (*cartilage-derived retinoic acid-sensitive protein*)**
c-*erb*B2	**Gene celular para receptor do fator de crescimento epitelial humano 2 ou EGFR-2 (*epidermal growth factor receptor-2*) ou HER2 (*human epidermal growth factor receptor 2*)**
ChM-1	**Proteína condromodulina 1 matriz-cartilagem específica (*cartilage-specific matrix chondromodulin-1*)**
c-myc	**Oncogene celular da mielocitomatose *(myelocytomatosis celular oncogene)***
COOH	**Porção carbóxi-terminal protéica**
***CTNNB*1**	**Gene da proteína catenina associada a caderina beta 1 *(catenin cadherin-associated protein β1)***
DAB	**Diaminobenzidina**
DNA	**Ácido desoxirribonucléico (*desoxi ribonucleic acid*)**
GSK-3β	**Proteína quinase (serina-treonina) sintetizadora de glicogênio (*serine-threonine glycogen synthetase kinase-3β*)**
h-caldesmona	**Caldesmona de cadeia pesada**
HMGIC	**Gene da proteína do grupo de alta mobilidade (não histona cromossomal) – isoforma I-C (*High-mobility group nonhistone chromosomal protein isoform I-C*)**
I	**Glândula mamária canina Inguinal**

Ki-67	**Antígeno marcador de proliferação celular Ki-67**
K-ras	**Oncogene celular homólogo do oncogene viral do sarcoma em ratos Kirsten (*Kirsten rat sarcoma viral oncogene)***
LEF	**Fator aumentador de linfócitos (*lynfocyte enhancer factor*)**
mdm2	**Proteína de célula transformada (*transformed 3T3 cell double minute 2*)**
MUC	**Mucina**
N-CAM	**Molécula de adesão celular neuronal (*neural-cell adhesion molecule*)**
NH_2^-	**Porção amino-terminal protéica**
NOS	**Sem outra especificação (*not otherwise specified*)**
p	**Braço curto de cromossomo (*petit*)**
p105	**Proteína p105**
p16	**Proteína p16**
p21	**Proteína p21**
p53	**Proteína p53**
p63	**Proteína p63**
PAAF	**Punção aspirativa por agulha fina**
PCNA	**Antígeno nuclear de proliferação celular (*proliferating cell nuclear antigen*)**
PIP	**Proteína prolactina induzível**
***PLAG*1**	**Gene do adenoma pleomórfico 1**

q	**Braço longo de cromossomo**
RB	**Proteína do gene *RB* (retinoblastoma)**
RE	**Receptor de estrógeno**
RNAm	**Ácido ribonucléico mensageiro *(ribonucleic acid)***
RT-PCR	**Reação em cadeia da polimerase - transcritase reversa (*reverse transcriptase polymerase chain reaction)***
S-100	**Proteína cérebro específica (*brain-specific protein*)**
SMMHC	**Miosina de músculo liso de cadeia pesada (*smooth-muscle myosin heavy chain*)**
T1	**Glândula mamária canina torácica-cranial**
T2	**Glândula mamaria canina torácica-caudal**
TA	**Isoforma transativadora da proteína p63**
Tcf	**Fator de células T (linfócitos) (*T-cell factor*)**
***TP*53**	**Gene p53**
Wnt	**Via celular transdutoa de sinais (*Wingless*)**

LISTA DE ILUSTRAÇÕES

INTRODUÇÃO

I. A CONTRIBUIÇÃO DA PATOLOGIA COMPARADA

Uma estratégia que tem permitido avançar no conhecimento dos diferentes aspectos da carcinogênese comparada tem sido a utilização de modelos animais (FERNANDES, 1996). O objetivo final do estudo das neoplasias em modelos animais é a busca de maior entendimento dos fatores responsáveis pela doença no homem e a expectativa de que estes possam ser identificados, controlados e até mesmo, eliminados. Informações sobre a ocorrência de tumores espontâneos originam-se principalmente das pesquisas veterinárias de tumores em animais domésticos, selvagens e naqueles criados em zoológicos e laboratórios. O estudo dos tumores mais freqüentes em animais pode fornecer dados epidemiológicos e indicações para a melhor compreensão de sua etiologia e desenvolvimento, bem como material para investigação biológica e terapêutica (MARCHANT, 1987).

I.1. Tumores Mamários Humanos *versus* Tumores Mamários Caninos

As glândulas mamárias da espécie canina (*Canis familiaris*) originam-se embriologicamente pela invaginação de brotos ectodérmicos para o interior do mesoderma subjacente. Elas se desenvolvem em cinco pares a partir de linhas ou cristas mamárias bilaterais nas regiões torácica, abdominal e inguinal. A denominação de cada par de mamas no sentido crânio-caudal da superfície ventral é: torácico-cranial (T1), torácico-

caudal (T2), abdominal-cranial (A1), abdominal-caudal (A2) e inguinal (I) (CASSALI, 2002) (Figura 1).

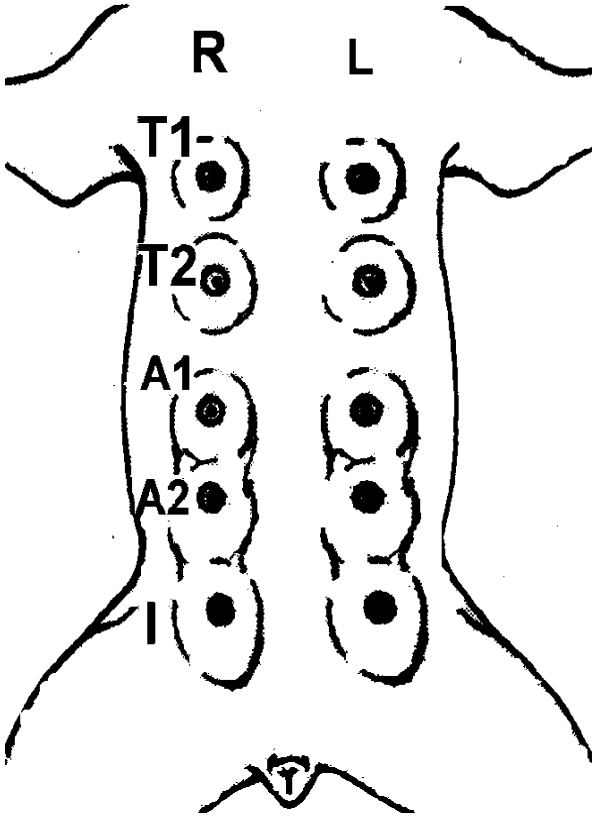


Figura 1 – Representação esquemática dos cinco pares de mama canina e suas denominações.

Os tumores da glândula mamária canina despertam especial interesse nos pesquisadores que trabalham com neoplasias por causa de suas similaridades com os tumores de mama humanos, tendo inclusive sido proposto, em muitos trabalhos, o estudo destas lesões como modelo comparativo para a espécie humana (*Homo sapiens*) (SCHNEIDER, 1970; STRANDBERG & GOODMAN, 1974; MARTIN et al., 1984; MOTTOLESE et al., 1994; PELETEIRO, 1994; SCHAFER et al., 1998; CASSALI, 2000).

Os tumores mamários caninos espontâneos apresentam várias características epidemiológicas, clínicas, biológicas e aparentemente genéticas, semelhantes aos dos tumores mamários da espécie humana. Entre estas podemos citar: a faixa etária de aparecimento (SCHNEIDER et al., 1969; MOULTON, 1970; ELLING & UNGEMACH, 1983; MIALOT & LAGADIC, 1990), a morfologia histológica (HAMILTON et al., 1977; MOULTON, 1990; FLORES ALÉS, 1997), o efeito protetor da ovariectomia (SCHNEIDER et al., 1969; ELLING & UNGEMACH, 1983; MILLER, 1991), a presença de receptores de estrógeno (RE) e progesterona (SCHNEIDER et al., 1969; EVANS & PIERREPOINT, 1975; HAMILTON et al., 1977; ELLING & UNGEMACH, 1983; FLORES ALÉS, 1997; GRAHAM et al., 1999; GERALDES et al., 2000; NIETO et al., 2000), os órgãos alvo de metástases (HAMILTON et al., 1977; FLORES ALÉS, 1997), a evolução clínica das neoplasias (SCHNEIDER et al., 1969; HAMILTON et al., 1977; MOULTON, 1990) e a hereditariedade em alguns casos (TAYLOR et al., 1976; MOULTON et al., 1996). Também foi demonstrado que as neoplasias mamárias caninas podem apresentar um fenótipo antigênico comparável àquele observado em lesões de mama humana (MOTTOLESE et al., 1994) e há homologia entre o gene *BRCA*1 humano e do cão (SZABÓ et al., 1996).

A incidência dos tumores mamários caninos espontâneos é duas a três vezes superiores à observada na mulher e 16 vezes superior no cão em relação ao homem (SCHNEIDER, 1970). De maneira similar à espécie humana, os tumores de mama também são raros nos machos (STRANDBERG & GOODMAN, 1974). Como em humanos o câncer de mama afeta usualmente indivíduos mais idosos, sendo a média de idade de cerca de dez anos, raramente ocorrendo antes dos cinco anos (COHEN et al., 1974). Quando se utiliza a tabela de Lebeau (1953), verifica-se que ocorrem em faixas etárias correspondentes (SCHNEIDER, 1970). No que se refere aos tumores malignos, os

carcinomas mais freqüentes na mama humana e na mama canina são, respectivamente, os carcinomas ductais invasivos e as neoplasias descritas genericamente como adenocarcinomas (STRANDBERG & GOODMAN, 1974; MARTIN et al., 1984). Finalmente, quando se considera a freqüência de outras lesões neoplásicas malignas da mama como, por exemplo, os sarcomas, verifica-se que estes são igualmente raros nas duas espécies (STRANDBERG & GOODMAN, 1974; ROSEN & OBERMAN, 1993).

I.2. Tumores Mamários Humanos *versus* Tumores de Glândula Salivar Humanos

Já em 1968, foi relatada uma associação peculiar entre tumores de glândula salivar e tumores de mama humanos: em um estudo de sobrevida observou-se que mulheres com tumores de mama tendem significativamente a desenvolver tumores de glândula salivar ou vice-versa (BERG et al., 1968).

Atualmente, admite-se que existam similaridades bem conhecidas entre as glândulas mamária e salivar na espécie humana devido à homologia estrutural básica entre essas glândulas exócrinas (BERG et al., 1968; DUNN et al., 1972; PRIOR & WATERHOUSE, 1977; ABBEY et al., 1984). Notáveis semelhanças morfológicas entre certos tumores de glândula salivar e algumas neoplasias de mama têm sido descritas, tais como aquelas existentes entre o adenocarcinoma polimorfo de baixo grau de malignidade e o carcinoma lobular invasivo (FREEDMAN & LUMERMAN, 1983; ABERLE et al., 1985; WENIG & GNEPP, 1991; NICOL & ISKANDAR, 2000), entre o carcinoma de células acinares e o carcinoma secretor invasivo (DAMIANI et al., 2000; HIROKAWA et al., 2002), entre o carcinoma epitelial-mioepitelial e o adenomioepitelioma (SEIFERT, 1998; NAGAO et al., 1998; SUGANO et al., 2001). Carcinomas ductais (WICK et al., 1998; HOANG et al., 2001; SKÁLOVÁ et al., 2001), carcinomas adenóides císticos (DORI et al., 2000; PIA-FOSCHINI et al., 2003) e os tumores mistos benignos e malignos

(adenomas pleomórficos - AP e carcinomas ex-adenomas pleomórficos - Ca ex-AP, respectivamente) (CHEN, 1990; MORAN et al., 1990; REID-NICHOLSON et al., 2003; KUMAR et al., 2005; HAYES et al., 2005) podem ser encontrados em ambas as estruturas, ainda que os prognósticos sejam diferentes.

Alguns aspectos genéticos como expressão de c-*erb*B2 (CHO et al., 1999; SKALOVÁ et al., 2001; SCHOLL et al., 2001) e PIP (*prolactin-inducible protein*) (CLARK et al., 1999), além de alterações alélicas nos cromossomos 6q, 16q, 17p e 17q (HOANG et al., 2001) também são compartilhados pelos tumores de mama e glândula salivar. Sobre a presença de RE em glândula salivar humana, permanece a controvérsia sobre sua expressão e significado biológico (JEANNON et al., 1999; DORI et al., 2000; LEIMOLA-VIRTANEN et al., 2000; KUMAGAMI & ONITSUKA, 2001; BJORLING et al., 2002).

É válido ressaltar que, como na mama humana, o avanço da idade diminui o volume do parênquima das glândulas salivares, principalmente devido à perda de tecido acinar e sua substituição por tecido fibroso e adiposo (SCOTT, J. 1977a e b).

I.3. Tumores Mamários Caninos *versus* Tumores de Glândula Salivar Humanos

Os tumores mistos, lesões incomuns na mama humana, são os mais freqüentes na mama canina (CASSALI, 2000). Esta, certamente, é uma das importantes diferenças verificadas entre os tumores mamários do homem e do cão, mas é também uma característica semelhante ao dos tumores de glândula salivar.

Os tumores mistos caninos são compostos de uma mistura de células epiteliais e mioepiteliais dispersas em um estroma “mesenquimatoso” (ZHUANG et al., 1997; SANTOS, 1979), como visto na glândula salivar humana. Os tumores mistos malignos são menos freqüentes que os benignos e, como na glândula salivar humana, aparecem mais

tardiamente (SANTOS, 1979). Para MOULTON et al. (1990) alguns tumores mistos diagnosticados histologicamente como benignos podem ser potencialmente malignos. Se tiverem tempo suficiente para crescimento, os tumores mistos caninos podem sofrer transformação maligna como a que acontece com os AP humanos, numa relação direta com o tempo de evolução da doença (GNEPP, 1993).

A diversidade histológica vista nos tumores mistos caninos correspondem às mesmas observadas nos tumores mistos da glândula salivar humana. As células epiteliais e mioepiteliais perdem um pouco da arquitetura ductal-lobular, ficando dispersas em um estroma caracteristicamente mixóide, condróide ou ósseo (HAMPE & MISDORP, 1974; MOULTON, 1990). Os estudos realizados para determinação da histogênese das células tumorais vêm demonstrando que, provavelmente, estas possuam origem em uma mesma célula-mãe, dita "intermediária", geneticamente instável, a qual assumiria expressão fenotípica comum aos dois tipos, epitelial e miopitelial, e que as células mioepiteliais seriam responsáveis pela formação de tecidos "estromais" (PELETEIRO, 1994). Essa hipótese, também levantada sobre a origem dos tumores mistos da glândula salivar humana (DARDICK et al, 1982; KUMAR et al, 2005), foi reforçada por GÄRTNER et al. (1999) em um estudo usando imuno-histoquímica e análise de DNA por citometria estática. Por algum mecanismo ainda desconhecido, uma célula totipotente com capacidade de diferenciação divergente sofre transformação metaplásica, resultando na aparência histológica heterogênea desses tumores.

Tais evidências nos levaram a perguntar se a glândula mamária canina não poderia também ser utilizada como o modelo comparativo para o estudo da carcinogênese nas glândulas salivares, como proposto para os tumores de mama humana. Para testar essa hipótese fez-se necessária a avaliação de critérios significativos, tais como, aspectos

clínicos, morfologia microscópica e expressão de antígenos relevantes para definição histogênica nesses dois grupos de lesões.

II. RECONHECIMENTO DO FENÓTIPO TRANSFORMADO E DOS COMPONENTES TUMORAIS ATRAVÉS DE MARCADORES MOLECULARES

O uso de marcadores moleculares que possam auxiliar na identificação dos componentes tumorais, compreensão da histogênese e dos processos envolvidos na carcinogênese é um importante instrumento da patologia humana e veterinária. A classificação precisa de muitas neoplasias depende essencialmente do encontro de marcadores antigênicos, só identificáveis na prática, pelas reações com seus anticorpos específicos (BRASILEIRO-FILHO, 2004; CASSALI et al, 1999a; CASSALI, 2000).

A utilização da técnica de imuno-histoquímica teve enorme impacto no diagnóstico dos tumores tanto da área humana quanto animal. Vários anticorpos já foram empregados no diagnóstico histogenético de neoplasias morfologicamente indiferenciadas, na caracterização da origem e tipo das células constituintes tumorais, na discriminação entre natureza “benigna” ou “maligna” de proliferações celulares e na avaliação prognóstica de determinados tumores (LEONG & WRIIGHT, 1987; ALVES et al, 1999; CASSALI et al, 1999b).

Um painel utilizando principalmente marcadores mesenquimais, epiteliais e mioepiteliais parece ser efetivo para distinguir os diferentes tipos de tumores de glândula salivar, cujo diagnóstico histomorfológico é, sabidamente, um desafio para o patologista (ARAÚJO et al., 2000). O mesmo pode-se afirmar para os tumores animais. Vários autores já demonstraram a reatividade de anticorpos monoclonais humanos para diversos antígenos

das glândulas mamárias caninas, estendendo o potencial da imuno-histoquímica também para a patologia veterinária (MOTTOLESE et al, 1994, FERNANDES, 1996; CASSALI, 2000).

II.1. Marcadores Mesenquimais

A vimentina é o mais ubíquo dos filamentos intermediários do citoesqueleto e se expressa amplamente desde o início do desenvolvimento embrionário dos mamíferos, podendo ser detectada tanto em tecidos de origem ectodérmica quanto mesodérmica. Entretanto, sua expressão vai progressivamente se restringindo a alguns tipos celulares. O alto grau de insolubilidade da vimentina sugere a sua função estrutural no citoplasma (MACHADO & FIGUEIREDO, 1996). A vimentina é típica das células mesenquimais, como fibroblastos, células endoteliais, linfócitos, macrófagos e outras células derivadas do mesoderma, ainda que algumas de maneira mais escassa (ALVES et al, 1999) e, até o aparecimento de marcadores mais específicos e sensíveis, era utilizado como marcador clássico de células mioepiteliais.

Nas células mioepiteliais tanto das glândulas salivares humanas quanto nas glândulas mamárias caninas, vimentina é co-expressada com algumas citoqueratinas (CASSALI, 2000). A vimentina está presente nas neoplasias originadas das células mesenquimais, mas muitos carcinomas humanos podem apresentar positividade, principalmente aqueles com componente mioepitelial (DUPREY & PAULIN, 1995). Uma possível explicação para o fato das células mioepiteliais expressarem vimentina seria a “reversão” para um tipo celular mais primitivo ou embrionário (FERNANDES, 1996).

II.3. Marcadores Epiteliais

As citoqueratinas, proteínas constituintes dos filamentos intermediários que integram o citoesqueleto, são marcadores epiteliais de eleição em mamíferos. Em humanos são conhecidas pelo menos 20 citoqueratinas cuja expressão varia de acordo com o tipo de célula, período de desenvolvimento embrionário, grau de diferenciação e microambiente de crescimento celular, o que permite considerá-las como marcadores de diferenciação. São utilizadas na caracterização dos carcinomas de origem glandular ou escamosa, bem como na tentativa de indicar um sítio primário para tumores metastáticos. As células mioepiteliais também expressam citoqueratinas, mas em menor quantidade que as epiteliais (ALVES et al., 1999). O anticorpo clone AE1/AE3 reage com um *pool* de citoqueratinas humanas (1-8, 10, 14-16 e 19) e outras caninas (MARGARITESCU et al., 1999-2004; FERNANDES, 1996). Este é o preparado mais amplamente usado na demonstração da natureza epitelial de tumores morfologicamente indiferenciados (ALVES et al., 1999). Outras citoqueratinas específicas podem auxiliar também o diagnóstico diferencial de carcinomas primários e metástases carcinomatosas (ARAÚJO et al., 2000; NIKITAKIS et al., 2004).

II.4. Marcadores Mioepiteliais

O mioepitélio neoplásico desempenha um papel fundamental na histogênese e nos processos morfogenéticos responsáveis pela variável aparência histológica dos tumores mistos (AP e Ca ex-AP) de glândulas salivares humanas (DARDICK et al., 1982; ZARBO et al., 2000; SAVERA & ZARBO, 2004). BATSAKIS et al., (1983) chegaram a afirmar que os tumores mistos não ocorrem em tecidos nos quais as células mioepiteliais estejam ausentes. Além disso, a camada de células mioepiteliais parece ter uma grande significância como inibidor parácrino da progressão tumoral (STERNLICHT et al., 1997;

LAKHANI & O'HARE, 2000). A identificação das células mioepiteliais é de particular valor diagnóstico nos tumores de mama humanos, pois elas são retidas na maioria das lesões benignas e perdidas no processo de malignização e invasão (YAZIGI et al., 2000; BARBARESCHI et al., 2001; KALOF et al., 2004). É de se esperar um comportamento semelhante nos tumores de glândula salivar e nos tumores mamários caninos, dadas as já comentadas semelhanças biológicas entre estes grupos de neoplasias.

As células mioepiteliais, como o próprio nome indica, exibem características de ambas as células epiteliais e da musculatura lisa, além de que, sua heterogeneidade citomorfológica (células claras, epitelióides, alongadas, plasmocitóides) dificulta sua correta distinção (SIMPSON, 2002). Essa heterogeneidade se dá, provavelmente, em decorrência de diferentes estágios de diferenciação (ARAÚJO & RAITZ, 2004). Existem três tipos de marcadores de células mioepiteliais. O primeiro tipo inclui marcadores de proteínas de músculo liso tais com como α-SMA (α-*smooth muscle actin*), SMMHC (*smooth muscle myosin-heavy chain*), h-caldesmona e calponina. O segundo tipo é expresso também nas células epiteliais ductais e inclui citoqueratinas 14, 5 e 17, α1-β1 integrina e metalotioneína. Vimentina, CD10 e S-100 estão no terceiro grupo, cujas proteínas são expressas também nas células mesenquimais (SAVERA et al., 1997; FOSCHINI et al., 2000; KALOF et al., 2004; FURUSE et al., 2005). Como visto, nenhum marcador de células mioepiteliais exibe perfeita sensibilidade e especificidade, sendo recomendável que uma combinação de marcadores seja usada em casos de dificuldades de identificação (KALOF et al., 2004).

Investigações recentes mostraram que a proteína p63, além de essencial na manutenção da população de células precursoras (*stem cells*) em vários tecidos epiteliais, é um excelente marcador imuno-histoquímico de células mioepiteliais tanto nos tecidos glandulares humanos quanto caninos (REIS-FILHO & SCHMITT, 2002; WEBER et al.,

2002; BILAL et al., 2003; GAMA et al, 2003; EDWARDS et al., 2004). p63 demonstra sensibilidade comparável à de outros marcadores mioepiteliais como α-SMA, SMMHC e calponina, mas uma maior especificidade: as células estromais tais como os miofibroblastos, as células neurais e as células endoteliais são consistentemente negativas (BARBARESCHI et al., 2001; WERLING et al., 2002).

A transcrição do gene para p63 se dá a partir de dois promotores distintos e *splicings* alternativos responsáveis por duas classes de proteínas que, a despeito de sua homologia estrutural, possuem funções aparentemente distintas e, pelo menos, seis isoformas. Três contêm um domínio de transcrição N-terminal, semelhante à p53. Supõe-se que essa classe transativadora (TA) tenha a habilidade de ativar o gene *TP*53 induzindo apoptose e regulando o ciclo celular, agindo como um gene supressor de tumor não clássico. As outras três isoformas não possuem o domínio NH_2^- terminal sendo denominadas ΔN. Essas agiriam através de mecanismos alternativos driblando o controle do ciclo celular e apoptose em oposição à via TAp63/p53. Os domínios COOH distintos (α, β e γ) possuem papéis pobremente conhecidos (YANG et al., 1998; LITTLE & JOCHEMSEN, 2002).

Algumas evidências de que as isoformas ΔN-p63 se associavam às moléculas de β-catenina, do complexo de adesão celular E-caderina/β-catenina no processo da carcinogênese *in vitro* foram descritas por PATTURAJAN et al. (2002) No entanto, essa hipótese não se confirmou quando foram realizados estudos com neoplasias humanas (KOGA et al., 2003; REIS-FILHO et al., 2003).

II.5. Complexo E-caderina/β-catenina

Um dos primeiros processos que levam um tumor à invasão e metástase é a perda de adesão celular. Este fato é atribuído a uma desregulação de um ou mais genes e seus produtos, responsáveis pela adesão intercelular, dentre eles as caderinas e cateninas (SHIOZAKI et al., 1996). A E-caderina é a principal molécula de adesão do epitélio. Através de seu domínio extra-citoplasmático, é responsável pela adesão homotípica, em presença de cálcio, com outra molécula de E-caderina da célula vizinha. Através do seu domínio citoplasmático, interage diretamente com a β-catenina (HARRINGTON & SYRIGOS, 2000).

O complexo E-caderina/β-catenina está intimamente envolvido no controle da diferenciação morfológica (organogênese) e proliferação celular durante o desenvolvimento, exercendo função primordial na organização e manutenção das estruturas tissulares (HARRINGTON & SYRIGOS, 2000). Quando a β-catenina não participa do complexo de adesão, a molécula livre é seqüestrada por um complexo formado pelos produtos do gene *APC* (*adenomatous polyposis coli*), pela molécula GSK-3β (*serine-threonine glycogen synthetase kinase-3β*) e por uma proteína adaptadora, inabilitando a fosforilação e degradação da β-catenina pelo sistema proteolítico celular (JANKOWISKI et al., 1997). Dessa maneira β-catenina também participa da transdução de sinais, pela via Wnt (*Wingless*) (JANKOWSKI et al., 1997; SHIEH et al., 2003). Aumento nas concentrações de β-catenina livre no citoplasma estimula sua ligação com proteínas tais como LEF (*lynfocyte enhancer factor*) e Tcf (*T-cell factor*). Estes complexos dirigem-se para o núcleo e agem como co-fatores de transcrição de genes relacionados à proliferação celular (JANKOWISKI et al., 1997).

Pouco se conhece sobre a participação do complexo E-caderina/β-catenina nos tumores de glândula salivar, mas existem fortes evidências de que esteja associado ao fenótipo agressivo de muitos desses tumores (ZHANG et al., 2000). Baixos níveis de expressão E-caderina e β-catenina foram correlacionado positivamente com a desdiferenciação das células tumorais (ECONOMOPOULOU et al., 2000).

Nos tumores mamários caninos a redução da expressão de E-caderina e sua distribuição anormal estão relacionadas ao baixo grau de diferenciação desses tumores e diminuição do tempo de sobrevida (REIS et al, 2003; RESTUCCI et al, 1992; GOMES, 2004).

Vários fatores podem modular a interação do complexo E-caderina/β-catenina levando a uma redistribuição da β-catenina. Dessa maneira, pode-se dizer que a β-catenina é uma proteína com dupla função, a qual é determinada por sua localização na membrana ou no núcleo. Na membrana, representa um importante papel na adesão célula-célula. β-catenina nuclear aumenta a transcrição de genes ligados à proliferação celular tumoral.

II.6. Receptor de Estrógeno

Células possuidoras de receptores de estrógeno (RE) e sensíveis à sua ação podem induzir a secreção de fatores mitogênicos e reguladores de crescimento e estimular a produção de proteases, as quais degradam matriz extracelular e estimulam invasão e metástase (MILLER, 1990). As células mioepiteliais, tanto das glândulas salivares quanto das mamárias, humanas e caninas, respondem a estímulos hormonais para expelir saliva e leite (HAYWARD et al., 1996). Além de que, o *status* hormonal para RE em alguns tumores de mama humanos, tem um importante significado prognóstico e terapêutico (GOBBI et al, 2005).

Também nos tumores mamários caninos, a presença de RE é indicativa da dependência hormonal dessas neoplasias (HAMILTON et al, 1977), havendo correlação com as características patológicas e comportamento biológico (MAC EWEN et al, 1982).

As glândulas mamária e salivar humanas compartilham as mesmas estruturas ducto-acinares básicas e uma co-existência de carcinomas em ambos os sítios tem sido reportada (BERG et al., 1968; DUNN et al., 1972; PRIOR & WATERHOUSE, 1977; ABBEY et al., 1984; MILLER et al., 1994). Assim, vários estudos procuraram estabelecer uma possível associação entre a presença de RE nas glândulas salivares e o desenvolvimento de tumores.

Poucos trabalhos foram capazes de demonstrar a presença de RE em glândulas salivares. Dentre estes podemos citar um estudo bioquímico (DIMERY, 1987), dois ensaios imuno-histoquímicos (JEANNON et al., 1999; GLAS et al., 2002) e outro, utilizando RT-PCR (*Reverse Transcriptase - Polymerase Chain Reaction*). LEIMOLA-VIRTANEN et al. (2000) conseguiram mostrar atividade transcricional do gene RE através da presença abundante de RNAm. Entretanto, a imuno-histoquímica pode deixar de detectar RE devido a múltiplas razões tais como: eficiência relativa na tradução da proteína, processamento pós-traducional, flutuações na estabilidade protéica e sua degradação, expressão em níveis muito baixos ou por dificuldades no reconhecimento de epitopos (LAMEY et al., 1987; MILLER et al., 1994; SHICK et al., 1995; GAFFNEY et al., 1995; DORI et al., 2000; NASSER et al., 2003).

Assim, os critérios histomorfológicos de diagnóstico, quando associados a biomarcadores como proteínas reguladoras do ciclo celular, moléculas de adesão e receptores hormonais podem fornecer novos *insights* sobre a histogênese, o comportamento biológico e prognóstico dos tumores em estudo e, em última instância, direcionar condutas terapêuticas.

OBJETIVOS

Considerando a importância da patologia comparada utilizando modelos animais, propomos abordar o tema estabelecendo os seguintes objetivos:

OBJETIVO GERAL

- Analisar os aspectos histomorfológicos e imuno-histoquímicos dos tumores mamários caninos (tumores mistos e carcinomas metaplásicos) e compará-los aos tumores de glândula salivar da espécie humana (adenoma pleomórfico e carcinoma ex-adenoma pleomórfico) com a finalidade de averiguar seu valor como modelo de estudo comparativo.

OBJETIVOS ESPECÍFICOS

- Caracterizar imuno-histoquimicamente os elementos epiteliais e mesenquimais dos tumores mistos da mama canina (tumores mistos benignos e carcinomas metaplásicos) e dos tumores mistos de glândula salivar humana (adenoma pleomórfico e carcinoma ex-adenoma pleomórfico) através da expressão dos seguintes marcadores: AE1-AE-3, vimentina, p63, β-catenina, E-caderina e receptor de estrógeno.

- Comparar os aspectos histomorfológicos e o perfil imuno-histoquímico dos tumores mamários caninos (tumores mistos e carcinomas metaplásicos) com os tumores de glândula salivar humana (adenoma pleomórfico e carcinoma ex-adenoma pleomórfico) a fim de melhor compreender a histogênese e comportamento biológico dos mesmos.

MATERIAIS E MÉTODOS

1. Caracterização das Amostras

As amostras incluídas em parafina dos tumores mamários de cães sem raça definida foram obtidas nos arquivos do Laboratório de Patologia Comparada do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais. Na análise das amostras caninas foi empregada a classificação histológica para mama humana (ROSEN & OBERMAN, 1993) para efeito de comparação. As amostras de tumores mistos de glândula salivar humana foram obtidas nos departamentos de Anatomia Patológica da Faculdade de Medicina da UFMG, Centro de tratamento e Pesquisa Hospital do Câncer A. C. Camargo e Instituto Nacional do Câncer (INCA). O critério de seleção foi baseado no diagnóstico histopatológico das amostras com exame clínico conhecido. Estas amostras foram revisadas independentemente por dois observadores e os tumores humanos foram classificados e subtipados de acordo com a morfologia histológica (SEIFERT & SOBIN, 1991). Os dados clínicos foram obtidos dos arquivos médicos e veterinários e incluíram idade, gênero e glândula salivar ou mamária afetada.

As amostras foram caracterizadas clinicamente segundo a idade de aparecimento dos tumores em ambas as espécies e de acordo com o tamanho da glândula acometida. As idades dos animais foram convertidas utilizando-se a tabela de Lebeau (1953). De acordo com essa tabela, os primeiros 2 anos de vida do cão correspondem aos primeiros 24 anos de vida na espécie humana. Após o segundo ano de vida no animal, cada ano corresponde a 4 anos na espécie humana.

Para efeito de comparação, os cincos pares de mama de cadela foram classificados de acordo com seu tamanho e relacionados às glândulas salivares de acordo com a tabela 1.

Categoria	Glândulas salivares da espécie humana	Glândulas mamárias caninas
Classe 1 (menores)	Sublingual e glândulas menores	Torácica-cranial (T1) Torácica-caudal (T2)
Classe 2 (intermediárias)	Submandibular	Abdominal-cranial (A1) Abdominal-caudal (A2)
Classe 3 (maiores)	Parótida	Inguinal (I)

Tabela 1 – Classificação comparativa entre as glândulas salivares humanas e as glândulas mamárias caninas, segundo o tamanho das mesmas.

2. Ensaio imuno-histoquímico

Após a revisão dos casos, foram selecionadas 10 amostras de cada uma das entidades patológicas a serem estudadas para o ensaio imuno-histoquímico, a saber: 10 tumores mistos de mama da cadela, 10 carcinomas metaplásicos de mama da cadela, 10 adenomas pleomórficos, 10 carcinomas ex-adenoma pleomórfico. Na seleção do material foram priorizadas as amostras com exame clínico conhecido. Os anticorpos foram utilizados em secções de 4μm de tecido mamário canino normal e neoplásico e tecido de glândula salivar normal e neoplásico incluídos em parafina. Foi utilizada a técnica de

estreptavidina-biotina-peroxidase com recuperação antigênica pelo calor úmido (banho-maria) empregando-se solução recuperadora própria. As lâminas foram incubadas por 60 minutos com cada um dos anticorpos primários listados na tabela 2 e por 10 minutos para as demais etapas como: bloqueio da peroxidase endógena, bloqueio de anticorpos teciduais, incubação do anticorpo secundário, conjugação com estreptavidina-biotina-peroxidase, revelação da reação com diaminobenzidina (DAB) e contra-coloração com hematoxilina de Mayer. Como controles positivos foram usadas amostras de neoplasias de mama humana previamente testadas e os controles negativos foram obtidos pela omissão do anticorpo primário e substituição por água destilada.

Anticorpo	**Característica**	**Clone**	**Fonte**	**Diluição**
Anti-pan-citoqueratina	Monoclonal	NCL-AE1/AE3	Novocastra	1:100
Anti-vimentina	Monoclonal	V9	DAKO	1:50
Anti-p63	Monoclonal	4A4	Santa Cruz	1:100
Anti-β-catenina	Monoclonal	E-5	Santa Cruz	1:400
Anti-E-caderina	Monoclonal	4A2C7	Zymed	1:40
Anti-receptor de estrógeno	Monoclonal	CC4-5	Novocastra	1:50

Tabela 2 – Anticorpos primários, fontes e diluições (mg/ml) utilizados nos ensaios imuno-histoquímicos.

A expressão dos marcadores foi analisada nos diferentes componentes dos tumores. Para avaliação qualitativa e semiquantitativa, foram empregados critérios morfológicos e pontos de corte utilizados em estudos prévios sobre tais marcadores: 10% para citoqueratinas e vimentina, (ARAÚJO et al, 2000; GRAHAM et al, 1999) β-catenina e E-caderina (SHIEH et al, 2003) e receptor de estrógeno (DORI et al, 2000); para o antígeno p63 o ponto de corte utilizado foi de 5% (REIS-FILHO et al, 2002b).

3. Análise estatística

Foram realizados testes estatísticos apropriados a cada um dos estudos relatados nos resultados como artigos.

4. Ética

O projeto de pesquisa para a realização do presente trabalho foi submetido ao Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais (COEP/UFMG) que emitiu parecer favorável à sua execução: ETIC 193/04 (**Anexo 1**).

RESULTADOS

Os resultados, obtidos a partir das amostras de tumores animais e humanos e dos ensaios realizados para atingir os objetivos propostos, serão apresentados de forma alternativa, como artigos científicos submetidos a periódicos internacionais e nacionais Qualis A.

Cada artigo está estruturado com base nas normas do periódico onde foi publicado. À exceção do primeiro artigo, uma revisão bibliográfica estruturada conforme as normas do Jornal Brasileiro de Patologia e Medicina Laboratorial (ISSN: 1676 2444), todos os outros possuem introdução, objetivos, materiais e métodos, resultados, discussão, conclusão e numeração de páginas próprios.

Após a apresentação desses artigos, seguir-se-á uma conclusão geral, que atende aos objetivos propostos no início deste trabalho, considerações finais e referências bibliográficas referentes à introdução geral.

Artigo 1 – Adenoma pleomórfico e carcinoma ex-adenoma pleomórfico de glândulas salivares – Revisão da literatura

ADENOMA PLEOMÓRFICO E CARCINOMA EX-ADENOMA PLEOMÓRFICO DE GLÂNDULAS SALIVARES – REVISÃO DA LITERATURA

Pleomorphic adenoma and carcinoma ex-pleomorphic adenoma of salivary glands. A review

Marisa Genelhu[1] Helenice Gobbi[2]; Geovanni Dantas Cassali[3]

1. Mestre e doutoranda em Patologia pela Faculdade de Medicina da Universidade Federal de Minas Gerais (UFMG); professora adjunta e pesquisadora do Núcleo de Pesquisa em Imunologia da Universidade Vale do Rio Doce (UNIVALE).
2. Professora adjunta e doutora do Departamento de Anatomia Patológica e Medicina Legal da Faculdade de Medicina da UFMG.
3. Professor doutor do Laboratório de Patologia Comparada do Instituto de Ciências Biológicas da UFMG.

Esta revisão é parte da tese de doutorado “Tumores mistos e carcinomas metaplásicos da glândula mamária canina: aspectos comparativos com tumores de glândula salivar da espécie humana”, desenvolvida no Laboratório de Patologia Comparada, ICB/UFMG.
Apoio: Coordenação para Aperfeiçoamento de Pessoal de Nível Superior através do Programa de Intercâmbio Internacional Brasil/Portugal (CAPES/GRICES 112/04) e Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG).

Resumo

A despeito dos inúmeros trabalhos na literatura com relação aos adenomas pleomórficos e sua contraparte maligna, os carcinomas ex-adenomas pleomórficos de glândula salivar, a grande maioria é representada por relatos de caso, devido à raridade do tumor maligno, suas peculiaridades histomorfológicas e controvérsias sobre histogênese, mecanismos de transformação maligna e metástases. O presente trabalho tem por objetivo reunir as informações mais importantes já descritas a respeito dessas lesões, de um modo sistemático e conciso a fim de facilitar a compreensão global dessas intrigantes neoplasias.

Unitermos: carcinoma ex-adenoma pleomórfico, neoplasias de glândulas salivares, revisão

Abstract

Despite several works in the literature with regard to pleomorphic adenomas (PA) and their malign counterpart, salivary gland's carcinoma ex pleomorphic adenomas (Ca ex-PA), most of them are represented by case reports, given how rare malign tumors are, their histomorphological peculiarities and controversies on histogenesis, malign transformation and metastasis mechanisms. The present work has the purpose of gathering the most important pieces of information about lesions, in a systematic and concise way to make it easy to understand these intriguing neoplasias.

Key Words: carcinoma ex-pleomorphic adenoma, salivary gland tumors, review

Introdução - Aspectos Gerais dos Tumores de Glândula Salivar

As glândulas salivares humanas possuem uma morfologia normal relativamente simples. Entretanto, originam uma variedade surpreendente de tumores malignos e benignos, sendo que mais de 90% desses são de origem epitelial.[49] Essa variedade tem sido identificada, categorizada e dinamicamente modificada numa grande diversidade de tipos histológicos que dificultam a avaliação de casos antigos.[39] De modo geral esses neoplasmas são relativamente incomuns (representam menos de 2-3% de todos os tumores e 7% dos tumores de cabeça e pescoço),[36] mas a despeito da baixa incidência, assumem substancial importância. Apesar de a grande maioria ser de natureza benigna, quando malignos apresentam um variado comportamento biológico. Conseqüentemente, muitos destes tumores representam um desafio diagnóstico e terapêutico, como é o caso dos tumores mistos.

O Tumor Misto ou Adenoma Pleomórfico

Os tumores mistos benignos ou adenomas pleomórficos (AP) são os mais freqüentes tumores das glândulas salivares humanas (cerca de 90%). Ocorrem mais comumente na parótida (85%), seguida pelas glândulas submandibular (10%), sublingual e menores (5%); acometem adultos jovens (4ª-5ª décadas), com leve predileção pelo gênero feminino,[88, 33]. Clinicamente, é uma lesão de crescimento lento, aparentemente bem delimitada, que raramente excede 6 cm em seu maior diâmetro. Causa aumento de volume indolor e pode ser facilmente palpável como uma massa discreta.

Histologicamente, os AP caracterizam-se pela notável diversidade histológica: uma mistura de células epiteliais ductais e mioepiteliais dispersas em um estroma com variados graus de tecido mixóide, hialino, condróide, ósseo e até mesmo, adiposo. [74, 42, 94]

A Histogênese

O fato de os AP se originarem em glândulas localizadas em uma zona transicional ontogenética, uma região onde o endoderma e ectoderma se encontram, poderia ser uma das razões para sua freqüente variabilidade histopatológica.[88, 92] Entretanto, a maioria dos autores sustenta a hipótese de que, o que representa o princípio básico da heterogeneidade tecidual desses tumores seja a "estromatização" e transformação do epitélio em mesênquima.[68, 62, 49] Assim, mesmo que o tumor tenha freqüentemente um componente "estromal", com aparência mesenquimatosa, não é verdadeiramente misto, pois não deriva de mais de uma camada germinativa. [62] As mudanças "estromais" características, seriam produzidas por células mioepiteliais com propriedades multipotenciais ou epiteliais progenitoras dos ductos basais que, embora imunofenotipicamente diversas, compartilhariam as mesmas alterações citogenéticas.[14, 17] Ou seja, as células neoplásicas parecem possuir natureza clonal, com uma única célula de origem.[26]

A presença de tecidos mixóide, hialino, condróide e ósseo, foi associada à superexpressão de algumas proteínas pelas células neoplásicas tais como, glicosaminoglicanos e agregan,[98] BMPs (*bone morphogenetics proteins*),[55, 99, 50] ChM-1 (*cartilage-specific matrix chondromodulin-1*),[50] CD-RAP (*cartilage-derived retinoic acid-sensitive protein*),[19] lumican[51] e tenascina.[30] Um achado ocasional é a presença de metaplasia epitelial em elementos escamosos, sebáceos ou oncocíticos.[68]

A Recorrência

A excisão cirúrgica com margens adequadas de ressecção é o tratamento indicado para se evitar recorrências que ocorrem em cerca de 10% dos casos. A origem da recorrência dos AP após tratamento cirúrgico é discutível. Além do difícil manejo cirúrgico devido ao risco de agressão ao nervo facial e/ou profundidade do tumor,[11] a

cápsula dos AP parece não ser completamente desenvolvida.[81] O crescimento expansivo produz projeções digitiformes, o que torna difícil a enucleação do tumor e facilita a ocorrência de recidivas.[25, 40]

A atividade proliferativa avaliada através da expressão imuno-histoquímica de PCNA e Ki-67 é marcadamente mais alta no componente epitelial de AP recorrentes quando comparada à dos tumores não recorrentes, o que sugere que o componente epitelial seja a origem provável das recorrências.[59]

Com relação ao conteúdo nuclear, os AP com evolução clínica de menos de um ano geralmente possuem uma população de células diplóide. Em contraste, os tumores com mais de cinco anos de evolução mostram uma população celular aneuplóide, semelhante à dos carcinomas.[24, 56] Criscuolo *et al.*[12] encontraram uma significância prognóstica para a expressão de AgNORs (*silver-stained nucleolar organizer regions*) em AP recorrentes e Hamada *et al.*[38] atribuíram ao tamanho do tumor (mais que 3 cm) e à expressão de mucinas (MUC1) um fator de risco para a recorrência de AP.

Múltiplas recidivas locais associadas à incompleta excisão cirúrgica e/ou longo tempo de evolução do tumor estão diretamente relacionados à transformação maligna do AP.[60, 66, 52]

O Tumor Misto Maligno ou Carcinoma ex-adenoma Pleomórfico

Relatado pela primeira vez no início dos anos 50,[32] o desenvolvimento de um novo tumor com propriedades malignas a partir de um AP benigno pré-existente foi denominado tumor misto maligno, carcinoma em tumor misto ou carcinoma ex-adenoma pleomórfico (Ca ex-AP).[7, 25] A freqüência com que esse evento de transformação maligna acontece varia em diferentes séries de 2% a 25%.[85, 70]

O Ca ex-AP é um tumor raro, agressivo, tipicamente de alto grau que, em geral, acomete pacientes acima da 5ª década de vida. A média de idade dos pacientes com este tumor é em torno de 15 a 20 anos acima da dos pacientes com AP.[54, 85, 68, 62] Usualmente, ocorre em glândulas salivares maiores (ao contrário da maioria dos outros tumores malignos de glândula salivar) devido à associação ou recorrência do tumor benigno (AP).[53, 65] Ao exame macroscópico, as lesões são firmes, com evidências de infiltração, necrose ou hemorragia, dependendo da proporção entre os elementos benigno e maligno. Nem sempre a porção benigna pode ser identificada. Ao exame microscópico mostram-se como neoplasias infiltrativas, às vezes com alta celularidade, invasão de parênquima glandular e extensão para estruturas adjacentes. As células podem formar arranjos sólidos eosinofílicos, com núcleos pleomórficos e hipercromáticos, cromatina disposta irregularmente e nucléolos proeminentes.[54, 28] É freqüente o envolvimento perineural, invasão vascular, áreas de necrose e mitoses atípicas.[53]

A Transformação Maligna

Além do fato de o risco de malignização aumentar com a duração do tumor e o número de recorrências, outros fatores patológicos de valor preditivo para AP são pobremente definidos. Os principais achados clínicos no diagnóstico inicial correlacionados à transformação maligna são idade avançada do paciente e maior tamanho do tumor.[5] Análise retrospectiva de parâmetros histológicos (angiogênese e angiotropismo, hialinização, hipercelularidade, necrose, anaplasia celular, maior atividade mitótica) não são consenso na literatura como critérios preditivos confiáveis para transformação maligna e metastatização de AP.[93, 5, 59, 64]

Alguns fatores genéticos já foram associados à malignização do AP. Avaliações citogenéticas demonstraram que a maioria das alterações são translocações e perda de

heterozigose que se dão nos cromossomos 8 e 12. O gene alvo no cromossomo 8 é o *PLAG*1 (8q12) e no cromossomo 12 é o *HMGIC* (12q15).[22, 4, 17, 70, 57, 67] A mais comum alteração encontrada foi t(3;8)(p21;q12),[89, 90] uma translocação capaz de interferir também na transcrição do gene *CTNNB*1 (*catenin cadherin-associated protein β1*).[44, 26] Outras anormalidades recorrentes que podem ser importantes são: aquisição de cópias extras do cromossomo 7, deleções nos cromossomos 5 e 17[8] e alterações no cromossomo 6q.[61] Mutações e amplificações de genes relacionados ao controle do ciclo celular tais como, *RB*, *K-ras*, *c-myc*, p21,[18, 95, 27] p16,[83] p105,[56] mdm2[3, 70] e, principalmente *TP*53[69, 95, 96, 3, 64] têm sido encontradas em Ca ex-AP. A despeito dos inúmeros estudos sobre a expressão imuno-histoquímica da proteína p53 mutada em tumores de glândula salivar, ainda não há consenso sobre o significado dessa expressão na discriminação do comportamento biológico nessas lesões.[79]

Mutação no gene c-*erb*-B2 e superexpressão da proteína receptora para fator de crescimento epitelial (EGFR-2) ou HER-2/neu também está implicada na iniciação e progressão tumoral e associada a um pior prognóstico em carcinomas de ducto salivar (como nos tumores de mama), o que poderia sugerir que os primeiros também pudessem se beneficiar de imunoterapia com o transtuzumab.[78] Com relação ao Ca ex-AP, o que se sabe é que este expressa HER-2/neu e AP não.[31, 71] Entretanto, os poucos estudos de avaliação não foram conclusivos a respeito da utilização da imunoterapia para CA ex-AP.[36, 21].

A diminuição na expressão de proteínas constituintes da membrana basal, tais como laminina e colágeno tipo IV,[29] proteínas da matriz extracelular como a tenascina,[30] de moléculas de adesão tais como N-CAM[72] e as do complexo E-caderina-β-catenina[76] podem também estar relacionadas aos mecanismos de malignização do AP.

O Diagnóstico

Como visto, após o surgimento do tumor benigno e sua evolução com o tempo, as células podem sofrer transformação maligna sob ação de vários fatores. É possível que, mesmo os Ca ex-AP diagnosticados como *de novo*[15] possam se originar de tumores benignos muito pequenos, profundos e clinicamente indetectáveis. Ou seja, mesmo quando os tumores primários benignos não são diagnosticados previamente, estes poderiam, em hipótese, já estar presentes, fazendo com que uma pergunta perdure até hoje: no caso de diagnóstico de Ca ex-AP, o tumor primário era realmente benigno ou o carcinoma já existia e não foi detectado?[53, 65]

Para minimizar essa dúvida, um diagnóstico acurado de Ca ex-AP requer a presença de evidência histológica do tumor benigno em associação com o tumor maligno e/ou a história clínica de recorrências no mesmo sítio anatômico.[23, 84] A punção aspirativa por agulha fina (PAAF) é um procedimento diagnóstico bastante usado no pré-operatório de AP, mas o Ca ex-AP dificilmente é identificado por ele.[46, 2, 87] O exame anátomo-patológico é obrigatório. FOOTE & FRAZELL[32] estabeleceram que 100 cortes podem ser necessários para se encontrar um pequeno carcinoma em um AP.

A malignidade dos Ca ex-AP está relacionada à extensão da invasão, à infiltração de estruturas subjacentes[28] e ao subtipo histológico[86] Incorreções no diagnóstico são comuns por causa do reduzido resíduo ou ausência do tumor misto benigno e a variedade de subtipos apresentados[65]. O Ca ex-AP pode manifestar um amplo e diferente espectro de malignidades fenotípicas (**Tabela 1**) e a identificação do subtipo histológico é determinante importante para compreensão do comportamento biológico e sua implicação no prognóstico.[86] Além disso, esses subtipos podem ser agrupados em dois grupos: Ca ex-AP com diferenciação apenas epitelial (75% dos casos) e Ca ex-AP com um componente mioepitelial (25%).[1]

Tabela 1 – Exemplos de subtipos histológicos encontrados em carcinomas ex-adenomas pleomórficos (Ca ex-AP) e respectivas referências na literatura.

SUBTIPOS DE Ca ex-AP	REFERÊNCIA BIBLIOGRÀFICA
Carcinoma adenóide cístico	93, 65
Carcinoma mucoepidermóide	48, 65
Carcinoma de ducto salivar	31, 2, 65
Adenocarcinoma polimorfo de baixo grau	16
Carcinoma de células escamosas	46
Mioepitelioma maligno	97
Adenocarcinoma NOS (*not otherwise specified*)	65
Carcinoma epitelial-mioepitelial	65
Carcinoma mioepitelial	65, 77
Carcinoma indiferenciado	65, 37
Carcinoma Sebáceo	10
Carcinoma sarcomatóide	65, 86
Carcinossarcoma verdadeiro	39
Histiocitoma fibroso maligno	62
Rabdomiossarcoma	35
Neurossarcoma	45
Oncocitoma	58

As Categorias de Ca ex-AP

Os Ca ex-AP podem ainda ser divididos em três categorias: o Ca ex-AP intracapsular, o tumor misto metastatizante, e o verdadeiro carcinossarcoma.[54, 37] A categoria conhecida por Ca ex-AP intracapsular, *in situ* ou não invasiva[54, 28, 21] é caracterizada por áreas malignas completamente circunscritas por AP e sem infiltração. Teoricamente, essa categoria possuiria um melhor prognóstico. Entretanto, já existe um

relato de Ca ex-AP totalmente encapsulado cujo curso clínico foi agressivo com disseminação metastática.[28]

Os tumores mistos metastatizantes podem metastatizar como verdadeiros carcinomas, mas os implantes metastáticos apresentam-se com inequívocas características histológicas benignas, semelhantes ao tumor primário.[93, 29] Por causa da aparência benigna das metástases, elas podem por vezes, ser erroneamente diagnosticadas como tumores primários. Por isso, é importante ressaltar que os pacientes acometidos invariavelmente apresentam um AP primário e a metástase é usualmente precedida de múltiplos episódios de recorrência.[13] O mecanismo de metástase desse tipo de tumor permanece desconhecido. Existe a hipótese de que a manipulação cirúrgica possa deslocar células neoplásicas para espaços vasculares, de onde sairiam para se implantar em estruturas adjacentes ou à distância.[75] Suportando esse conceito está o fato de que tecidos de AP têm sido transplantados com sucesso para camundongos, onde as células continuam a crescer.[9, 6]

O verdadeiro carcinossarcoma ou verdadeiro tumor misto maligno é muito raro e caracterizado pela transformação em ambos os componentes epitelial e mesenquimal.[82, 15, 39] Histologicamente, o componente sarcomatoso também pode apresentar-se com fenótipos variáveis como fibrossarcoma, condrossarcoma, osteossarcoma, lipossarcoma, leiomiossarcoma, rabdomiossarcoma e histiocitoma fibroso maligno.[35,62] Excepcionalmente a transformação envolve apenas o componente mioepitelial.[77, 20, 97]

As Metástases

Com relação às metástases, suas localizações (principalmente linfonodos, ossos e pulmões) indicam ambas as vias de disseminação, linfática e hematogênica. Essas são compostas, na maioria das vezes, de elementos carcinomatosos, exceção feita às metástases

de carcinossarcomas verdadeiros cujas metástases podem ter tanto elementos de origem epitelial ou mesenquimal e do tumor misto metastatizante, cujo implante metastático é histologicamente idêntico ao tumor primário benigno.[93, 29] Sabe-se também que as características cariotípicas dos tumores mistos metastatizantes diferem das características citogenéticas encontradas em AP e outros Ca ex-AP.[43]

O Prognóstico

O Ca ex-AP, uma vez diagnosticado, determina um tempo de sobrevida curto (37% em 5 anos) e alta taxa de mortalidade (55%).[53, 65] podendo ser considerado o terceiro tumor de glândula salivar com pior prognóstico em sobrevida, sendo superado apenas pelo carcinoma indiferenciado e adenocarcinoma NOS.[91] Li Volsi & Persin[54] e Spiro *et al.*[90] sugeriram que a presença de linfonodos positivos possa ser o principal fator prognóstico.

Seja qual for sua aparência, categoria ou subtipo, esses tumores devem ser cuidadosamente manipulados, pois as evidências são argumentos definitivos para considerar todas as variantes de Ca ex-AP como neoplasmas potencialmente metastáticos e agressivos.[47, 34, 73, 13, 28]

A Terapêutica

Apesar de que, a possibilidade de recorrência ou de transformação maligna de um AP dependa não apenas do procedimento cirúrgico, mas de propriedades biológicas intrínsecas de cada tumor[59] a melhor prevenção contra recorrências e malignização ainda é a adequada remoção cirúrgica dos tumores benignos o mais precocemente possível, com margens de segurança e seguida de um prolongado *follow-up* dos pacientes.[63, 66]

A terapêutica para os tumores malignos inclui excisão total da glândula afetada, dissecção dos linfonodos cervicais, ressecção de estruturas adjacentes (como por exemplo,

o nervo facial), radioterapia e quimioterapia.[84, 65, 41] Um estudo recente mostra um decréscimo na incidência de Ca ex-AP que pode ser conseqüência do diagnóstico precoce e rápida remoção da lesão benigna.[91]

A raridade dos Ca ex-AP e sua variabilidade têm dificultado a avaliação do uso de imunoterapia, mesmo conhecendo o fato de que estes expressam HER-2/neu, ao contrário dos AP. O benefício, se é que exista, estaria associado ao subtipo histológico dos Ca ex-AP: tumores que se manifestassem como aqueles originados no ducto intercalar (carcinoma adenóide cístico, adenocarcinoma NOS, carcinoma de células acinares, adenocarcinoma polimorfo de baixo grau e carcinoma mioepitelial) não responderiam tão bem ao transtuzumab quanto aqueles semelhantes aos originados nos ductos secretórios (carcinoma de ducto salivar, carcinoma mucoepidermóide e carcinoma de células escamosas).[36]

Conclusão

Em conclusão, a presente revisão mostra os vários fatores até então associados à transformação maligna do AP em Ca ex-AP, mas ressalta que apenas a precoce e adequada remoção cirúrgica do tumor benigno é consenso na literatura como procedimento preventivo da malignização. Além disso, este estudo reforça o conceito de que a carcinogênese é um complexo processo *multi-steps* que envolve uma série de fenômenos moleculares, muitos deles ainda pouco conhecidos.

Referencias Bibliográficas

1. ALTEMANI, A. et al. Carcinoma ex-pleomorphic adenoma (CXPA): immunoprofile of the cells involved in the carcinomatous progression. Histopathology, v.46, n.6, p.635-41, 2005.
2. ANAND, A.; BROCKIE E.S. Cytomorphological features of salivary duct carcinoma ex pleomorphic adenoma: diagnosis by fine-needle aspiration biopsy with histologic correlation. Diagn Cytopathol, v.20, n.6, p.375-8, 1999.
3. ARAÚJO, V.C. et al. Immunohistochemical Mdm2 expression in minor salivary gland tumours and its relationship to p53 gene status. Oral Oncol, v.36, n.1, p.67-9, 2000.
4. ASTRÖM, A.K. et al. Conserved mechanism of PLAG1 activation in salivary gland tumors with and without chromosome 8q12 abnormalities: identification of SII as a new fusion partner gene. Cancer Res, v.59, n.4, p.918-23, 1999.
5. AUCLAIR, P.L.; ELLIS G.L. Atypical features in salivary gland mixed tumors: their relationship to malignant transformation. Mod Pathol, v.9, n.6, p.652-7, 1996.
6. BARFOED, C. et al. Human pleomorphic adenomas transplanted to nude mice. Arch Otolaryngol Head Neck Surg, v.112, n.9, p.946-8, 1986.
7. BEAHRS, O.H. et al. Carcinomatous transformation of mixed tumors of the parotid gland. AMA Arch Surg, v.75, n.4, p.605-13, 1957.
8. BULLERDIEK, J. et al. Cytogenetic investigation on cell line derived from a carcinoma arising in a salivary pleomorphic adenoma. Cancer Genet Cytogenet, v.44, n.2, p.253-62, 1990.

9. CASELITZ, J. et al. The pleomorphic adenoma of salivary glands transplanted on athmymic mice. A lightmicroscopical and immunohistochemical investigation. Virch Arch A Pathol Anat Histopathol, v.408, n.2-3, p.191-209, 1985.

10. COHN, M.L. et al. Sebaceous carcinoma ex pleomorphic adenoma: a rare phenotypic occurrence. Ann Diagn Pathol, v.8, n.4, p.224-6, 2004

11. CONLEY, J.; CLAIRMONTT A.A. Facial nerve in recurrent benign pleomorphic adenoma. Arch Otolaryngol, v.105, n.5, p.247-51, 1979.

12. CRISCUOLO, M. et al. Prognostic significance of nucleolar organizer regions in recurrent pleomorphic adenomas of salivary glands. Pathologica, v.86, n.6, p.606-11, 1994.

13. CZADER, M. et al. Metastasizing mixed tumor of the parotid: initial presentation as a solitary kidney tumor and ultimate carcinomatous transformation at the primary site. Am J Surg Pathol, v.24, n.8, p.1159-64, 2000.

14. DARDICK, I. et al. Histogenesis of salivary gland pleomorphic adenoma (mixed tumor) with an evaluation of the role of the myoepithelial cell. Hum Pathol, v.13, n.1, p.62-75, 1982.

15. DARDICK, I. et al. Ultrastructural contributions to the study of morphological differentiation in malignant mixed (pleomorphic) tumour of salivary gland. Head Neck, v.11, n.1, p.5-21, 1989.

16. DARLING, M.R. et al. Polymorphous low-grade adenocarcinoma and adenoid cystic carcinoma: a review and comparison of immunohistochemical markers. Oral Oncol, v.38, n.7, p.641-5, 2002.

17. DEBIEC-RYCHTER, M. et al. Histologic localization of PLAG1 (pleomorphic adenoma gene 1) in pleomorphic adenoma of the salivary gland: cytogenetic

evidence of common origin of phenotypically diverse cells. Lab Invest, v.81, n.9, p.1289-97, 2001.

18. DEGUSHI, H. et al. c-myc, ras p21 and p53 expression in pleomorphic adenoma and its malignant form of the human salivary glands. Acta Pathol Jpn, v.43, n.7-8, p.413-22, 1993.
19. DEVLIN, H, SLOAN P. Immunolocalisation of cartilage-derived retinoic acid-sensitive protein in pleomorphic adenoma of the parotid salivary gland. J Oral Pathol Med, v.30, n.2, p.87-90, 2001.
20. DI PALMA, S. et al. Malignant myo-epithelioma of the parotid gland arising in a pleomorphic adenoma. Histopathology, v.19, n.3, p.273-5, 1991.
21. DI PALMA, S. et al. Non-invasive (intracapsular) carcinoma ex pleomorphic adenoma: recognition of focal carcinoma by HER-2/neu and MIB1 immunohistochemistry. Histopathology, v.46, n.2, p.144-52, 2005.
22. EL-NAGGAR, A.K. et al. Concurrent cytogenetic, interphase fluorescence in situ hybridization and DNA flow cytometric analyses of a carcinoma ex-pleomorphic adenoma of parotid gland. Cancer Genet Cytogenet, v.107, n.2, p.132-6, 1998.
23. ELLIS G.L.; AUCLAIR P.L. Tumors of the salivary glands. In *Atlas of Tumor Pathology*. Third Series, fascicle 17. Washington, DC: Armed Forces Institute of Pathology, 1996.
24. ENEROTH, C.M.; ZETTERBERG, A. Malignancy in pleomorphic adenoma. A clinical and microspectrophotometric study. Acta Otolaryngol, v.77, n.6, p.426-32, 1974.
25. ENEROTH, C.M. et al. Carcinoma in pleomorphic adenoma of the parotid gland. Acta Otolaryngol, v.66, n.6, p.477-92, 1968.

26. ENLUND, F. et al. Expression of PLAG1 and HMGIC proteins and fusion transcripts in radiation-associated pleomorphic adenomas. Int J Oncol, v.20, n.4, p.713-6, 2002.

27. ETGES, A. et al. Immunohistochemical expression of retinoblastoma pathway proteins in normal salivary glands in salivary glands tumors. Oral Oncol, v.40, n.3, p.326-31, 2004.

28. FÉLIX, A. et al. Intracapsular carcinoma ex pleomorphic adenoma. Report of case with unusual metastatic behaviour. Oral Oncol. v.38, n.1, p.107-10, 2002.

29. FÉLIX, A. et al. Laminin and collagen IV in pleomorphic adenoma and carcinoma ex-pleomorphic adenoma: an immunohistochemical study. Hum Pathol, v.30, n.8, p.964-9, 1999.

30. FÉLIX, A. et al. Pleomorphic adenoma and carcinoma ex-pleomorphic adenoma: immunohistochemical demonstration of the association between tenascin expression and malignancy. Histopathology, v.45, n.2, p.187-92, 2004.

31. FÉLIX, A. et al. Prognostic significance of biomarkers (c-*erb*B-2, p53, proliferating cell nuclear antigen, and DNA content) in salivary duct carcinoma. Hum Pathol, v.27, n.6, p.561-6, 1996.

32. FERNANDES, MMG. Estudo da patologia dos tumores mamários caninos através de técnicas de imunoistoquímica e citometria estática. Faculdade de Medicina da Universidade do Porto, Portugal, 1996. 93p. Dissertação (Mestrado em Oncobiologia).

33. FOOTE F.W. JR; FRAZELL E.L. Tumors of the major salivary glands. Cancer, v.6, n.6, p.1065-133, 1953.

34. FRIEDRICH, R.E. et al. Pleomorphic adenoma of the salivary glands: analysis of 94 patients. Anticancer Res, v.25, n.3A, p.1703-5, 2005.

35. FUJIMURA, M. et al. Carcinomatous change in the cranial metastasis from a metastasizing mixed tumor of the salivary gland – case report. Neuro Med Chir, v.37, n.7, p.546-50, 1997.

36. GANDOUR-EDWARDS, R.F. et al. Carcinosarcoma (malignant mixed tumor) of the parotid: report of a case with a pure rhabdomyosarcoma component. Head Neck, v.16, n.4, p.379-82, 1994.

37. Geraldes M, Gartner F, Schmitt F. Immunohistochemical study of hormonal receptors and cell proliferation in normal canine mammary glands and spontaneous mammary tumours. Vet Rec. 2000; 146(14): 403-6.

38. GLISSON, B. et al. HER2 expression in salivary gland carcinomas: dependence on histological subtype. Clin Cancer Res, v.10, n.3, p.944-6, 2004.

39. GNEPP, D.R. Malignant mixed tumors of the salivary glands: a review. Pathol Annu, v.28, n.1, p.279-328, 1993.

40. HAMADA, T. et al. Mucin expression in pleomorphic adenoma of salivary gland: a potential role for MUC1 as a marker to predict recurrence. J Clin Pathol, v.57, n.8, p.813-21, 2004.

41. HARADA, H. Histomorphological investigation regarding to malignant transformation of pleomorphic adenoma (so-called malignant mixed tumor) of the salivary gland origin: special reference to carcinosarcoma. Kurume Med J, v.47, n.4, p.307-23, 2000.

42. HENRIKSSON, G. Recurrent primary pleomorphic adenomas of salivary gland origin. Intrasurgical rupture, histopathologic features, and pseudopodia. Cancer, v.82, n.4, p.617-20, 1998.

43. HODGE, C.W. et al. Role of radiotherapy for pleomorphic adenoma. Am J Clin Oncol, v.28, n.2, p.148-51, 2005.

44. JIN, Y. et al. Pleomorphic adenoma with extensive adipose content. Case report. Histopathology. v.28, n.1, p.87-9, 1996.

45. JIN, Y. et al. Unbalanced chromosomal rearrangements in a metastasizing salivary gland tumor with benign histology. Cancer Genet Cytogenet, v.102, n.1, p.59-64, 1998.

46. KAS, K. Et al. Promoter swapping between the genes for a novel zinc finger protein and beta-catenin in pleomorphic adenomas with t(3;8)(p21;q12) translocations. Nat Genet, v.15, n.2, p.170-4, 1997. Erratum in: Nat Genet, v.15, n.4, p.411.

47. KHOCHTALI, H. et al. Neurosarcome de la gland submandibulaire développé sur un adénome pléomorphe. A propos d'un cas. Rev Stomatol Chir Maxillofac, v.100, n.2, p.85-7, 1999.

48. KIM, T. et al. Fine needle aspiration diagnosis of malignant mixed tumor (carcinosarcoma) arising in pleomorphic adenoma of the salivary gland. A case report. Acta Cytol, v.42, n.4, p.1027-31, 1998.

49. KLIJANIENKO, J. et al. Clinically aggressive metastasizing pleomorphic adenoma: report of two cases. Head Neck, v19, n.7, p.629-33, 1997.

50. KLIJANIENKO, J. et al. Mucoepidermoid carcinoma ex pleomorphic adenoma: nonspecific preoperative cytologic findings in six cases. Cancer, v.84, n.4, p.231-4, 1998.

51. KUMAR, V. et al. Cabeça e Pescoço. In: KUMAR V.; KINGEN M.W. *Robbins & Cotran Patologia – Patológicas das Doenças*. 7ed. Rio de Janeiro: Elsevier; 2005. p. 831-36.

52. KUSAFUKA, K. et al. Cartilage-specific matrix protein chondromodulin-I is associated with chondroid formation in salivary pleomorphic adenomas: immunohistochemical analysis. Am J Pathol, v.158, n.4, p.1465-72, 2001.

53. KUSAFUKA, K. et al. Lumican expression is associated with formation of mesechyme-like elements in salivary pleomorphic adenomas. Pathol, v.203, n.4, p.953-60, 2004.

54. LEONETTI, J.P. et al. Recurrent pleomorphic adenoma of the parotid gland. Otolayngol Head Neck Surg, v.133, n.3, p.319-22, 2005.

55. LEWIS, J.E. et al. Carcinoma ex pleomorphic adenoma: pathologic analysis of 73 cases. Hum Pathol, v.32, n.6, p.596-604, 2001.

56. LI VOLSI, V.A.; PERZIN K.H. Malignant mixed tumors arising in salivary glands. 1. Carcinomas arising in benign mixed tumors: a clinicopathologic study. Cancer, v.39, p.2209-30, 1977.

57. LIANJIA, Y. et al. An immunohistochemical study of bone morphogenetic protein in pleomorphic adenoma of the salivary gland. Virch Arch A Pathol Anat Histopathol. v.422, n.6, p.439-43, 1993.

58. MARTIN, A.R. et al. Proliferative activity and aneuploidy in pleomorphic adenomas of the salivary glands. Arch Pathol Lab Med, v.118, n.3, p.252-9, 1994.

59. MARTINS, C. et al. PLAG1 gene alterations in salivary gland pleomorphic adenoma and carcinoma ex-pleomorphic adenoma: a combined study using chromosome banding, in situ hybridization and immunocytochemistry. Mod Pathol, v.18, n.8, p.1048-55, 2005.

60. MATSUZAKA, K. et al. Oncocytic tumor in myoepithelioma arising from grossopalatine gland. Oral Oncol, v.39, n.3, p.306-8, 2003.

61. MATTURRI, L. et al. Cell Kinetics of pleomorphic adenomas of the parotid gland. Oral Oncol Eur J Cancer, v.32B, n.3, p.154-7, 1996.

62. MORBERG, J.G.; ENEROTH C..M. Malignant mixed tumors of the major salivary glands. Special reference to the histologic structure in metastases. Cancer, v.21, n.6, p.1198-211, 1968.

63. MORIO, T. DNA copy number changes in carcinoma in pleomorphic adenoma of the salivary gland: a comparative genomic hybridization study. Pathol Int, v.52, n.8, p.501-7, 2002.

64. NEVILLE, B.W. et al. Tumores das glândulas salivares. In: NEVILLE B.W. et al. *Patologia Oral e Maxilofacial*. 2ed. Rio de Janeiro: Guanabara-Koogan; 2004. p.369- 417.

65. NIPARKO, J.K. et al. Surgical treatment of recurrent pleomorphic adenoma of the parotid gland. Arch Otolaryngol Head Neck Surg, v.112, n.11, p.1180-4, 1986.

66. OHTAKÉ, S. et al. Precancerous foci in pleomorphic adenoma of the salivary gland: recognition of focal carcinoma and atypical tumor cells by p53 immunohistochemistry. J Oral Pathol Med, v.31, n10, p.590-7, 2002.

67. OLSEN, K.D., LEWIS J.E. Carcinoma ex pleomorphic adenoma: a clinicopathologic review. Head Neck, v.23, n.9, p.705-12, 2001.

68. PATEL, N.; POOLE A. Recurrent benign parotid tumours: the lesson not learnt yet? Aust N Z J Surg, v.68, n.8, p.562-4, 1998.

69. POETSCH, M. et al. Loss of heterozygosity occurs predominantly, but not exclusively, in epithelial compartment of pleomorphic adenoma. Neoplasia, v.7, n.7, p.688-95, 2005.

70. REGEZI, J.A.; SCIUBBA J.J. Doenças das glândulas salivares. In: REGEZI. J.A.; SCIUBBA J.J. *Patologia Bucal. Correlações Clinicopatológicas*. 3ed. Rio de Janeiro: Guanabara-Koogan, 2000. p.213-243.

71. RIGHI, P.D. et al. The role of the p53 gene in the malignant transformation of pleomorphic adenomas of the parotid gland. Anticancer Res, v.14, n.5B, p.2253-7, 1994.

72. RÖIJER, E. et al. Translocation, deletion/amplification, and expression of HMGIC and MDM2 in a carcinoma ex pleomorphic adenoma. Am J Pathol, v.160, n.2, p.433-40. 2002.

73. ROSA, J.C. et al. Immunohistochemical study of c-*erb*B-2 expression in carcinoma ex-pleomorphic adenoma. Histopathology, v.28, n.3, p.247-52, 1996.

74. SALEH, E.R.M. et al. Neural adhesion molecule (N-CAM) in pleomorphic adenoma and carcinoma ex-pleomorphic adenoma. J Oral Pathol Med, v.32, n.9, p.562-7, 2003.

75. SAMPSON, B.A. et al. Metastasizing mixed tumor of the parotid gland: a rare tumor with unusually rapid progression in a cardiac transplant recipient. Mod Pathol, v.11, n.11, p.1142-5, 1998.

76. SEIFERT, G.; SOBIN, L.H. The World Health Organization. International classification of tumors. Histological typing of salivary gland tumors, 2nd ed. New York: Spring-Verlag, 1991.

77. SHAABAN, H. et al. Recurrent pleomorphic adenoma of the palate in a child. Br J Plast Surg, v.54, n.3, p.245-7, 2001.

78. SHIBUYA, Y. et al. Ultrastructural localization of E-cadherin and alpha-/beta-catenin in adenoid cystic carcinoma. Histopathology, v.35, n.5, p.423-31, 1999.

79. SINGH, R.; CAWSON, R.A. Malignant myoepithelial carcinoma (myoepithelioma) arising in a pleomorphic adenoma of the parotid gland. An immunohistochemical study and review of the literature. Oral Surg Oral Med Oral Pathol, v.66, n.1, p.65-70, 1988.

80. SKÁLOVÁ, A. et al. Salivary duct carcinoma: a highly aggressive salivary gland tumor with HER-2/neu oncoprotein overexpression. Pathol Res Pract, v.197, n.9, p.621-6, 2001.

81. SOUZA, K.C.N. et al. Expressão imuno-histoquímica de p53 na discriminação do comportamento biológico dos tumores de glândula salivar. J Bras Patol Med Lab, v.41, n.3, p.189-95, 2005.

82. SPIRO, R.H. et al. Malignant mixed tumor of salivary origin. A clinicopathologic study of 146 cases. Cancer, v.39, n.2, p.388-96, 1977.

83. STENNERT, E. et al. Recurrent pleomorphic adenoma of the parotid gland: a prospective histopathological and immunohistochemical study. Laryngoscope, v.114, n.1, p.148-63, 2004.

84. STEPHENS, J. et al. True malignant mixed tumors (carcinosarcoma) of salivary glands. Oral Surg Oral Med Oral Pathol, v.61, n.6, p.597-602, 1986.

85. SUZUKI, H.; FUJIOKA, Y. Deletion of the p16 gene and microsatellite in carcinoma in pleomorphic adenoma of the parotid gland. Diagn Mol Pathol, v.7, n.4, p.224-31, 1998.

86. TERHAARD, C.H. et al. The role of radiotherapy in the treatment of malignant salivary gland tumors. Int J Radiat Oncol Biol Phys, v.61, n.1, p.103-11, 2005.

87. THACKRAY, A.C.; LUCAS, R.B. Tumors of the salivary glands. In: *Atlas of Tumor Pathology*. Washington DC: Armed Forces Institute of Pathology; 1983. p107-117.

88. TORTOLEDO, M.E. et al. Carcinomas ex pleomorphic adenoma and malignant mixed tumors. Histomorphologic indexes. Arch Otolaryngol, v.110, n.3, p.172-6, 1984.

89. VERMA, K., KAPILA K. Role of needle aspiration cytology of pleomorphic adenomas. Cytopathol, v.13, n.2, p.121-7, 2002.

90. VOZ, M.L. et al. First insight into the molecular basis of pleomorphic adenomas of the salivary glands. Adv Dent Res, v.14, p.81-3, 2000.

91. VOZ, M.L. et al. PLAG1, the main translocation target in pleomorphic adenoma of the salivary glands, is a positive regulator of IGF-II. Cancer Res, v.60, n.1, p.106-13, 2000.

92. VOZ, M.L. et al. The recurrent translocation t(5;8)(p13;q12) in pleomorphic adenomas results in upregulation of PLAG1 gene expression under control of the LIFR promoter. Oncogene, v.16, n.11, p.1409-16, 1998.

93. WAHLBERG, P. et al. Carcinoma of the parotid and submandibular glands – a study of survival in 2465 patients. Oral Oncol, v.38, n.7, p.706-13, 2002.

94. WEBER, A. et al. Expression profiles of p53, p63, and p73 in benign salivary gland tumors. Virch Arch, v.441, n.5, p.428-36, 2002.

95. WENIG, B.M. et al. Metastasizing mixed tumor of salivary glands. A clinicopathologic and flow cytometric analysis. Am J Surg Pathol, v.16, n.9, p.845-58, 1992.

96. XU, H. et al. Pleomorphic adenoma of the submandibular salivary gland with marked ossification. J Oral Pathol Med, v.32, n.8, p.499-501, 2003.

97. YAMAMOTO, Y. et al. DNA analysis at p53 locus in carcinomas arising from pleomorphic adenomas of salivary glands: comparison of molecular study and p53 immunostaining. Pathol Int, v.48, n.4, p.265-72, 1998.

98. YAMAMOTO, Y. et al. Mutations associated with carcinomas arising from pleomorphic adenomas of the salivary glands. Hum Pathol, v.27, n.8, p.782-6, 1996.

99. YOSHIZAKI, T. Malignant myoepithelioma arising from recurrent pleomorphic adenoma of minor salivary gland. Auris Nasus Larynx, v.29, n.1, p.91-4, 2002.

100. ZHAO, M. et al. Biosynthesis of glycosaminoglycans and agregan by tumor cells in salivary pleomorphic adenoma: ultrastructural evidence. J Oral Pathol Med, v.28, n.10, p.442-50, 1999.

101. ZHAO, M. et al. Immunohistochemical demonstration of bone morphogenetic protein-2 (BMP-2) and type II collagen in pleomorphic adenoma of salivary glands. J Oral Pathol Med, v.27, n.7, p.293-6, 1998.

Artigo 2 - Immunohistochemical expression of p63 in pleomorphic adenomas and carcinomas ex-pleomorphic adenomas of salivary glands

Oral Oncology (2006) **42,** 154–160

ELSEVIER

ORAL ONCOLOGY

http://intl.elsevierhealth.com/journals/oron/

Immunohistochemical expression of p63 in pleomorphic adenomas and carcinomas ex-pleomorphic adenomas of salivary glands

Marisa Cristina Leite Santos Genelhu [a,b,d], Helenice Gobbi [b], Fernando Augusto Soares [c], Antônio Hugo José Froes de Marques Campos [c], Carlos Alberto Ribeiro [b], Geovanni Dantas Cassali [a,*]

[a] *Laboratório de Patologia Comparada, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*
[b] *Departamento de Anatomia Patológica e Medicina Legal, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*
[c] *Departamento de Anatomia Patológica, Hospital do Câncer A. C. Camargo, São Paulo, Brazil*
[d] *Núcleo de Pesquisa em Imunologia, Faculdade de Ciências da Saúde, Universidade Vale do Rio Doce, Governador Valadares, Brazil*

Received 23 May 2005; accepted 29 June 2005

KEYWORDS
p63;
Pleomorphic adenoma;
Carcinoma ex-pleomorphic adenoma;
Salivary gland tumor;
Immunohistochemistry

Summary Alteration of the p63 protein expression has been associated with several human cancers including salivary gland tumors. We sought to assess the p63 expression in pleomorphic adenomas (PA) and carcinoma ex-pleomorphic adenomas (Ca ex-PA), since this protein has been related to myoepithelial phenotype and the biological behavior of cancer. Ten samples of PA and ten of Ca ex-PA were submitted to immunohistochemistry using a monoclonal anti-p63 antibody, clone 4A4. p63 was regularly expressed in myoepithelial cells' nuclei of the normal glandular parenchyma and in PA, but it was completely absent in five out of the ten samples of Ca ex-PA. ($P < 0.05$ by Fisher's exact test). Apparently, only those cases depicting some myoepithelial differentiation were positive. Our data suggest that loss of myoepithelial differentiation is important in the evolution of Ca ex-PA, and corroborate the hypothesis that p63 antigen may be a useful marker of myoepithelial cells in salivary glands neoplasms.

* Corresponding author. Address: Laboratorio de Patologia Comparada, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, Belo Horizonte, Minas Gerais, Brazil. Tel.: +55 31 34992891; fax: +55 31 34992879.
E-mail address: cassalig@icb.ufmg.br (G.D. Cassali).

1368-8375/$ - see front matter
doi:10.1016/j.oraloncology.2005.06.026

Introduction

The p63 protein has been shown to play an essential role in epithelial development and in normal cellular differentiation and may be associated with tumorigenesis in epithelial tissues.[1–5] It is also considered a highly sensitive and specific immunohistochemical marker of myoepithelial cells.[3,4,6–8] Recent studies have reported alterations of p63 expression in several human cancers including salivary gland carcinomas.[1–9]

Pleomorphic adenomas (PA) are the most frequent benign tumors of the salivary glands. Although monoclonal, PA are composed of epithelial, myoepithelial, and stromal components.[10,11] Malignant transformation of PA have been reported, and it is usually associated with recurrent or longstanding lesions.[12–14] In carcinomas ex-pleomorphic adenomas (Ca ex-PA), malignant transformation is associated with epithelial and/or myoepithelial component resulting in several histological patterns.[10–15] The transformation rarely involves only the myoepithelial component.[16] These tumors usually present an aggressive course and a poor clinical prognosis.[14,17,18]

There is no consensus regarding the histological features of PA that are predictive for malignization.[19,20] Moreover, no conclusive finding was observed in several studies of the molecular events associated with this transformation.[21–25]

The aim of this study was to assess the p63 immunohistochemical expression in PA and Ca ex-PA in order to better define the possible role of p63 in histogenesis, differentiation and aspects of the transformation from benign to malignant phenotype.

Materials and methods

Case selection

Ten cases of PA and 10 cases of Ca ex-PA were retrieved from Department of Pathology, School of Medicine, Federal University of Minas Gerais, the A. C. Camargo Cancer Hospital, and the National Institute of Cancer. The diagnosis of all cases were confirmed by two experienced pathologists (A.H.J.F.M.C. and C.A.R.) and further subtyped according to the World Health Organization (WHO) and Armed Forces Institute of Pathology (AFIP) criteria.[10,11] Accurate Ca ex-PA diagnosis required the presence of histological evidence of the benign tumor in association with the malignant tumor and/or the clinical history of recurrences in the same anatomical site (Fig. 1).

Immunohistochemistry

All samples were fixed in buffered formalin and embedded in paraffin. Briefly, four-micrometer sections were obtained from each tumor and dispensed on silane-coated slides. They were submitted to antigen retrieval solution (DAKO, pH 6.0) before applying the anti-p63 (clone 4A4, dilution

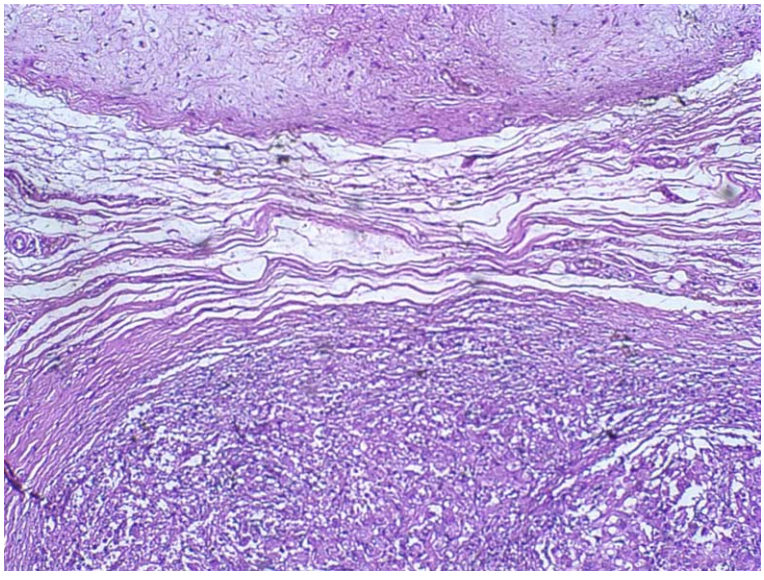


Figure 1 Carcinoma ex-pleomorphic adenoma. Note the benign component (at the top) and the malignant component (at the bottom) (HE 10x).

1:100, Santa Cruz, CA) and anti-AE1/AE3 antibodies, a pan-cytokeratin employed to assert the presence of epithelial component in the lesions (clone NCL-AE1/AE3, dilution 1:100, Novocastra, UK). Both antibodies were incubated for one hour at room temperature, and the reaction was amplified using a streptavidin-biotin-peroxidase immunostaining method. Diaminobenzidine was used as a chromogen followed by counterstaining with Mayer's hematoxilyn. The primary antibody was omitted as a negative control.

Cytoplasmic immunostaining was evaluated qualitatively for AE1/AE3 and semi-quantitatively for nuclear p63 localization, using the score proposed by Reis-Filho et al.:[8] −, negative nuclear staining of neoplastic cells; +, focal (<5%) positivity of neoplastic cells; ++, moderate (5—50%) positivity of neoplastic cells; and +++, diffuse (>50%) positivity of neoplastic cells.

Statistical analysis

The Fisher's exact test was used. The *P* value was considered significant when it was less than 0.05.

Results

The clinical, histopathological and immunohistochemical features of the tumors are summarized in Tables 1 and 2.

The mean age of detection was 39.6 years old (SD ± 11.0) for PA, and 51.7 years old (SD ± 26.9) for Ca ex-PA. Therefore, a difference of 12.1 years was noted between the incidence of these tumors. Female patients were more prevalent in the PA sampling (6:4), while for the Ca ex-PA, the proportion was of 1:1. Parotid was the most affected gland. Histologically, the majority of PA consisted dominantly of myxoid stroma.

All tumors depicted positivity for AE1/AE3 (Fig. 2A) in both normal glandular parenchyma adjacent and tumoral tissue, confirming the presence of epithelial component in the cases analyzed.

Immunostaining for p63 was restricted to the nuclei of myoepithelial cells (Fig. 2B). In PA, the ductal/lobular architecture was lost, but p63 was positive in cells with myoepithelial differentiation (Fig. 2C), varying from weak (four cases) to

Table 1 Clinical and immunohistochemical characterization of AE1/AE3 and p63 antigens in pleomorphic adenomas

Case	Age	Gender	Gland	AE1/AE3	p63
1	30	M	minor (lip)	+	++
2	35	F	parotid	+	+++
3	42	M	parotid	+	+
4	53	F	parotid	+	+
5	29	F	parotid	+	+++
6	51	F	parotid	+	+
7	32	F	parotid	+	+++
8	39	F	submandibular	+	+++
9	58	M	parotid	+	+++
10	27	M	parotid	+	+

Table 2 Clinical, histopathological, and immunohistochemical characterization of AE1/AE3 and p63 antigens in carcinomas ex-pleomorphic adenomas

Case	Age	Gender	Gland	AE1/AE3	p63	Histologic subtype
1	33	F	minor (palate)	+	−	adenocarcinoma NOS
2	43	M	Parotid	+	+++	myoepithelial carcinoma
3	53	F	minor (palate)	+	+++	myoepithelial carcinoma
4	71	M	parotid	+	+++	undifferentiated carcinoma
5	65	F	parotid	+	++	adenoid cystic carcinoma (solid)
6	92	F	submandibular	+	−	polymorphous low-grade adenocarcinoma
7	66	M	parotid	+	−	mucoepidermoid carcinoma
8	02	M	parotid	+	−	myoepithelial carcinoma
9	70	M	minor (palate)	+	+++	adenoid cystic carcinoma (tubular)
10	22	F	submandibular	+	−	adenocarcinoma NOS

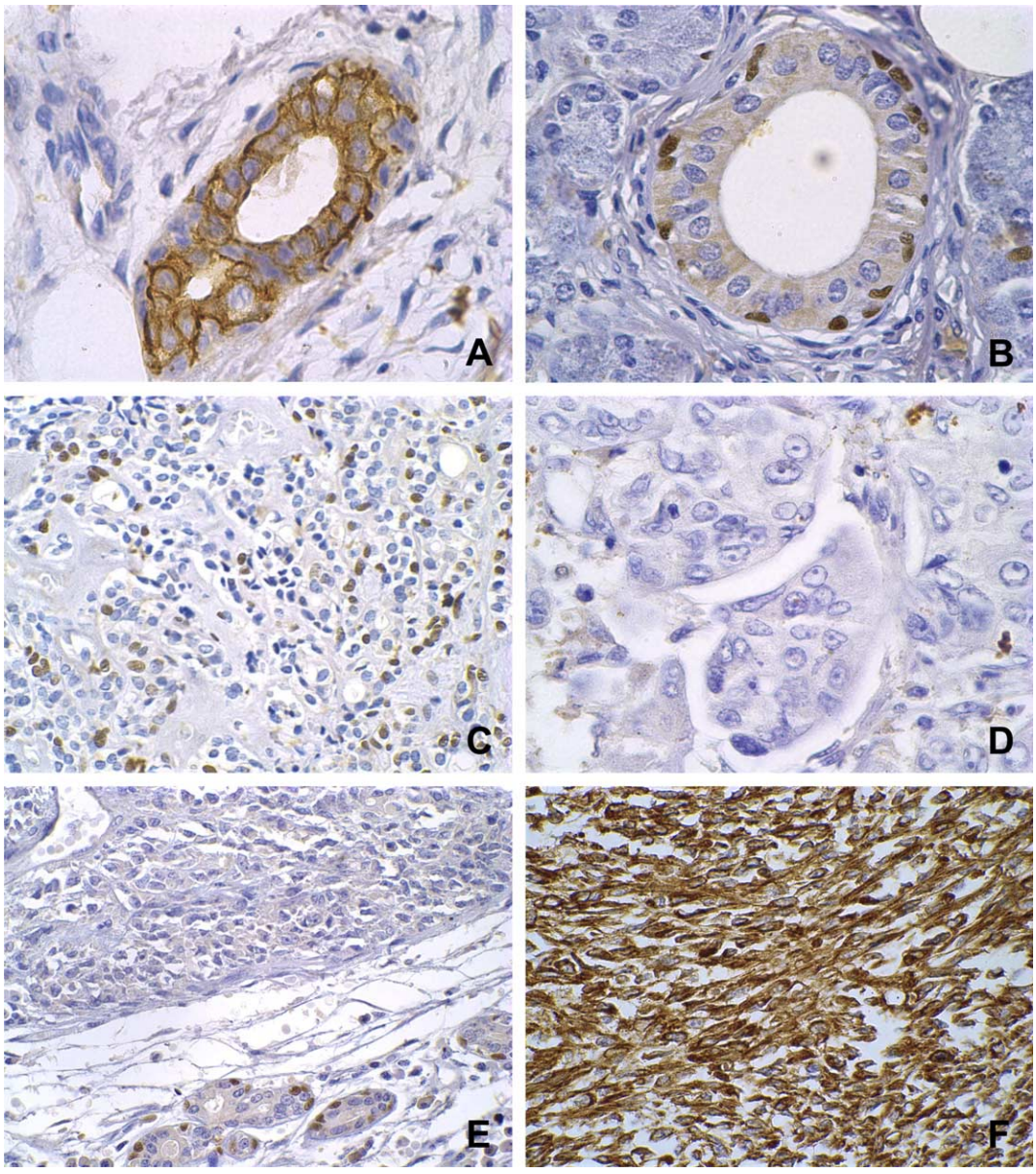


Figure 2 (A) Positive immunostaining for AE1/AE3 in cytoplasm of epithelial cells of normal salivary gland (100x). (B) Positive immunostaining for p63 in nuclei of myoepithelial cells of normal salivary gland (100x). (C) Positive immunostaining for p63 in myoepithelial cell of PA (60x). (D) Absence of immunostaining for p63 in nuclei of myoepithelial cells of Ca ex-PA (100x). (E) Absence of immunostaining for p63 in myoepithelial carcinoma (60x). (F) Positive immunostaining for vimentin in myoepithelial carcinoma (60x).

moderate/diffuse (six samples). In Ca ex-PA, our most interesting finding was that immunohistochemical staining of the p63 antigen was solely observed in five out of ten lesions (Fig. 2D). Statistical analysis was thought to confirm that absence of p63 immunolocalization was associated with an increased risk of malignancy ($P < 0.05$). In p63 positive Ca ex-PA, the protein expression was also moderate to diffuse. No mesenchymal, neural, endothelial, smooth muscle, adipose cells or fibroblasts stained for p63.

One case classified by hematoxilyn-eosin (HE) as myoepithelial carcinoma was negative for p63 (Fig. 2E). In an additional immunohistochemical study, this tumor was positive for vimentin (clone V9, DAKO) (Fig. 2F), and negative for S-100 (polyclonal, DAKO), GFAP (clone 6F2, DAKO), and CD68 (clone KP1, DAKO). Although the

immunohistochemical profile provided was not conclusive, this case was classified as p63 negative myoepithelial carcinoma based upon its morphological features.

Discussion

Although PA is the most frequent benign tumor of salivary glands, the Ca ex-PA, its malignant counterpart, is rare. This is due to the fact that time of tumor evolution and the number of recurrences are involved in the mechanisms of malignant transformation of PA into Ca ex-PA as well as being associated with clinical disease progression.[12–14] The recurrences are mostly related to incomplete surgical excision, mainly due to pseudopodia pattern of those tumors.[26] Recently, PA have been earlier diagnosed and appropriate surgeries reduced malignant transformation of these benign lesions.[27]

In this series, clinical characteristics such as age, gender, and type of affected salivary gland were similar to those reported in the literature, in addition to the gap in time between the emergence of PA and of the Ca ex-PA.

Neoplastic myoepithelium is a key cellular participant in the morphogenesis of the variable histologic appearances of many salivary gland tumors.[28] Batzakis et al.[29] proposed that mixed tumors do not occur in tissues where the myoepithelial cells are absent. Barbareschi et al.[6] showed that in breast tumors, the identification of myoepithelial cells has a diagnostic value because, they are retained in benign lesions and lost during the process of malignization and invasion.

Recent research has shown that p63 is a good marker of myoepithelial cells even in the presence of cytomorphological heterogeneity (clear, spindle, or plasmacytoid cells). The anti-p63 antibody demonstrates sensitivity comparable to other myoepithelial markers, such as α-smooth muscle actin (α-SMA), smooth muscle myosin heavy chain (SMM-HC), and calponin, but it has a greater specificity. Stroma cells are consistently negative.[3,4,6–8] However, there are few studies evaluating p63 expression in salivary gland tumors.[2,4,30]

The transcription of the p63 gene begins from two different promoters and further alternative splicing. The two main isoforms, TAp63 and ΔNp63, have opposite functions, being responsible for cell-cycle arrest and cell proliferation, respectively.[31,32] In our study, using the 4A4 clone, that recognizes all p63 isoforms, all PA showed p63 expression in myoepithelial cells nuclei, although those positive cells became dispersed in the tumoral mass.

Our results showed significant variation in p63 expression in Ca ex-PA: only 5 cases of malignant tumors were immunostaining to p63, opposite to all cases of PA. This finding allows us to suggest that Ca ex-PA subtypes can be subdivided into two main groups: tumors with myoepithelial differentiation (myoepithelial carcinomas and adenoid cystic carcinoma) and those devoid of or with scanty myoepithelial differentiation (mucoepidermoid carcinoma, adenocarcinoma NOS, and polymorphous low-grade adenocarcinoma) as it was accomplished by Pia-Foschini et al.[33] with salivary gland-like tumors of the breast.

Coupled with the rarity of myoepithelial carcinoma, the diagnosis is complicated by the considerable variability in morphologic features.[15] In case of 8, classified as myoepithelial carcinoma by HE, the spindle cells were negative for p63. The immunohistochemical profile demonstrated the mesenchymal nature of part of the tumor's malignant component (vimentin positive). We considered this case a p63 negative myoepithelial carcinoma: myoepithelial carcinomas may show an abortive myoepithelial phenotype.[28]

It is possible that p63 positive undifferentiated carcinoma (case 4) can be also a myoepithelial carcinoma. High-grade carcinomas may show myoepithelial differentiation even without evident myoepithelial morphological features.[28] However, for further conclusions an additional immunohistochemical panel including other myoepithelial markers such as α-SMA, SMMH, calponin and caldesmon would be necessary.

The identification of Ca ex-PA subtypes is very important due to prognostic implications. The worst survival rates are related to the undifferentiated carcinoma, followed by adenocarcinoma NOS.[14] However, in our study, immunostaining for p63 was not useful to identify Ca ex-PA subtypes. In Edwards et al.[30] study, p63 staining pattern also was not able to distinguish between malignant (adenoid cystic carcinoma, polymorphous low-grade adenocarcinoma) and benign (basal cell adenoma) salivary gland tumors. In that study, most cases of adenoid cystic carcinoma and polymorphous low-grade adenocarcinoma were uniformly positive for p63. Our case of Ca ex-PA classified as polymorphous low-grade adenocarcinoma was shown to be negative p63 expression. Therefore, p63 is not an ideal marker to distinguish the different subtypes of Ca ex-PA. In the present study, this distinction was accomplished using morphologic

parameters. Reis-Filho et al.[35] suggest that, poorly differentiated carcinomas can be better characterized by p63 when used together with cytokeratins 5/6 and 14.

In spite of the fact that no formal analysis of specificity and sensitivity was carried out in the present study regarding other myoepithelial markers, our results showed interesting points in common with another authors' study. Prasad et al.[34] studied 135 benign and malignant tumors of salivary glands using α-SMA, SMMH and calponin. They found negative results for these markers in polymorphous low-grade adenocarcinoma, mucoepidermoid carcinomas, and adenocarcinoma no other specification (NOS), but results were positive for adenoid cystic carcinomas and myoepithelial carcinomas. These findings are in agreement with those of our study, using p63 in these Ca ex-PA subtypes.

We also tried to analyze the results considering the dual nature of p63 as an oncoprotein through its inhibition of p53 transactivation functions (ΔN isoforms) and apoptosis-inducing agent (TA isoforms).[31,32] We can suppose that preservation of myoepithelial cells in PA could be related to the histogenesis of benign tumors, because they were able to act as progenitors of neoplastic cells (if these cells are expressing ΔNp63). Another possibility is that myoepithelial cells of PA could be expressing TAp63, with its tumor suppressor functions. One has indeed invested the myoepithelium with great significance as a paracrine inhibitor of invasion and thus an inhibitor of tumor progression.[36] This hypothesis can be observed in the transformation from in situ to invasive mammary carcinoma.[37]

In the cases of Ca ex-PA we can suppose that the loss of p63 expression could implicate the loss of the TAp63 isoforms in the myoepithelial cells or, as already proposed, the tumoral progression was addressed for tumors without myoepithelial differentiation. However, our data are insufficient to relate the loss of the expression of this antigen in some Ca ex-PA to the process of malignant transformation, since this study does not identify which isoforms were lost or maintained in the tumors.

In conclusion, our results favor the participation of the myoepithelial cell in the histogenesis of mixed tumors of salivary gland and suggest that significant loss of p63 expression is probably involved in the differentiation of several subtypes of Ca ex-PA. In addition, they corroborate the hypothesis that the study of p63 antigen may be a useful marker of myoepithelial cells in salivary glands neoplasms. However, our data are insufficient to involve this fact in the malignant transformation from benign PA to the malignant phenotype of Ca ex-PA. Further studies using antibodies against the different isoforms are necessary for understanding the biological significance of p63 in salivary tumorigenesis.

Acknowledgements

We thank Heitor Paiva, MD, Lucília Zardo, MD, from National Institute of Cancer (INCA), Rio de Janeiro, Brazil, for providing cases of Ca ex-PA, Lúcia Porto Fonseca de Castro, MD, from School of Medicine of the Federal University of Minas Gerais, Brazil, for her scientific assistance, and Sérgio Cardoso, PhD, from State University of Montes Claros for having critically reviewed the paper.
This work was supported in part by Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

1. Di Como CJ, Urist MJ, Babayan I, Drobnjak M, Hedvat CV, Teruya-Felstein J, et al. p63 expression profiles in human normal and tumor tissues. *Clin Cancer Res* 2002;**8**:494—501.
2. Weber A, Langhanki L, Schütz A, et al. Expression profiles of p53, p63, and p73 in benign salivary gland tumors. *Virchows Arch* 2002;**441**:428—36.
3. Reis-Filho JS, Schmitt FC. Taking advantage of basic research: p63 is a reliable myoepithelial and stem cell marker. *Adv Anat Pathol* 2002;**9**(5):280—9.
4. Bilal H, Handra-Luca A, Bertrand JC, Fouret PJ. p63 is expressed in basal and myoepithelial cells of human normal and tumor salivary gland tissues. *J Histochem Cytochem* 2003;**51**:133—9.
5. Bortoluzzi M, Yurgel LS, Dekker NP, Jordan RCK, Regezi JA. Assessment of p63 expression in oral squamous cell carcinomas and dysplasias. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004;**98**(6):698—704.
6. Barbareschi M, Pecciarini L, Cangi MG, Macrì E, Rizzo A, Viale G, et al. p63, a p53 homologue, is a selective nuclear marker of myoepithelial cells of the human breast. *Am J Surg Pathol* 2001;**25**(8):1054—60.
7. Werling RW, Hwang H, Yaziji H, Gown AM. Immunohistochemical distinction of invasive from noninvasive breast lesions: a comparative study of p63 versus calponin and smooth muscle myosin heavy chain. *Am J Surg Pathol* 2003;**27**(1):82—90.
8. Reis-Filho JS, Torio B, Albergaria A, Schmitt F. p63 expression in normal skin and usual cutaneous carcinomas. *J Cutan Pathol* 2002;**29**:517—23.
9. Park BJ, Lee SJ, Kim JI, Lee SJ, Lee CH, Chang SG, et al. Frequent alteration of p63 expression in human bladder carcinomas. *Cancer Res* 2000;**60**:3370—4.
10. Seifert G, Sobin LH. *WHO International classification of tumors. Histological Typing of Salivary Gland Tumors*. 2nd Ed. Berlin Heidelberg: Spring-Verlag; 1991.

11. Ellis GL, Auclair PL. *Tumors of the salivary glands. Atlas of tumor pathology*. 3rd Ed. Washington, DC: Armed Forces Institute of Pathology; 1996.
12. Gnepp DR. Malignant mixed tumors of the salivary glands: a review. *Pathol Annu* 1993;**28**(Pt 1):279—328.
13. Li Volsi VA, Perzin KH. Malignant mixed tumors arising in salivary glands. 1. Carcinomas arising in benign mixed tumors: a clinicopathologic study. *Cancer* 1977;**39**: 2209—30.
14. Spiro RH, Huvos AG, Strong EW. Malignant mixed tumor of salivary origin. A clinicopathologic study of 146 cases. *Cancer* 1977;**39**:388—96.
15. Said S, Campana J. Myoepithelial carcinoma ex pleomorphic adenoma of salivary glands: a problematic diagnosis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2005;**99**: 196—201.
16. Singh R, Cawson RA. Malignant myoepithelial carcinoma (myoepithelioma) arising in a pleomorphic adenoma of the parotid gland. An immunohistochemical study and review of the literature. *Oral Surg Oral Med Oral Pathol* 1988;**66**(1): 65—70.
17. Lewis JE, Olsen KD, Sebo TJ. Carcinoma ex pleomorphic adenoma: pathologic analysis of 73 cases. *Hum Pathol* 2001; **32**:596—604.
18. Olsen KD, Lewis JE. Carcinoma ex pleomorphic adenoma: a clinicopathologic review. *Head Neck* 2001;**23**(9):705—12.
19. Auclair PL, Ellis GL. Atypical features in salivary gland mixed tumors: their relationship to malignant transformation. *Mod Pathol* 1996;**9**(6):652—7.
20. Tortolodo ME, Luna MA, Batsakis JG. Carcinomas ex-pleomorphic adenoma and malignant mixed tumors. Histomorfologic indexes. *Arch Otolaryngol* 1984;**110**(3):172—6.
21. Righi PD, Li YQ, et al. The role of the p53 gene in the malignant transformation of pleomorphic adenomas of the parotid gland. *Anticancer Res* 1994;**14**(5B):2253—7.
22. Costa-Rosa J, Fonseca I, Félix A, Soares J. Immunohistochemical study of c-erbB-2 expression in carcinoma ex-pleomorphic adenoma. *Histopathology* 1996;**28**:247—52.
23. Röijer E, Nordkvist A, Ström AK, Ryd W, Behrendt M, Bullerdiek J, et al. Translocation, deletion/amplification, and expression of HMGIC and MDM2 in a carcinoma ex pleomorphic adenoma. *Am J Pathol* 2002;**160**:433—40.
24. Voz ML, Mathys J, Hensen K, Pendeville H, Van Valckenborgh I, Van Huffel C, et al. Microarray screening for target genes of the proto-oncogene PLAG1. *Oncogene* 2004;**23**(1): 179—91.
25. El-Naggar AK, Callender D, Coombes MM, Hurr K, Luna MA, Batsakis JG. Molecular genetic alterations in carcinoma ex-pleomorphic adenoma: a putative progression model? *Genes Chromosomes Cancer* 2000;**27**(2):162—8.
26. Henriksson G, Westrin KM, Carlsöö B, Silfverswärd C. Recurrent primary pleomorphic adenomas of salivary gland origin. Intrasurgical rupture, histopathologic features, and pseudopodia. *Cancer* 1998;**82**:617—20.
27. Stennert E, Wittekindt C, Klussmann JP, Arnold G, Guntinas-Lichius O. Recurrent pleomorphic adenoma of the parotid gland: a prospective histopathological and immunohistochemical study. *Laryngoscope* 2004;**114**(1):148—63.
28. Savera AT, Zarbo RJ. Defining the role of myoepithelium in salivary gland neoplasia. *Adv Anat Pathol* 2004;**11**(2): 69—85.
29. Batzakis JG, Kraemer B, Sciubba JJ. The pathology of head and neck tumors: the myoepithelial cell and its participation in salivary gland neoplasia, Part 17. *Head Neck Surg* 1983;**5**(3):222—33.
30. Edwards PC, Bhuiya T, Kelsch RD. Assessment of p63 expression in the salivary gland neoplasms adenoid cystic carcinoma, polymorphous low-grade adenocarcinoma, and basal cell and canalicular adenomas. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004;**97**(5):613—9.
31. Yang A, Kaghad M, Wang Y, Gillett E, Fleming MD, Dötsch V, et al. p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. *Molecular Cell* 1998;**2**: 305—16.
32. Little NA, Jochemsen AG. P63. *Int J Biochem Cell Biol* 2002; **34**:6—9.
33. Pia-Foschini M, Reis-Filho JS, Eusebi V, Lakhani SR. Salivary gland-like tumours of the breast: surgical and molecular pathology. *J Clin Pathol* 2003;**56**:497—506.
34. Prasad AR, Savera AT, Gown AM, Zarbo RJ. The myoepithelial immunophenotype in 135 benign and malignant salivary gland tumors other than pleomorphic adenoma. *Arch Pathol Lab Med* 1999;**123**:801—6.
35. Reis-Filho JS, Simpson PT, Martins A, Preto A, Gartner F, Schmitt FC. Distribution of p63, cytokeratins 5/6 and cytokeratin 14 in 51 normal and 400 neoplastic human tissue samples using TARP-4 multi-tumor tissue microarray. *Virchows Arch* 2003;**443**(2):122—32.
36. Stemlicht MD, Kedeshian P, Shao ZM, Safarians S, Barsky SH. The human myoepithelial cell is a natural tumor suppressor. *Clin Cancer Res* 1977;**3**:1949—58.
37. Kalof AN, Tam D, Beatty B, Cooper K. Immunostaining of myoepithelial cells in breast lesions: a comparison of CD10 and smooth muscle myosin heavy chain. *J Clin Pathol* 2004;**57**:625—9.

Artigo 3 - Immunolocalization of β-catenin in pleomorphic adenomas and carcinomas ex-pleomorphic adenomas of salivary glands

Immunolocalization of β-Catenin in Pleomorphic Adenomas and Carcinomas Ex-pleomorphic Adenomas of Salivary Glands

Marisa C. L. S. Genelhu, PhD,† ‡ Helenice Gobbi, PhD,† Diele C. B. Arantes, PhD,§ Sérgio V. Cardoso, PhD,‖ and Geovanni D. Cassali, PhD*†*

Abstract: β-catenin plays a central role in cadherin/catenin cell-cell adhesion complex and is involved in cell signaling pathway. Change in β-catenin distribution has been associated with several human cancers including salivary gland tumors. We studied the immunolocalization of β-catenin in a series of pleomorphic adenomas (PA) and carcinomas ex-pleomorphic adenomas (Ca ex-PA). Ten samples of PA and ten of Ca ex-PA were evaluated by immunohistochemistry using streptavidin-biotin-peroxidase technique and a monoclonal antibody against β-catenin (E-5). Cell membrane/cytoplasmic staining of β-catenin was observed in normal gland parenchyma, PA, and in well-differentiated Ca ex-PA. Cytoplasmic/nuclear β-catenin staining was observed in poorly differentiated carcinomas and, interestingly, in one case of PA. Our data showed decreased cell membrane β-catenin expression in higher-grade tumors suggesting that β-catenin may play an important role in histologic differentiation and transition to malignant phenotype of Ca ex-PA.

Key Words: β-catenin, pleomorphic adenoma, carcinoma, salivary gland, immunohistochemistry

(*Appl Immunohistochem Mol Morphol* 2007;15:273–278)

Several studies have suggested that E-cadherin/catenins cell-cell adhesion complex is damaged in cancer cells.[1–4] E-cadherins, mainly responsible for cell-cell adhesion in most epithelial tissues, are calcium-dependent transmembrane glycoproteins, located in adherent junctions. Adhesion occurs through homotypical bindings between their extracellular amino-terminal domains. Nevertheless, by means of its cytoplasmic carboxy-terminal tail, E-cadherin is linked to a series of actinic microfilaments of cell cytoskeleton through α-catenins, β-catenins, γ-catenins and p120.[5]

In addition to its well-defined role in regulating cell-cell adhesion via interactions with E-cadherin, β-catenin, a 92-kd protein, has a critical role in the highly conserved *Wnt* (*Wingless*) signaling pathway.[6,7] When β-catenin does not take part in the adhesion complex, the free molecule is sequestered by a complex formed by APC (*adenomatous polyposis coli*) gene products, by GSK-3β (*serine-threonine glycogen synthetase kinase-3*β) molecule and by an axin adapting protein, disqualifying β-catenin phosphorylation and its degradation by ubiquitin-dependent proteolitic system.[4,6] Increased free β-catenin concentrations in cytosol stimulate their link with proteins such as *lymphocyte enhancer factor* and T-cell factor. These complexes are transferred to the nucleus and act as cofactors of genes transcription related to cell proliferation including well-established oncogenes, such as c-*myc*, *Cyclin D1*, and *matrix metalloproteinase-7*.[8]

This signaling pathway is important during embryogenesis when *Wnt* gene is required to establish and maintain normal epithelial morphology and tissue and organ differentiation processes.[9] However, in adult tissues, it plays an important role in effective neoplastic transformation when inappropriately activated.[6,10]

Consistent with β-catenin's ostensibly independent functions in cell adhesion and signaling, distinct pools of β-catenin exist in cells, including a cadherin-associated (membrane-associated) pool and a cytoplasmic and/or nuclear pool involved in *Wnt* signaling.[8]

Adhesion loss is an important step in the progression of several epithelial tumors.[11] Cellular redistribution of β-catenin with reduction of membrane expression and nuclear β-catenin accumulation is observed in tumors with aggressive histopathologic characteristics defined by loss of normal tissue architecture, high grade, infiltrative growth, lymph node involvement and metastases.[4,5,12–15]

A variety of human malignancies have already been associated with β-catenin redistribution.[3] In salivary gland tumors, studies focusing β-catenin role are scarce

Received for publication October 18, 2005; accepted May 3, 2006.

From the *Laboratory of Comparative Pathology, Biological Sciences Institute; †Department of Anatomic Pathology and Forensic Medicine, School of Medicine, Federal University of Minas Gerais; §Newton Paiva University Center, Belo Horizonte; ‖Oral Diagnosis Division, Department of Dentistry, State University of Montes Claros, and Oral Pathology Laboratory, School of Dentistry, Federal University of Uberlândia; and ‡Immunology Research Laboratory, Vale do Rio Doce University, Governador Valadares, Minas Gerais, Brazil.

Supported in part by grants from CAPES, CNPq, and FAPEMIG, Brazil.

Reprints: Dr Marisa Genelhu, PhD, Núcleo de Pesquisa em Imunologia, Universidade Vale do Rio Doce, Rua Israel Pinheiro, 2000, Governador Valadares, Minas Gerais, Brazil, CEP: 35020-220 (e-mail: genelhumc@uol.com.br).

TABLE 1. Clinical Features, Histologic Subtypes, and Localization of β-Catenin Expression of Human Salivary Glands Carcinomas Ex-pleomorphic Adenomas

Case	Age (y)	Sex	Gland	Histologic Subtype	Differentiation Grade	Localization of β-Catenin Expression
1	33	F	Minor (palate)	Adenocarcinoma NOS		Cytoplasmic/nuclear
2	22	F	Submandibular	Adenocarcinoma NOS	Group 1	Cytoplasmic/nuclear
3	71	M	Parotid	Undifferentiated carcinoma	Poorly differentiated tumors	Cytoplasmic/nuclear
4	66	M	Parotid	Mucoepidermoid carcinoma		Cytoplasmic/nuclear
5	65	F	Parotid	Adenoid cystic carcinoma (solid)		Cytoplasmic/nuclear
6	70	M	Minor (palate)	Adenoid cystic carcinoma (tubular)	Group 2	Cytoplasmic/nuclear
7	53	F	Minor (palate)	Myoepithelial carcinoma	Well-differentiated tumors	Membrane/cytoplasmic
8	43	M	Parotid	Myoepithelial carcinoma		Membrane/cytoplasmic
9	02	M	Parotid	Myoepithelial carcinoma		Membrane/cytoplasmic
10	92	F	Submandibular	Polymorphous low-grade adenocarcinoma		Membrane/cytoplasmic

F indicates female; M, Male; NOS, not otherwise specified.

and the concept of dysregulation of E-cadherin/β-catenin complex in the progression of low to high-grade malignant neoplasms was little studied. Previous studies have shown disorders in β-catenin cell distribution in mucoepidermoid carcinomas[7] and cystic adenoid carcinomas.[16,17] In the latter, a clear association was observed between mutation in β-catenin gene (*CTNNB*1) with tumorigenesis and tumor morphologic features.[18,19]

Pleomorphic adenomas (PA) are the most common benign tumors of salivary glands. They occur more frequently in parotid, in young adults, slightly tending to females. They are characterized by a remarkable histomorphologic heterogeneity: a mixture of ductal epithelial and myoepithelial cells dispersed in stroma with several grades of myxoid, hyaline, chondroid, and even bone tissues. Pleomorphism is more frequent among tumor components than among neoplastic cells.[20,21] Despite their benign appearance, these tumors may change resulting in their malignant counterpart, carcinomas ex-pleomorphic adenomas (Ca ex-PA).[22–24] PA change into Ca ex-PA is closely related to evolution time and number of benign tumor recurrences.[21] Although the possibility of PA recurrence and malignant change depends on tumor's intrinsic biologic properties,[25] most of the times, relapses are related to an incomplete surgical excision.[21] Distinguishing criteria between benign and malignant components of Ca ex-PA have already been appropriately described,[26] but there is no consensus on histologic features of predictive values for malignization. Molecular mechanisms through which adenoma-carcinoma progression occurs remain to be elucidated.[27] Therefore, we need to know more biologic details of Ca ex-PA onset and progression, a neoplasm characterized by an aggressive course and a poor clinical prognosis and excessive morbidity and mortality.[23,24]

Because of β-catenin molecule importance as a gatekeeper in tumorigenesis and malignant progression, the purpose of this study was to localize, through immunohistochemistry, β-catenin in PA and Ca ex-PA and relate this localization to histomorphologic features of these tumors.

MATERIALS AND METHODS

Ten samples of PA and 10 of Ca ex-PA were obtained from the Department of Pathology, School of Medicine, Federal University of Minas Gerais (UFMG, Belo Horizonte, Minas Gerais, Brazil), A. C. Camargo Cancer Hospital (São Paulo, São Paulo, Brazil), and National Cancer Institute (INCA, Rio de Janeiro, Rio de Janeiro, Brazil). Ca ex-PA diagnosis was restricted to cases of clinical or histopathologic evidence of evolution from benign lesion. Malignant components of Ca ex-PA were further identified and subtyped according to Armed Forces Institute of Pathology[20] and World Health Organization[21] criteria and are summarized in Table 1.

All samples were fixed in buffered formalin and embedded in paraffin. Briefly, 4-mm-thick histologic sections were obtained from each tumor and dispensed on silane-coated slides. After deparaffinization with xylol and dehydration in a graded ethanol series, they were submitted to antigen retrieval solution (*Target Retrieval Solution*, Dako, Carpinteria, CA) pH 6.0, in a double boiler (90°C) for 20 minutes. Endogenous peroxidase activity and nonspecific binding were blocked by incubation with 3% hydrogen peroxide in methanol and nonimmune serum (*Ultra V Block*, LabVision, Fremont, CA) for 15 minutes, respectively. Slides were incubated for 1 hour at room temperature with anti–β-catenin primary antibody (E-5, dilution 1:400, Santa Cruz Biotechnology, Santa Cruz, CA). After two 5-minute washes in phosphate buffered saline, tissue sections were treated with a biotinylated secondary antibody for 15 minutes and the reaction was amplified using a streptavidin-biotin-peroxidase immunostaining method (*UltraVision Large Volume Detection System*, LabVision, Fremont, CA). Slides were then stained with 3,3-diaminobenzidine chromogen, counterstained with Mayer hematoxylin, dehydrated, and coverslipped with Entellan (Merck). Additional positive control of normal salivary gland was included and the primary antibody was omitted as negative control.

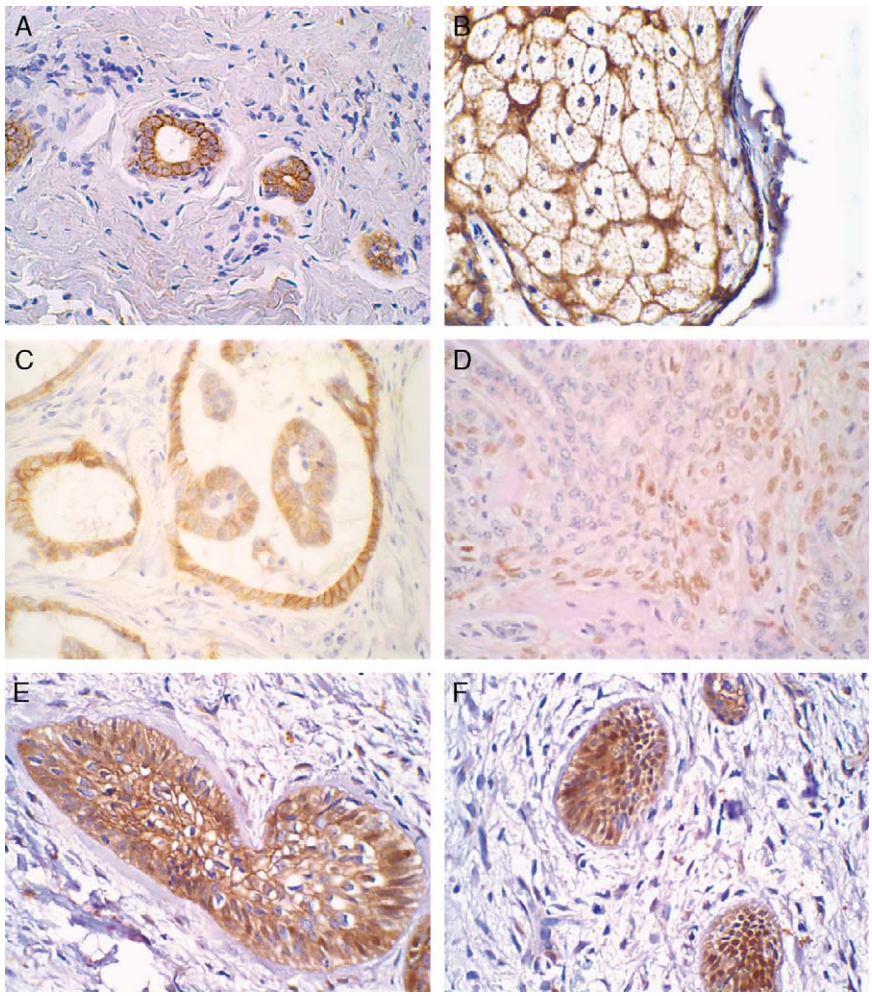


FIGURE 1. β-Catenin in pleomorphic adenomas (PA) and carcinoma ex-pleomorphic adenomas (Ca ex-PA) of salivary glands. A, Membrane/cytoplasmic localization of β-catenin in normal glandular epithelium (60 ×). B, Membrane localization with high expression of β-catenin in sebaceous metaplasia area of PA (100 ×). C, Membrane/cytoplasmic localization of β-catenin in well-differentiated Ca ex-PA (polymorphous low-grade adenocarcinoma subtype) (60 ×). D, Cytoplasmic and nuclear localization of β-catenin in Ca ex-PA (60 ×). E, Loss of β-catenin membrane immunostaining and cytoplasmic/nuclear β-catenin immunostaining in Ca ex-PA (60 ×). F, Cytoplasmic/nuclear β-catenin immunostaining in solid areas of cellular proliferation of PA (60 ×).

β-catenin cellular localization was evaluated qualitatively. Membrane, cytoplasmic, and nuclear immunoreactivity patterns were simply recorded as positive or negative. Negative staining pattern represented the absence of immunoreaction or positivity in less than 10% of neoplastic cells.[7]

RESULTS

Ca ex-PA subtypes found in our series were classified in to 2 different groups: (1) well-differentiated tumors (3 myoepithelial carcinomas, 1 solid adenoid cystic carcinoma, 1 tubular adenoid cystic carcinoma, 1 polymorphous low-grade adenocarcinoma, and 1 mucoepidermoid carcinoma); (2) poorly differentiated tumors (2 adenocarcinomas not otherwise specified and 1 undifferentiated carcinoma), with invasive and aggressive phenotype (Table 1).

Normal gland parenchyma showed linear membranous β-catenin staining around both serous and mucous acinar cells of intralobular and excretory ducts, but no labeling was detected in basal and/or myoepithelial cells (Fig. 1A). β-catenin immunoexpression was observed in all PA, but its distribution was irregular (due to loss of lobular architecture), occasionally with membrane/cytoplasmic localization. Membrane expression was also observed in PA with an area of sebaceous metaplasia, as shown in Figure 1B. In 4 out of 7 Ca ex-PA, called well-differentiated tumors (3 myoepithelial carcinomas and 1 polymorphous low-grade adenocarcinoma), cytoplasmic redistribution of β-catenin was observed (Fig. 1C). Mucoepidermoid carcinoma (Fig. 1D) and adenoid cystic carcinomas subtypes of Ca ex-PA showed cytoplasmic/nuclear staining in a frequency apparently lower than that of tumors considered poorly differentiated or undifferentiated (Fig. 1E). Unexpectedly, 1 case of benign PA showed nuclear expression of β-catenin in a specific area of solid cell proliferation (Fig. 1F). β-catenin was not expressed in sites with a greater number of myoepithelial cells and in chondromyxoid stroma.

DISCUSSION

Cadherin-catenin complex, a primary cell-cell adhesion mediator, is closely involved in maintaining tissue integrity, morphogenesis, cell differentiation, and tumor

progression cells.[7] Function loss leads malign lesions to escape from their primary sites, degrading extracellular matrix, acquiring an invasive phenotype and, eventually, metastasizing.[11] In addition, cadherin-catenin complex is part of signal-transduction pathway *Wingless*/*Wnt*.[2,4,7] β-catenin is an important member of this complex, with at least 2 functions, which are determined by its localization, in the membrane or nucleus. It plays an important role in cell adhesion in the membrane. Nuclear β-catenin increases *Wnt* gene transcription responsive to cell growth.[28]

Several authors have reported β-catenin subcellular compartmentalization in different types of cancer, but the role of this redistribution still has not been studied in PA and its malignant counterpart, Ca ex-PA. It is known that such event is associated with the initiation and progression of tumors but, mechanisms by which β-catenin molecules are redistributed, remain elusive. This redistribution may be a consequence of mutations in APC tumor suppressor gene[29,30] or mutations in exon 3 of β-catenin gene itself.[31] Posttranslational changes of any E-cadherin/catenin complex element may also be the cause of β-catenin nuclear/cytoplasmic expression in neoplasms.[32–34]

The present manuscript is the first exploratory study to report association between immunoexpression of β-catenin in PA and Ca ex-PA of salivary glands. Although the number of cases of Ca ex-PA was limited because of the scarcity of malignant lesions, we report interesting data. β-catenin immunolocalization analysis in all PA and, in 4 out of 7 well-differentiated malignant tumors have shown predominantly membrane/cytoplasmic expressions. In contrast, in 3 malignant tumors considered poorly differentiated or undifferentiated, membrane expression loss and, in some cells, nuclear β-catenin accumulation were observed.

Despite considering well-differentiated malignant tumors, adenoid cystic carcinomas and mucoepidermoid carcinoma subtypes of Ca ex-PA, have shown cytoplasmic/nuclear staining similarly to poorly differentiated or undifferentiated tumors. Our results have been shown to be similar to those obtained in immunohistochemical studies of mucoepidermoid carcinomas and cystic adenoid carcinomas carried out by Shieh et al[7] and Daa et al,[16] respectively. This may be related to the most aggressive biologic behavior of these tumors, which is possibly due to abnormalities in cellular mechanisms of cell adhesion and signaling (such as those associated with β-catenin), in addition to several other regulatory factors of proliferative and invasive activity regardless *Wnt* pathway.

In this work, an inverse correlation was found between membrane expression of β-catenin and low-grade differentiation and/or more aggressive biological behavior of tumor cells.[35] This result, membrane expression loss with subcellular (nuclear and perinuclear) β-catenin localization, is in accordance with those found in the literature for other tumors such as oral,[12,14,33] larynx,[34] and esophageal[35] squamous cells carcinomas, pancreatic adenocarcinomas,[36] gastric carcinomas,[37,38] lung carcinomas,[39] colorectal carcinomas,[40,41] endometrial carcinomas,[42,43] hepatocellular carcinomas,[44] and bladder,[45] renal,[46] skin (melanoma),[47] thyroid,[31] and breast cancers.[48,49]

We may suppose that in neoplastic cell membrane of PA and well-differentiated Ca ex-PA, β-catenin maintains its role as cell adhesion and tumor suppressor molecule. Cytoplasmic localization may both indicate that after the posttranslational process, the molecule is driven to the membrane where it links to E-cadherin, and that β-catenin is free in the cytoplasm, at disposal of APC/GSK-3β/axin complex which restrains its degradation and makes its nucleus targeting easier.[50] In this case, evidences suggest that β-catenin plays a critical role in the signaling pathway accounting for gene transcription linked to cell proliferation in malignant tumors. In addition, the absence of membrane expression may be related to tumor progression and Ca ex-PA invasive properties.[37,51]

An interesting datum in this study was the nuclear localization of β-catenin in one of the benign tumors. This finding is very important because it may mean an early molecular event in the malignant transformation of PA, similar to what may be inferred for malignant tumors. This fact may be relevant in the prognostic of some PA regarding recurrence and malignant transformation.

The role of β-catenin in both *Wnt* signaling and cell adhesion also suggests that targeted disruption of β-catenin-mediated T-cell factor signaling is a possible strategy for early chemopreventive intervention to the tumor progression. Some data indicate that sulindac, sulindac sulfone, and indomethacin may modulate subcellular localization of β-catenin in vivo, and resulting in either decreased nuclear compartmentalization or enhanced localization of β-catenin in plasma membrane.[52]

Finally, our results suggest that β-catenin immunohistochemical expression evaluation is of great diagnostic and prognostic value for PA and Ca ex-PA studies, as it is for several other tumors.[53] Changes in β-catenin localization may be explored, along with conventional clinical and histopathologic characteristics, distinguishing benign tumors with malignant potential from malignant tumors with more aggressive characteristics of invasiveness and metastases.[35,53]

In conclusion, we have demonstrated changes in β-catenin immunolocalization in PA and Ca ex-PA. Our data showed decreased cell membrane β-catenin expression in higher-grade tumors suggesting that β-catenin may play an important role in histologic differentiation and transition to malignant phenotype of Ca ex-PA. The presence of nuclear β-catenin in a benign tumor suggests a possible association of this molecule with early molecular events of PA malignant transformation into Ca ex-PA. We believe that β-catenin may become a significant prognostic marker for tumors, biologic behavior and occurrence of local and distant metastases. Responses to some extracellular modulators may favorably change β-catenin localization and lead to new therapeutic approaches for these tumors.

ACKNOWLEDGMENTS

The authors are grateful to Fernando Soares, PhD, Antônio Campos, PhD, from Department of Anatomic Pathology, A.C. Camargo Cancer Hospital, São Paulo, Brazil and Heitor Paiva, MD, Lucília Zardo, MD, from National Institute of Cancer (INCA), Rio de Janeiro, Brazil, for providing cases of Ca ex-PA.

REFERENCES

1. Wheelock MJ, Johnson KR. Cadherins as modulators of cellular phenotype. *Annu Rev Cell Dev Biol*. 2003;19:207–235.
2. Nollet F, Geert B, Van Roy F. The role of the E-cadherin/catenin adhesion complex in the development and progression of cancer. *Mol Cell Biol Res Commun*. 1999;2:77–85.
3. Van Aken E, De Wever O, Correia da Rocha AS. Defective E-cadherin/catenin complexes in human cancer. *Virchows Arch*. 2001;439:725–751.
4. Shiozaki H, Oka H, Inoue M, et al. E-cadherin mediated adhesion system in cancer cells. *Cancer*. 1996;77:1605–1613.
5. Efstathiou JA, Liu D, Wheeler JM, et al. Mutated epithelial cadherin is associated with increased tumorigenicity and loss of adhesion and of responsiveness to the motogenic trefoil factor 2 in colon carcinoma cells. *Proc Natl Acad Sci USA*. 1999;96:2316–2321.
6. Jankowski JA, Bruton R, Shepherd N, et al. Cadherin and catenin biology represent a global mechanism for epithelial cancer progression. *Mol Pathol*. 1997;50:289–290.
7. Shieh YS, Chang LC, Chiu KC, et al. Cadherin and catenin expression in mucoepidermoid carcinoma: correlation with histopathologic grade, clinical stage, and patient outcome. *J Oral Pathol Med*. 2003;32:297–304.
8. Zhai Y, Wu R, Schwartz DR, et al. Role of β-catenin/T-cell factor-regulated genes in ovarian endometrioid adenocarcinomas. *Am J Pathol*. 2002;160:1229–1238.
9. Cadigan KM, Nusse R. Wnt signaling: a common theme in animal development. *Genes Devel*. 1997;11: F127–F147.
10. Harrington KJ, Syrigos KN. The role of E-cadherin-catenin complex: more than an intercellular glue? *Ann Surg Oncol*. 2000;7:783–788. Erratum in: *Ann Surg Oncol*. 2001;8:186.
11. Chow V, Yuen AP, Lam KY, et al. A comparative study of the clinicopathological significance of E-cadherin and catenins (alpha, beta, gamma) expression in the surgical management of oral tong carcinoma. *J Cancer Res Clin Oncol*. 2001;127:59–63.
12. Kudo Y, Kitajima S, Ogawa I, et al. Invasion and metastasis of oral cancer cells require methylation of E-cadherin and/or degradation of membranous beta-catenin. *Clin Cancer Res*. 2004;10:5455–5463.
13. Margulis A, Zhang W, Alt-Holland A, et al. E-cadherin suppression accelerates squamous cell carcinoma progression in three-dimensional, human tissue constructs. *Cancer Res*. 2005;65:1783–1791.
14. Lo Muzio L, Staibano S, Pannone G, et al. Beta- and gamma-catenin expression in oral squamous cell carcinomas. *Anticancer Res*. 1999;19:3817–3826.
15. Williams HK, Sanders DS, Jankowski JA, et al. Expression of cadherins and catenins in oral epithelial dysplasia and squamous cell carcinoma. *J Oral Pathol Med*. 1998;27:308–317.
16. Daa T, Kaku N, Kashima K, et al. Expression of beta-catenin, E-cadherin and Cyclin D1 in adenoid cystic carcinomas of the salivary gland. *J Exp Clin Cancer Res*. 2005;24:83–87.
17. Zhang ZY, Wu YQ, Zhang WG, et al. The expression of E-cadherin-catenin complex in adenoid cystic carcinoma of salivary glands. *Chin J Dent Res*. 2000;3:36–39.
18. Daa T, Kashima K, Kaku N, et al. Mutations in components of the Wnt signaling pathway in adenoid cystic carcinoma. *Mod Pathol*. 2004;17:1475–1482.
19. Shibuya Y, Ri S, Umeda M, et al. Ultrastructural localization of E-cadherin and α-/β-catenin in adenoid cystic carcinoma. *Histopathology*. 1999;35:423–431.
20. Ellis GL, Auclair PL. *Tumors of the Salivary Glands. Atlas of Tumor Pathology*. 3rd ed. Washington, DC: Armed Forces Institute of Pathology; 1996.
21. Seifert G, Sobin LH. *WHO International Classification of Tumors. Histological Typing of Salivary Gland Tumors*. 2nd ed. Berlin, Heidelberg: Springer-Verlag; 1991.
22. Gnepp DR. Malignant mixed tumors of the salivary glands: a review. *Pathol Annu*. 1993;28:279–328.
23. Lewis JE, Olsen KD, Sebo TJ. Carcinoma ex pleomorphic adenoma: pathologic analysis of 73 cases. *Hum Pathol*. 2001;32: 596–604.
24. Olsen KD, Lewis JE. Carcinoma ex pleomorphic adenoma: a clinicopathologic review. *Head Neck*. 2001;23:705–712.
25. Matturri L, et al. Cell kinetics of pleomorphic adenomas of the parotid gland. *Eur J Cancer B Oral Oncol*. 1996;32B:154–157.
26. Auclair PL, Ellis GL. Atypical features in salivary gland mixed tumors: their relationship to malignant transformation. *Mod Pathol*. 1996;9:652–657.
27. El Naggar AK, Lovell M, Callender DL, et al. Concurrent cytogenetic, interphase fluorescence in situ hybridization and DNA flow cytometric analyses of a carcinoma ex-pleomorphic adenoma of parotid gland. *Cancer Genet Cytogenet*. 1998;107:132–136.
28. Van Nhieu JT, Renard CA, Wei Y, et al. Nuclear accumulation of mutated β-catenin in hepatocellular carcinoma is associated with increased cell proliferation. *Am J Pathol*. 1999;155:703–710.
29. Rubinfeld B, Souza B, Albert I, et al. Association of the APC gene product with beta-catenin. *Science*. 1993 10;262:1731–1734.
30. Hulsken J, Birchmeier W, Behrens J. E-cadherin and APC compete for the interaction with beta-catenin and the cytoskeleton. *J Cell Biol*. 1994;127:2061–2069.
31. Garcia-Rostan G, Camp RL, Herrero A, et al. β-catenin dysregulation in thyroid neoplasms: down-regulation, aberrant nuclear expression, and CTNNB1 exon 3 mutations are markers for aggressive tumor phenotypes and poor prognosis. *Am J Pathol*. 2001;158:987–996.
32. Mareel M, Leroy A. Clinical, cellular, and molecular aspects of cancer invasion. *Physiol Rev*. 2003;83:337–376.
33. Tanaka N, Odajima T, Ogi K, et al. Expression of E-cadherin, alpha-catenin, and beta-catenin in the process of lymph node metastasis in oral squamous cell carcinoma. *Br J Cancer*. 2003; 89:557–563.
34. Lopez-Gonzalez JS, Cristerna-Sanchez L, Vasquez-Manriquez ME, et al. Localization and level of expression of β-catenin in human laryngeal squamous cell carcinoma. *Otolaryngol Head Neck Surg*. 2004;130:89–93.
35. De Castro J, Gamallo C, Palacios J, et al. beta-catenin expression pattern in oesophageal squamous cell carcinoma. Relationship with clinicopathologic features and clinical outcome. *Virchows Arch*. 2000;437:599–604.
36. Julkunen K, Makinen K, Karja V, et al. alpha-, beta- and chi-catenin expression in human pancreatic cancer. *Anticancer Res*. 2003;23:5043–5047.
37. Nakamura E, Sugihara H, Bamba M, et al. Dynamic alteration of E-cadherin/catenin complex during cell differentiation and invasion of undifferentiated-type gastric carcinomas. *J Pathol*. 2005;205: 349–358.
38. Kim HS, Hong EK, Park SY, et al. Expression of beta-catenin and E-cadherin in the adenoma-carcinoma sequence of the stomach. *Anticancer Res*. 2003;23:2863–2868.
39. Rodriguez-Salas N, Palacios J, de Castro J, et al. Beta-catenin expression pattern in small cell lung cancer: correlation with clinical and evolutive features. *Histol Histopathol*. 2001;16:353–358.
40. Hugh TJ, Dillon SA, O'Dowd G, et al. Beta-catenin expression in primary and metastatic colorectal carcinoma. *Int J Cancer*. 1999; 82:504–511.
41. Wong NACS, Pignatelli M. β-catenin—a linchpin in colorectal carcinogenesis? *Am J Pathol*. 2002;160:389–401.
42. Shih HC, Shiozawa T, Miyamoto T, et al. Immunohistochemical expression of E-cadherin and beta-catenin in the normal and malignant human endometrium: an inverse correlation between E-cadherin and nuclear beta-catenin expression. *Anticancer Res*. 2004;24:3843–3850.
43. Saegusa M, Hashimura M, Kuwata T, et al. β-catenin simultaneously induces activation of the p53-p21WAF1 pathway and

overexpression of cyclin D1 during squamous differentiation of endometrial carcinoma cells. *Am J Pathol*. 2004;164: 1739–1749.
44. Wong CM, Fan ST, Ng IO. beta-Catenin mutation and overexpression in hepatocellular carcinoma: clinicopathologic and prognostic significance. *Cancer*. 2001;92:136–145.
45. Shimazui T, Schalken JA, Giroldi LA, et al. Prognostic value of cadherin-associated molecules (α-, β-, γ-catenins and p120cas) in bladder tumors. *Cancer Res*. 1996;56:4154–4158.
46. Aaltomaa S, Lipponen P, Karja V, et al. The expression and prognostic value of alpha-, beta- and gamma-catenins in renal cell carcinoma. *Anticancer Res*. 2004;24:2407–2413.
47. Qi J, Chen N, Wang J, et al. Transendothelial migration of melanoma cells involves N-cadherin-mediated adhesion and activation of the β-catenin signaling pathway. *Mol Biol Cell*. 2005;16: 4386–4397.
48. Lin SY, Xia WY, Wang JC, et al. Beta-catenin, a novel prognostic marker for breast cancer: its roles in cyclin D1 expression and cancer progression. *Proc Natl Acad Sci U S A*. 2000;97:4262–4266.
49. Karayiannakis AJ, Nakopoulou L, Gakiopoulou H, et al. Expression patterns of β-catenin in situ and invasive breast cancer. *Eur J Surg Oncol*. 2001;27:31–36.
50. Klingelhofer J, Troyanovsky RB, Laur OY, et al. Exchange of catenins in cadherin-catenin complex. *Oncogene*. 2003;22:1181–1188.
51. Wijnhoven BP, Dinjens WN, Pignatelli M. E-cadherin-catenin cell-cell adhesion complex and human cancer. *Br J Surg*. 2000;87: 992–1005.
52. Clapper ML, Coudry J Chang WC. Beta-catenin-mediated signaling: a molecular target for early chemopreventive intervention. *Mutat Res*. 2004;555:97–105.
53. Montgomery E, Folpe AL. The diagnostic value of β-catenin immunohistochemistry. *Adv Anat Pathol*. 2005;12:350–356.

Artigo 4 - A comparative study between mixed-type tumours from human salivary and canine mammary glands

BMC Cancer

BioMed Central

Research article

A comparative study between mixed-type tumours from human salivary and canine mammary glands

Marisa CLS Genelhu[1,2,3], Sérgio V Cardoso[4], Helenice Gobbi[2] and Geovanni D Cassali*[1]

Address: [1]Laboratory of Comparative Pathology, Biological Sciences Institute, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, [2]Department of Pathology and Forensic Medicine, School of Medicine, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, [3]Immunology Research Laboratory, Vale do Rio Doce University, Governador Valadares, Minas Gerais, Brazil and [4]Department of Dentistry, State University of Montes Claros, and Oral Pathology Laboratory, School of Dentistry, Federal University of Uberlândia, Minas Gerais, Brazil

Email: Marisa CLS Genelhu - genelhumc@uol.com.br; Sérgio V Cardoso - cardososv@hotmail.com; Helenice Gobbi - hgobbi@medicina.ufmg.br; Geovanni D Cassali* - cassalig@icb.ufmg.br

* Corresponding author

Published: 28 November 2007

BMC Cancer 2007, **7**:218 doi:10.1186/1471-2407-7-218

Received: 21 November 2006
Accepted: 28 November 2007

This article is available from: http://www.biomedcentral.com/1471-2407/7/218

Abstract

Background: In comparative pathology, canine mammary tumours have special interest because of their similarities with human breast cancer. Mixed tumours are uncommon lesions in the human breast, but they are found most frequently in the mammary gland of the female dogs and in the human salivary glands. The aim of the study was to compare clinical, morphological and immunohistochemical features of human salivary and canine mammary gland mixed tumours, in order to evaluate the latter as an experimental model for salivary gland tumours.

Methods: Ten examples of each mixed tumour type (human pleomorphic adenoma and carcinomas ex-pleomorphic adenomas and canine mixed tumour and metaplastic carcinoma) were evaluated. First, clinical and morphologic aspects of benign and malignant variants were compared between the species. Then, streptavidin-biotin-peroxidase immunohistochemistry was performed to detect the expression of cytokeratins, vimentin, p63 protein, estrogen receptor, β-catenin, and E-cadherin.

Results: After standardization, similar age and site distributions were observed in human and canine tumours. Histological similarities were identified in the comparison of the benign lesions as well. Metaplastic carcinomas also resembled general aspects of carcinomas ex-pleomorphic adenomas in morphological evaluation. Additionally, immunohistochemical staining further presented similar antigenic expression between lesions.

Conclusion: There are many similar features between human salivary and canine mammary gland mixed tumours. This observation is of great relevance for those interested in the study and management of salivary gland tumours, since canine lesions may constitute useful comparative models for their investigations.

Background

Animal models have been widely used to investigate several forms of human neoplasias. Because of centuries of coexistence with humans in the same environment, dogs are of particular interest as they provide important evolutionary information. In addition, both species show great genotypic similarities [1]. Thus, spontaneous tumours of canine mammary glands have been proposed as comparative models for the study of human breast cancer, since these lesions share epidemiological, clinical, behavioural and antigenic features [2-5].

There is also a well-known relationship between the incidence of human mammary and salivary glands tumours [6-9]. Morphological similarities have been described between certain tumours of salivary glands and breast neoplasias such as those existing between polymorphous low-grade adenocarcinoma and invasive lobular carcinoma [10], between acinic cell carcinoma and invasive secretory carcinoma [11], and between epithelial-myoepithelial carcinoma and adenomyoepithelioma [12]. Ductal carcinomas [13,14], adenoid cystic carcinomas and mixed tumours with similar patterns may be found in both organs [15,16].

Mixed tumours are unusual lesions in the human breast [17], but they are frequent in both human salivary and canine mammary glands [18-20]. In a comparative evaluation of the available literature, pleomorphic adenoma (PA) and its malignant counterpart, the carcinomas expleomorphic adenomas (Ca ex-PA) have several interesting similarities to benign mixed tumours (MT) and to metaplastic carcinomas (MC) of canine mammary glands. First, all of them are derived from exocrine glands, which depict similar tissue architecture. Next, with few variations, both are microscopically characterized by a mixture of ductal and myoepithelial elements intermingling an apparently mesenchymal stroma of variable constitution [18-20]. In addition, malignant transformation is acknowledged for both for human PA and canine MT, particularly in lesions with long evolution and frequent recurrences [20-25]. In spite of these similar aspects, to the best of our knowledge no specific comparative investigation between human salivary and canine mammary glands tumours is available.

Thus, the present work aimed to perform objective morphological microscopic comparison between mixed tumours derived from human salivary and canine mammary glands, as well as to evaluate the immunohistochemical expression of some relevant antigens in order to characterize these two types of neoplastic alterations.

Methods

Samples

Ten samples of PA and 10 of Ca ex-PA were obtained from the Department of Pathology of School of Medicine, Federal University of Minas Gerais (UFMG, Belo Horizonte, Minas Gerais, Brazil), A. C. Camargo Cancer Hospital (São Paulo, São Paulo, Brazil), and the National Cancer Institute (Rio de Janeiro, Rio de Janeiro, Brazil). Ten samples of MT and 10 of MC of mammary glands of dogs without defined breed were obtained from the records of the Laboratory of Comparative Pathology, Biological Sciences Institute, UFMG. Ca ex-PA diagnosis was restricted to cases with clinical features (such us a previous benign tumour excised from a site in which recurrent malignant tumour), and/or histological evidence of arising in or from a benign lesion (identification of at least a focus benign tumour) [18]. The clinical analyses of the tumours studied are summarized in Tables 1, 2, 3, 4. The malignant components of Ca ex-PA were further identified and subtyped according to World Health Organization (WHO) and Armed Forces Institute of Pathology (AFIP) criteria [18,26] and are summarized in Table 2.

All samples were formalin-fixed, paraffin-embedded, and new histological sections were independently reviewed by two experienced observers to confirm the diagnosis. Clinical and demographic data (age, gender, affected salivary or mammary gland) from affected individuals were retrieved from medical and veterinary charts.

Immunohistochemistry

To further compare tumours, immunohistochemical assays were carried out to detect antigens related to cellular differentiation (cytokeratins, vimentin, and p63), adhesion (E-cadherin and β-catenin), and hormonal status (estrogen receptor), which have been shown to be relevant for the study of human salivary and breast cancer [27-36]. Streptavidin-biotin-peroxidase technique was used, employing the antibodies described in Table 5. Briefly, 3 μm thick histological sections were deparaffin-

Table 1: Clinical characterization of human salivary glands pleomorphic adenomas. (M – male; F – female)

Case	Age	Gender	Gland
1	30	M	minor (lip)
2	35	F	parotid
3	42	M	parotid
4	53	F	parotid
5	29	F	parotid
6	51	F	parotid
7	32	F	parotid
8	39	F	submandibular
9	58	M	parotid
10	27	M	parotid

Table 2: Clinical and histological subtypes of human salivary glands carcinomas ex-pleomorphic adenomas (M – male; F – female)

Case	Age (years)	Gender	Gland	Histological Subtype
1	33	F	minor (palate)	adenocarcinoma NOS
2	22	F	submandibular	adenocarcinoma NOS
3	71	M	parotid	undifferentiated carcinoma
4	53	F	minor (palate)	myoepithelial carcinoma
5	43	M	Parotid	myoepithelial carcinoma
6	02	M	parotid	myoepithelial carcinoma
7	65	F	parotid	adenoid cystic carcinoma (solid)
8	70	M	minor (palate)	adenoid cystic carcinoma (tubular)
9	92	F	submandibular	polymorphous low-grade adenocarcinoma
10	66	M	parotid	mucoepidermoid carcinoma

ised in xilol and dehydrated in decreasing alcohol concentrations. Next, they were submitted to antigenic retrieval (Target Retrieval Solution, pH 6.0, DakoCytomation, Carpinteria, USA) and endogenous peroxidase blocking (3% hydrogen peroxide in methanol). After incubation with primary antibodies (Table 5) and amplification (Ultra Vision Large Volume Detection System, Lab Vision, Fremont, USA), the reaction was revealed with diaminobenzidine as chromogen and Mayer haematoxylin as contrast. As positive controls, sections of normal human salivary and mammary glands with previously recognized positivity for the antigens studied were used. Substitution of primary antibody for normal human serum constituted the negative control.

Finally, morphological analysis of staining was performed, and then a semiquantitative protocol was

Table 3: Clinical characterization of benign mixed tumours of canine mammaryglands

Case	Age	Gender	Localization
1	5	F	Inguinal
2	7	F	Thoracic-cranial
3	9	F	Abdominal-caudal
4	4	F	Abdominal-caudal
5	6	F	Thoracic-cranial
6	3	F	Inguinal
7	7	F	Abdominal-caudal
8	5	F	Inguinal
9	7	F	Inguinal
10	8	F	Inguinal

Table 4: Clinical characterization of malignant mixed tumours of canine mammary glands

Case	Age	Gender	Localization
1	7	F	Thoracic-cranial
2	13	F	Thoracic-caudal
3	5	F	Abdominal-cranial
4	8	F	Inguinal
5	9	F	Thoracic-caudal
6	8	F	Inguinal
7	9	F	Inguinal
8	9	F	Thoracic-cranial
9	6	F	Thoracic-cranial
10	4	F	Abdominal-cranial

employed to segregate the cases. For this latter purpose the entire available tumoural tissue in the sections was evaluated. Next, it was determined whether the relative number of positive neoplastic cells was superior ("positive cases") or inferior ("negative cases") to 5% (for the analysis of p63 and estrogen receptor) [37,38] or 10% (cytokeratins, vimentin, E-cadherin, β-catenin) [29,32] from all of the neoplastic cells in the histological sections evaluated.

Statistical analysis

Frequency of positive immunostaining between the four groups of lesions was evaluated by Fisher's exact test with values of $p < 0.05$ considered statistically significant. Probability of α-error inferior to 5% was confirmed to be significant.

Results

In both species it was observed that the benign tumours occur in the younger individuals' group, while the malignant tumours are more frequent in older individuals' group. A slight predominance of female patients (six cases) was observed for PA, while a homogeneous distribution was observed among those patients with Ca ex-PA. In dogs, all lesions affected females.

Histomorphological comparative illustrations are exemplified in Figure 1. In general, both benign tumours presented formation of ductal structures and also cells with myoepithelial features, arranged in solid aggregations,

Table 5: Primary antibodies, resources and dilutions used in immunohistochemical assays

Antibody	Clone	Resource	Dilution
Anti-pan-cytokeratin	NCL-AE1/AE3	Novocastra	1:100
Anti-vimentin	V9	DAKO	1:50
Anti-p63	4A4	Santa Cruz	1:100
Anti-β-catenin	E-5	Santa Cruz	1:400
Anti-E-cadherin	4A2C7	Zymed	1:40
Anti-estrogen receptor	CC4–5	Novocastra	1:50

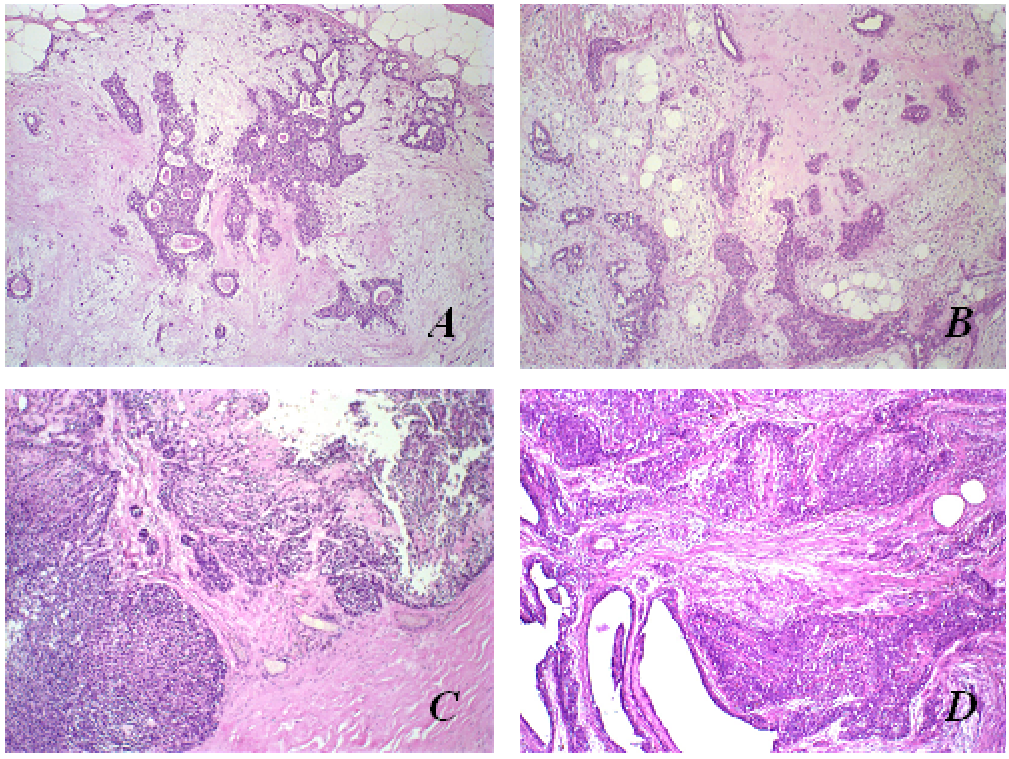


Figure 1
Histopathological aspects of benign and malignant mixed tumours of human salivary and canine mammary glands by haematoxylin and eosin (HE) stain; original magnification, 10×. **(A)** Pleomorphic adenoma in human salivary gland; **(B)** Mixed tumour in canine mammary gland; **(C)** Carcinoma ex-pleomorphic adenoma in human salivary gland; **(D)** Metaplastic carcinoma in canine mammary gland.

cords, nests, or even isolated, but irregularly dispersed in a predominantly myxoid or myxo-chondroid matrix. MC were observed to be histomorphologically similar to the adenocarcinoma NOS (not-otherwise specified) or to undifferentiated carcinoma-type Ca ex-PA, since both were infiltrative lesions with malignant degeneration areas, characterized by cell pleomorphism (ovoid to polyhedral cells with clear to hyaline cytoplasm), and hyperchromatic or vesiculated nuclei with conspicuous nucleoli.

Immunohistochemical assays displayed positive cytoplasmic localization of cytokeratins in all neoplastic cells from all lesions. Vimentin was identified in the cytoplasm of non-luminal cells of ductal formations, in plasmacytoid and spindle cells of PA and canine MT, in all MC cells, and was identified diffusely in Ca ex-PA with myoepithelial differentiation (those which the malignant component was described as myoepithelial, adenoid cystic, and polymorphous low-grade adenocarcinomas).

All PA presented positive p63 nuclear immunolocalization in neoplastic luminal, plasmacytoid and spindle cells, while p63 was found in only five samples of Ca ex-PA (two samples with diagnosis of myoepithelial carcinoma, two with adenoid cystic carcinomas, and one with undifferentiated carcinoma). All canine MT and MC depicted positive p63 expression, in a similar fashion to that seen in PA, while the malignant lesion had less positive cells and these presented less intense reaction (Figure 2). The decrease in immunopositivity frequency in Ca ex-PA was significantly different regarding all the other groups ($p < 0.05$).

β-catenin expressions in neoplastic epithelial cells of both PA of human salivary gland and MT of canine mammary glands have shown to be similar in location, to the expression in normal glandular parenchyma (membrane and/or cytoplasmic). Membrane and cytoplasmic β-catenin immunolocalization was especially frequent in cells of ductal formations in these benign tumours. In malignant lesions, nuclear expression of this protein was also identified (Figure 3). No β-catenin expression was observed in highly atypical areas of malignant tumours of both species or in those with rich myxo-chondroid stroma.

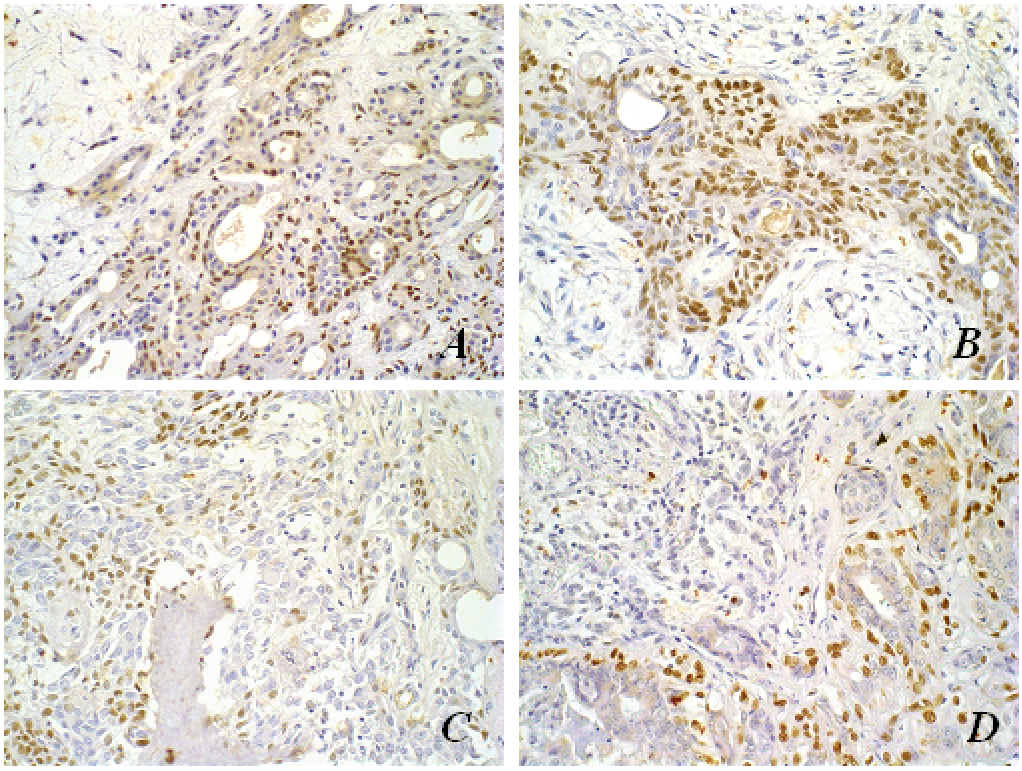


Figure 2
Immunohistochemical aspects of p63 antigen stain; original magnification, 40×. **(A)** Pleomorphic adenoma in human salivary gland; **(B)** Mixed tumour in canine mammary tumour; **(C)** Carcinoma ex-pleomorphic adenoma in human salivary gland; **(D)** Metaplastic carcinoma in canine mammary gland. Note myoepithelial p63-negative cells in malignant tumours (arrows).

Analysis of E-cadherin expression has shown that benign tumours of both species presented a similar linear membrane expression to that of normal gland parenchyma. Malignant neoplasias depicted less intense expression, which was also related to poor cell differentiation (Figure 4).

Estrogen receptor expression was not identified in tumours from the human salivary gland, while all the canine mammary tumours presented immunoreactivity for this marker ($p < 0.05$).

Discussion

The study of animal tumours may provide data to improve the understanding of similar lesions in humans, as well as tumour aetiology and development [39]. To date, there is not a universally accepted animal model for neoplastic pathology investigations. Transgenic mice, for instance, have received criticism as some of these animals are refractory to the development of certain types of tumours despite presentation of the same genetic lesion [40]. Animal cell culture is also an imperfect comparative model, as many of the events associated with carcinogenesis variably depend on a host [41]. One of the great advantages of canine breast model is that tumours are spontaneous in this organ. As its clinical evolution is natural, genetic and morphophysiological aspects may be better compared with some aspects of the human species [42].

Dogs represent a remarkable incidence of neoplasia development, usually associated with environmental exposure to important carcinogens for humans [1,43]. Tumours effecting the mammary glands, especially in females, are among the most frequent tumours observed in dogs. Finally, mixed tumours are one of the most frequent neoplasias in dogs [19] with remarkable features in common with human salivary gland tumours, justifying the investigation of other possible similarities between lesions of these two species.

The present study has confirmed that canine mammary gland MT and MC share some clinical characteristics with human salivary gland PA and Ca ex-PA, including age of emergence and several histopathological aspects. We have also demonstrated that commercially available antibodies for the study of human neoplasias are functional to detect antigenic expression in canine lesions. Moreover, similar

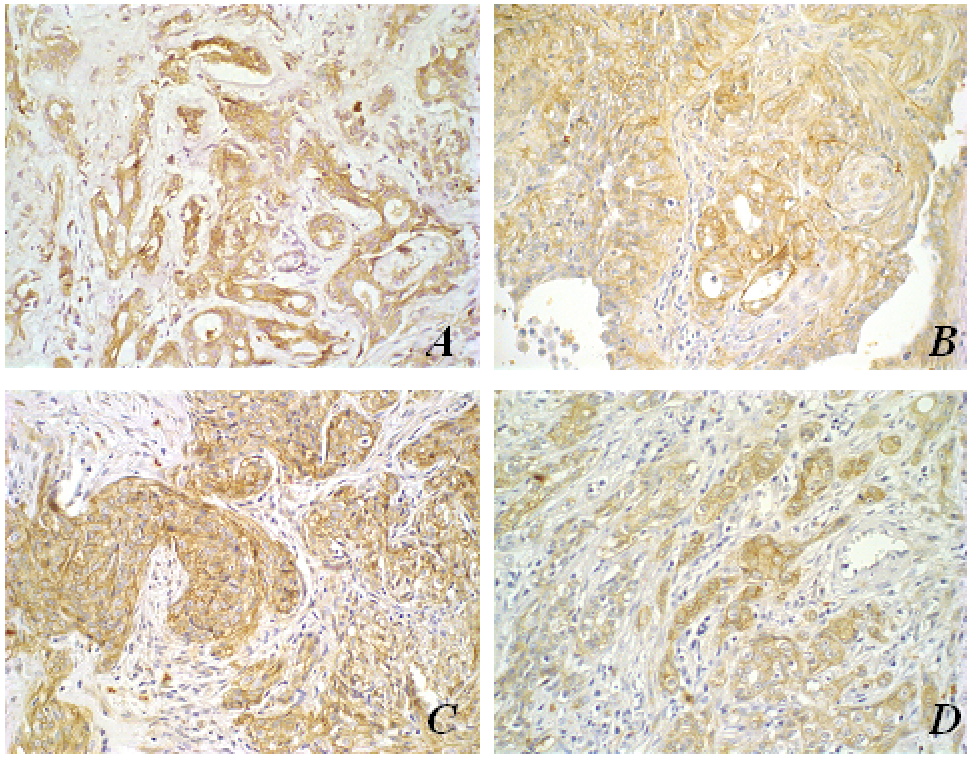


Figure 3
Immunohistochemical aspects of β-catenin antigen stain; original magnification, 40×. **(A)** Pleomorphic adenoma in human salivary gland with membrane and cytoplasmic β-catenin stain; **(B)** Mixed tumour in canine mammary tumour with membrane and cytoplasmic β-catenin stain; **(C)** Carcinoma ex-pleomorphic adenoma in human salivary gland showing β-catenin nuclear stain (arrows); **(D)** Metaplastic carcinoma in canine mammary gland showing β-catenin nuclear stain (arrows).

antigenic expressions (for cytokeratins, vimentin, β-catenin, and E-cadherin) were identified between the lesions, suggesting common pathogenetic mechanisms in the histogenesis of these tumours.

Vimentin and the pool of cytokeratins detected by the monoclonal antibody AE1/AE3 depicted parallel immunolocalization pattern, confirming the utility of this antigen to biologically characterize tumoural components.

p63 immunolocalization was restricted to cells with myoepithelial morphological features in PA, and to lesions of myoepithelial differentiation in Ca ex-PA, albeit the less intense staining in the latter suggests some loss of differentiation [44]. Similar observations were found for canine tumours, corroborating the use of this marker to demonstrate myoepithelial cells in both species. However, the analysis of the antigenic behaviour in tumours of both species is hindered by the fact that p63 possesses two different isoforms (TAp63 and ΔNp63) with opposite functions, being responsible for cell-cycle arrest and cell proliferation, respectively [45]. Clone 4A4, used in our study, recognizes all isoforms. It remains to be further evaluated by future works.

The expression of cell adhesion relating β-catenin and E-cadherin proteins was also similar in both species. In PA and canine MT, β-catenin and E-cadherin presented with predominantly membrane expression. β-catenin expression in MC and Ca ex-PA was either cytoplasmic/nuclear, or only nuclear, suggesting that changes in antigenic location may be related to the induction of gene transcription linked to cell proliferation in malignant tumours [34,35,45]. In addition, the loss of β-catenin and E-cadherin membrane expression may be associated with more aggressive tumour characteristics such as invasiveness and metastasis [46-49].

The most outstanding difference in antigenic expression was related to estrogen receptor. ER immunostaining was observed in all canine lesions, and it was not detected in any human neoplasia evaluated. Several previous studies showed the presence of ER in canine mammary tumours, suggesting this protein participates in lesion formation [50-52]. The lack of immunolocalization in salivary gland

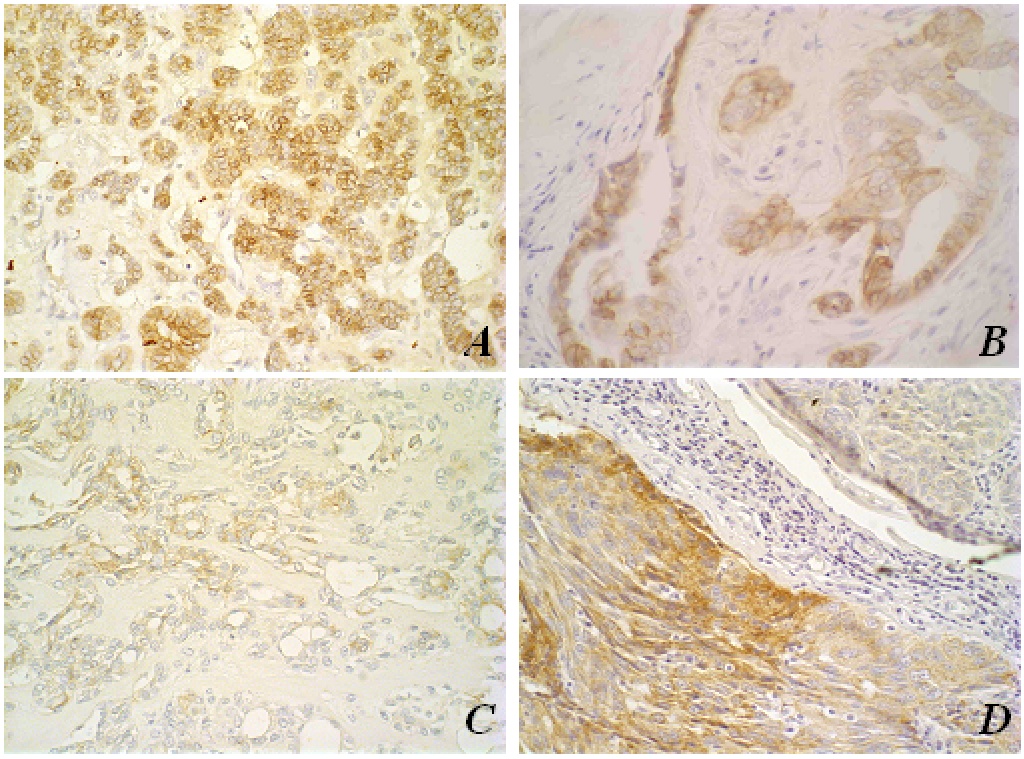


Figure 4
Immunohistochemical aspects of E-cadherin membrane antigen stain; original magnification, 40×.**(A)** Pleomorphic adenoma in human salivary gland with membrane E-cadherin stain; **(B)** Mixed tumour in canine mammary gland with membrane E-cadherin stain; **(C)** Carcinoma ex-pleomorphic adenoma in human salivary gland with evident loss of membrane E-cadherin marker; **(D)** Metaplastic carcinoma in canine mammary gland with evident loss of E-cadherin membrane marker. Note the loss of expression in malignant tumours.

tumours has been reported also by others [38,53-55]. One possible explanation would be the very low level expression of this protein [56], with an mRNA transcription being observed, which was shown by the study of Leimola-Virtanen *et al*. [57], or difficulties in recognition of epitopes through immunohistochemistry. In this work, the absence of ER expression in human salivary gland tumours suggests that these lesions not very responsive to estrogen, in contrast to the lesions in dogs, but further studies should be carried out to better define the role this protein in salivary gland tumorigenesis.

Conclusion

In the present work, some clinical, histopathological and antigenic similarities were confirmed between mixed-type tumours from human salivary and canine mammary glands. These data could suggest a hypothesis of similar histogenesis between these neoplasias. More interestingly, it encourages the use of spontaneous canine mammary gland tumours as animal models to study human salivary gland mixed neoplasias. However, differences were also identified and, therefore, additional studies should be carried out to better define advantages and disadvantages of a comparative assessment between these lesions.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

All authors contributed to the writing of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank the Instituto Nacional do Câncer (INCA), Hospital do Câncer A. C. Camargo, Brazil for providing cases of Ca ex-PA, and Luiz Cosme Malaquias, PhD, from Universidade Vale do Rio Doce for having critically reviewed the paper. This work was supported in part by Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

1. Lindblad-Toh K, Wade CM, Mikkelsen TS, Karlsson EK, Jaffe DB, Kamal M, Clamp M, Chang JL, Kulbokas EJ 3rd, Zody MC, Mauceli E, Xie X, Breen M, Wayne RK, Ostrander EA, Ponting CP, Galibert F,

Smith DR, DeJong PJ, Kirkness E, Alvarez P, Biagi T, Brockman W, Butler J, Chin CW, Cook A, Cuff J, Daly MJ, DeCaprio D, Gnerre S, Grabherr M, Kellis M, Kleber M, Bardeleben C, Goodstadt L, Heger A, Hitte C, Kim L, Koepfli KP, Parker HG, Pollinger JP, Searle SM, Sutter NB, Thomas R, Webber C, Baldwin J, Abebe A, Abouelleil A, Aftuck L, Ait-Zahra M, Aldredge T, Allen N, An P, Anderson S, Antoine C, Arachchi H, Aslam A, Ayotte L, Bachantsang P, Barry A, Bayul T, Benamara M, Berlin A, Bessette D, Blitshteyn B, Bloom T, Blye J, Boguslavskiy L, Bonnet C, Boukhgalter B, Brown A, Cahill P, Calixte N, Camarata J, Cheshatsang Y, Chu J, Citroen M, Collymore A, Cooke P, Dawoe T, Daza R, Decktor K, DeGray S, Dhargay N, Dooley K, Dooley K, Dorje P, Dorjee K, Dorris L, Duffey N, Dupes A, Egbiremolen O, Elong R, Falk J, Farina A, Faro S, Ferguson D, Ferreira P, Fisher S, FitzGerald M, Foley K, Foley C, Franke A, Friedrich D, Gage D, Garber M, Gearin G, Giannoukos G, Goode T, Goyette A, Graham J, Grandbois E, Gyaltsen K, Hafez N, Hagopian D, Hagos B, Hall J, Healy C, Hegarty R, Honan T, Horn A, Houde N, Hughes L, Hunnicutt L, Husby M, Jester B, Jones C, Kamat A, Kanga B, Kells C, Khazanovich D, Kieu AC, Kisner P, Kumar M, Lance K, Landers T, Lara M, Lee W, Leger JP, Lennon N, Leuper L, LeVine S, Liu J, Liu X, Lokyitsang Y, Lokyitsang T, Lui A, Macdonald J, Major J, Marabella R, Maru K, Matthews C, McDonough S, Mehta T, Meldrim J, Melnikov A, Meneus L, Mihalev A, Mihova T, Miller K, Mittelman R, Mlenga V, Mulrain L, Munson G, Navidi A, Naylor J, Nguyen T, Nguyen N, Nguyen C, Nguyen T, Nicol R, Norbu N, Norbu C, Novod N, Nyima T, Olandt P, O'Neill B, O'Neill K, Osman S, Oyono L, Patti C, Perrin D, Phunkhang P, Pierre F, Priest M, Rachupka A, Raghuraman S, Rameau R, Ray V, Raymond C, Rege F, Rise C, Rogers J, Rogov P, Sahalie J, Settipalli S, Sharpe T, Shea T, Sheehan M, Sherpa N, Shi J, Shih D, Sloan J, Smith C, Sparrow T, Stalker J, Stange-Thomann N, Stavropoulos S, Stone C, Stone S, Sykes S, Tchuinga P, Tenzing P, Tesfaye S, Thoulutsang D, Thoulutsang Y, Topham K, Topping I, Tsamla T, Vassiliev H, Venkataraman V, Vo A, Wangchuk T, Wangdi T, Weiand M, Wilkinson J, Wilson A, Yadav S, Yang S, Yang X, Young G, Yu Q, Zainoun J, Zembek L, Zimmer A, Lander ES: **Genome sequence, comparative analysis and haplotype structure of the domestic dog.** ***Nature*** 2005, **438(7609):**803-819.

2. Mottolese M, Morelli L, Agrimi U, Benevolo M, Sciarretta F, Antonucci G, Natali PG: **Spontaneous canine mammary tumors. A model for monoclonal antibody. The unique association between salivary gland cancer and breast cancer diagnosis and treatment of human breast cancer.** *Lab Invest* 1994, **71(2):**182-7.
3. Strandberg JD, Goodman DG: **Animal model of human disease: canine mammary neoplasia.** *Am J Pathol* 1974, **75(1):**225-8.
4. Schneider R: **Comparison of age, sex, and incidence rates in human and canine breast cancer.** *Cancer* 1970, **26(2):**419-26.
5. Owen LN: **A comparative study of canine and human breast cancer.** *Invest Cell Pathol* 1979, **2(4):**257-75.
6. Berg JW, Hutter RV, Foote FW Jr: **The unique association between salivary gland cancer and breast cancer.** *JAMA* **204(9):**771-4. 1968 May 27
7. Dunn JE Jr, Bragg KU, Sautter C, Gardipee C: **Breast cancer risk following a major salivary gland carcinoma.** *Cancer* 1972, **29(5):**1343-6.
8. Prior P, Waterhouse JA: **Second primary cancers in patients with tumours of the salivary glands.** *Br J Cancer* 1977, **36(3):**362-8.
9. Abbey LM, Schwab BH, Landau GC, Perkins ER: **Incidence of second primary breast cancer among patients with a first primary salivary gland tumor.** *Cancer* **54(7):**1439-42. 1984 Oct 1
10. Freedman PD, Lumerman H: **Lobular carcinoma of intraoral minor salivary gland origin. Report of twelve cases.** *Oral Surg Oral Med Oral Pathol* 1983, **56(2):**157-66.
11. Hirokawa M, Sugihara K, Sai T, Monobe Y, Kudo H, Sano N, Sano T: **Secretory carcinoma of the breast: a tumour analogous to salivary gland acinic cell carcinoma?** *Histopathology* 2002, **40(3):**223-9.
12. Seifert G: **Are adenomyoepithelioma of the breast and epithelial-myoepithelial carcinoma of the salivary glands identical tumours?** *Virchows Arch* 1998, **433(3):**285-8.
13. Skalova A, Starek I, Kucerova V, Szepe P, Plank L: **Salivary duct carcinoma: a highly aggressive salivary gland tumor with HER-2/neu oncoprotein overexpression.** *Pathol Res Pract* 2001, **197(9):**621-6.
14. Wick MR, Ockner DM, Mills SE, Ritter JH, Swanson PE: **Homologous carcinomas of the breasts, skin, and salivary glands. A histologic and immunohistochemical comparison of ductal mammary carcinoma, ductal sweat gland carcinoma, and salivary duct carcinoma.** *Am J Clin Pathol* 1998, **109(1):**75-84.
15. Foschini MP, Reis-Filho JS, Eusebi V, Lakhani SR: **Salivary gland-like tumours of the breast: surgical and molecular pathology.** *J Clin Pathol* 2003, **56(7):**497-506. Erratum in: *J Clin Pathol* 2003 Oct,56(10):804.
16. Hayes MM, Lesack D, Girardet C, Del Vecchio M, Eusebi V: **Carcinoma ex-pleomorphic adenoma of the breast. Report of three cases suggesting a relationship to metaplastic carcinoma of matrix-producing type.** *Virchows Arch* 2005, **446(2):**142-9.
17. Kumar PV, Sobhani SA, Monabati A, Talei AR, Shirazi B: **Cytologic findings of a pleomorphic adenoma of the breast: a case report.** *Acta Cytol* 2004, **48(6):**849-52.
18. Ellis GL, Auclair PL: **Tumors of the salivary glands.** In *Atlas of tumor pathology. 3rd Series, Fascicle 17 Volume 39–57*. Washington, DC: Armed Forces Institute of Pathology; 1996:228-251.
19. Cohen D, Reif JS, Brodey RS, Keiser H: **Epidemiological analysis of the most prevalent sites and types of canine neoplasia observed in a veterinary hospital.** *Cancer Res* 1974, **34(11):**2859-68.
20. Misdorp W, Else RW, Hellmen E, Limpiscomb TP: **Histological classification of the mammary tumors of the dog and the cat.** In *International Histological Classification of Tumors of domestic Animals. 2nd Series Volume 2*. Geneva: World Health Organization; 1999.
21. Auclair PL, Ellis GL: **Atypical features in salivary gland mixed tumors: their relationship to malignant transformation.** ***Mod Pathol*** 1996, **9(6):**652-7.
22. Li Volsi VA, Perzin KH: **Malignant mixed tumors arising in salivary glands. I. Carcinomas arising in benign mixed tumors: a clinicopathologic study.** *Cancer* 1977, **39(5):**2209-30.
23. Spiro RH, Huvos AG, Strong EW: **Malignant mixed tumor of salivary origin: a clinicopathologic study of 146 cases.** *Cancer* 1977, **39(2):**388-96.
24. Gnepp DR: **Malignant mixed tumors of the salivary glands: a review.** *Pathol Annu* 1993, **28 Pt 1:**279-328.
25. Lewis JE, Olsen KD, Sebo TJ: **Carcinoma ex pleomorphic adenoma: pathologic analysis of 73 cases.** *Hum Pathol* 2001, **32(6):**596-604.
26. Seifert G, Sobin LH: **Histological typing of salivary gland tumors.** In *The World Health Organization. International Classification of Tumors* 2nd edition. New York: Spring-Verlag; 1991.
27. de Araujo VC, de Souza SO: **Expression of different keratins in salivary gland tumours.** *Eur J Cancer B Oral Oncol* 1996, **32B(1):**14-8.
28. Reis-Filho JS, Simpson PT, Martins A, Preto A, Gartner F, Schmitt FC: **Distribution of p63, cytokeratins 5/6 and cytokeratin 14 in 51 normal and 400 neoplastic human tissue samples using TARP-4 multi-tumor tissue microarray.** *Virchows Arch* 2003, **443(2):**122-32.
29. Loyola AM, de Souza SO, Araujo NS, Araujo VS: **Study of minor salivary gland mucoepidermoid carcinoma differentiation based on immunohistochemical expression of cytokeratins, vimentin and muscle-specific actin.** *Oral Oncol* 1998, **34(2):**112-8.
30. Barbareschi M, Pecciarini L, Cangi MG, Macri E, Rizzo A, Viale G, Doglione C: **p63, a p53 homologue, is a selective nuclear marker of myoepithelial cells of the human breast.** *Am J Surg Pathol* 2001, **25(8):**1054-60.
31. Bilal H, Handra-Luca A, Bertrand JC, Fouret PJ: **p63 is expressed in basal and myoepithelial cells of human normal and tumor salivary gland tissues.** *J Histochem Cytochem* 2003, **51(2):**133-9.
32. Shieh YS, Chang LC, Chiu KC, Wu CW, Lee HS: **Cadherin and catenin expression in mucoepidermoid carcinoma: correlation with histopathologic grade, clinical stage, and patient outcome.** *J Oral Pathol Med* 2003, **32(5):**297-304.
33. Daa T, Kaku N, Kashima K, Nakayama I, Yokoyama S: **Expression of beta-catenin, E-cadherin and cyclin D1 in adenoid cystic carcinoma of the salivary gland.** *J Exp Clin Cancer Res* 2005, **24(1):**83-7.
34. Jankowski JA, Bruton R, Shepherd N, Sanders DS: **Cadherin and catenin biology represent a global mechanism for epithelial cancer progression.** *Mol Pathol* 1997, **50(6):**289-90.

35. Nollet F, Berx G, Van Roy F: **The role of the E-cadherin/catenin adhesion complex in the development and progression of cancer.** *Mol Cell Biol Res Commun* 1999, **2(2):**77-85.
36. Anderson E: **The role of oestrogen and progesterone receptors in human mammary development and tumorigenesis.** ***Breast Cancer Res*** 2002, **4(5):**197-201.
37. Reis-Filho JS, Torio B, Albergaria A, Schmitt F: **p63 expression in normal skin and usual cutaneous carcinomas.** *J Cutan Pathol* 2002, **29(9):**517-23.
38. Shick PC, Riordan GP, Foss RD: **Estrogen and progesterone receptors in salivary gland adenoid cystic carcinoma.** *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1995, **80(4):**440-4.
39. Porrelo A, Cardelli P, Spugnini EP: **Pet models in cancer research: general principles.** *J Exp Clin Cancer Res* 2004, **23(2):**181-93.
40. McLeod KF, Jacks T: **Insights into cancer from transgenic mouse models.** *J Pathol* 1999, **187(1):**43-60.
41. Rivera EM: **Mammary gland culture.** In *Methods in Mammalian Embryology* Edited by: Daniel JC Jr. San Francisco: WH Freeman Company; 1971.
42. Vail DM, MacEwen EG: **Spontaneously occurring tumors of companion animals as models for human cancer.** *Cancer Invest* 2000, **18(8):**781-92.
43. Schneider R, Dorn CR, Taylor DO: **Factors influencing canine mammary cancer development and postsurgical survival.** *J Natl Cancer Inst* 1969, **43(6):**1249-61.
44. Genelhu MC, Gobbi H, Soares FA, Campos AH, Ribeiro CA, Cassali GD: **Immunohistochemical expression of p63 in pleomorphic adenomas and carcinomas ex-pleomorphic adenomas of salivary glands.** *Oral Oncol* 2006, **42(2):**154-60.
45. Yang A, Kaghad M, Wang Y, Gillett E, Fleming MD, Dötsch V, Andrews NC, Caput D, McKeon F: **p63, a p53 homolog at 3q27–29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities.** *Molecular Cell* 1998, **2(3):**305-16.
46. Shih HC, Shiozawa T, Miyamoto T, Kashima H, Feng YZ, Kurai M, Konishi I: **Immunohistochemical expression of E-cadherin and beta-catenin in the normal and malignant human endometrium: an inverse correlation between E-cadherin and nuclear beta-catenin expression.** *Anticancer Res* 2004, **24(6):**3843-50.
47. Harrington KJ, Syrigos KN: **The role of E-cadherin-catenin complex: more than intercellular glue?** *Ann Surg Oncol* 2000, **7(10):**783-8. Erratum in: *Ann Surg Oncol* 2001 Mar, 8(2):186.
48. Shiozaki H, Oka H, Inoue M, Tamura S, Monden M: **E-cadherin mediated adhesion system in cancer cells.** *Cancer* **77(8 Suppl):**1605-13. 1996 Apr 15
49. Genelhu MC, Gobbi H, Arantes DC, Cardoso SV, Cassali GD: **Immunolocalization of beta-Catenin in Pleomorphic Adenomas and Carcinomas Ex-pleomorphic Adenomas of Salivary Glands.** *Appl Immunohistochem Mol Morphol* 2007, **15(3):**273-278.
50. Hamilton JM, Else RW, Forshaw P: **Oestrogen receptors in canine mammary tumours.** *Vet Rec* **101(13):**258-60. 1977 Sep 24
51. Graham JC, O'Keefe DA, Gelberg HB: **Immunohistochemical assay for detecting estrogen receptors in canine mammary tumors.** *Am J Vet Res* 1999, **60(5):**627-30.
52. Nieto A, Peña L, Perez-Alenza MD, Sanchez MA, Flores JM, Castano M: **Immunohistologic detection of estrogen receptor alpha in canine mammary tumors: clinical and pathologic associations and prognostic significance.** *Vet Pathol* 2000, **37(3):**239-47.
53. Miller AS, Hartman GG, Sow-Yeh C, Edmonds PR, Brightman SA, Harwick RD: **Estrogen receptor assay in polymorphous low-grade adenocarcinoma and adenoid cystic carcinoma of salivary gland origin. An immunohistochemical study.** *Oral Surg Oral Med Oral Pathol* 1994, **77(1):**36-40.
54. Dori S, Trougouboff P, David R, Buchner A: **Immunohistochemical evaluation of estrogen and progesterone receptors in adenoid cystic carcinoma of salivary gland origin.** *Oral Oncol* 2000, **36(5):**450-3.
55. Jeannon JP, Soames JV, Bell H, Wilson JA: **Immunohistochemical detection of oestrogen and progesterone receptors in salivary tumours.** *Clin Otolaryngol Allied Sci* 1999, **24(1):**52-4.
56. Gaffney EV, Pinkstone JA, Eidson JJ: **Estrogen receptors in parotid tumors.** *Endocr Res* 1995, **21(3):**635-43.
57. Leimola-Virtanen R, Salo T, Toikkanen S, Pulkkinen J, Syrjänen S: **Expression of estrogen receptor (ER) in oral mucosa and salivary glands.** *Maturitas* 2000, **36(2):**131-7.

Pre-publication history

The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-2407/7/218/prepub

CONCLUSÃO

- A análise histológica dos tumores mamários caninos e dos tumores de glândula salivar humana confirmou a notável semelhança morfológica entre essas neoplasias.

- As semelhanças histomorfológicas foram corroboradas pela caracterização imuno-histoquímica dos componentes tumorais. A transformação neoplásica do epitélio está associada com trocas antigênicas similares, o que sugere que, em ambos os sítios anatômicos (glândulas mamárias e glândulas salivares) e em ambas as espécies (canina e humana), a tumorigênese possa ser caracterizada por uma via patogenética comum.

- As disparidades encontradas na expressão dos antígenos p63 e receptor de estrógeno devem ser melhor investigadas, a fim de esclarecer a influência desses marcadores no comportamento biológico das neoplasias estudadas.

- Finalmente, os resultados deste trabalho sugerem que os tumores mistos da glândula mamária canina podem ser utilizados, em alguns aspectos, como modelo comparativo para o estudo dos tumores mistos da glândula salivar da espécie humana.

CONSIDERAÇÕES FINAIS

Este trabalho pretendeu contribuir para a caracterização de alguns aspectos moleculares (antigênicos) e demográficos das neoplasias mamárias caninas genericamente chamadas de tumores mistos, com a finalidade de averiguar o seu valor como modelo comparativo para o estudo dos tumores mistos das glândulas salivares humanas.

Assim, verificou-se que os marcadores correntemente utilizados para distinguir os diferentes componentes celulares dos adenomas pleomórficos e carcinomas ex-adenomas pleomórficos de glândula salivar humana (AE1/AE3 e vimentina) integram as mesmas estruturas na glândula mamária canina, permitindo reforçar a noção de uma mesma origem celular monoclonal para os dois grupos de neoplasias estudadas.

Entre os marcadores utilizados não foi possível identificar um que distinguisse lesões benignas e malignas, mas a utilização dos anticorpos p63, RE, E-caderina e β-catenina resultou na identificação de semelhanças (E-caderina e β-catenina) e diferenças (p63 e RE) de marcação entre os tumores mamários caninos e os tumores de glândula salivar da espécie humana.

Nos casos de E-caderina e β-catenina, os resultados permitiram inferir estreita relação de localização nuclear com malignidade e "pré-malignidade", tanto nos tumores mamários caninos quanto nos de glândula salivar humana. Por outro lado, p63 permitiu a distinção entre tumores bem diferenciados e pouco diferenciados/indiferenciados somente nos tumores malignos (Ca ex-AP) de glândula salivar humana.

Apesar de desempenhar um importante papel na carcinogênese mamária canina, o que pode dar suporte ao uso da terapia hormonal em medicina veterinária, RE parece não

ter relevância biológica para os tumores de glândula salivar humana. É importante ressaltar que devido à transcrição de RNAm, outros estudos com RE em tumores de glândula salivar humana precisam ser realizados até que se chegue a resultados conclusivos.

Tomados em conjunto, os dados demográficos, clínicos e imuno-histoquímicos deste estudo sugerem uma possível utilização dos tumores mistos das glândulas mamárias caninas como modelo de estudo comparativo para os tumores mistos das glândulas salivares da espécie humana.

REFERÊNCIAS BIBLIOGRÁFICAS

1. Abbey LM, Schwab BH, Landau GC, Perkins ER. Incidence of second primary breast cancer among patients with a first primary salivary gland tumor. Cancer 1984; 54(7): 1439-42.
2. Aberle AM, Abrams AM, Bowe R, Melrose RJ, Handlers JP. Lobular (polymorphous low-grade) carcinoma of minor salivary glands: a clinicopathologic study of twenty cases. Oral Surg Oral Med Oral Pathol. 1985; 60: 387-95.
3. Alves VAF, Bacchi CE, Vassalo J. Manual de Imuno-histoquímica. São Paulo: Sociedade Brasileira de Patologia, 1999.
4. Araújo VC, Raitz R. Estudo do estado de diferenciação da célula mioepitelial nas neoplasias de glândula salivar. Acta Scientiarum. 2004; 26(2): 345-50.
5. Araújo VC, Souza SOM, Carvalho YR, Araújo NS. Application of immunohistochemistry to the diagnosis of salivary gland tumors. Appl Immunohistoch Mol Morph. 2000; 8(2): 196-202.
6. Barbareschi M, Pecciarini L, Cangi MG, Macrì E, Rizzo A, Viale G, Doglione C. p63, a p53 homologue, is a selective nuclear marker of myoepithelial cells of the human breast. Am J Surg Pathol. 2001; 25(8): 1054-60.
7. Batzakis JG, Kraemer B, Sciubba JJ. The pathology of head and neck tumors: the myoepithelial cell and its participation in salivary gland neoplasia, Part 17. Head Neck Surg. 1983; 5(3): 222-33.

8. Berg JW, Hutter RV, Foote FW. The unique association between salivary gland cancer and breast cancer. JAMA 1968; 204: 771-4.
9. Bilal H, Handra-Luca A, Bertrand JC, Fouret PJ. p63 is expressed in basal and myoepithelial cells of human normal and tumor salivary gland tissues. J Histochem Cytochem. 2003; 51:133-9.
10. Björling DE, Beckman M, Clayton MK, Wang ZY. Modulation of nerve growth factor in peripheral organs by estrogen and progesterone. Neuroscience. 2002; 110(1): 155-67.
11. Brasileiro-Filho G, Barbosa AJA, Miranda D. Métodos de estudo em patologia. In: Brasileiro-Filho G. Bogliolo – Patologia. 6ed. Rio de Janeiro: Guanabara Koogan, 2004. p.6-18.
12. Cassali GD, Gobbi H, Gärtner F, Schmitt F. Secretory carcinoma of the canine mammary gland. Vet Pathol. 1999b, 36:601-03.
13. Cassali GD. Canine mammary tumours. A morphological and immunohistochemistry study: comparative aspects with human breast tumours. Virch Arch. 1999a; 435(3): 622.
14. Cassali GD. Estudo morfológico, imuno-histoquímico e citométrico de tumores mamários da cadela. Aspectos comparativos com neoplasias da mama humana. Escola de Veterinária da Universidade Federal de Minas Gerais. 2000, 73p. Tese (Doutorado em Ciência Animal).
15. Cassali GD. Patologias da glândula mamária In: Nascimento EF, Lima Santos R. Patologia da Reprodução dos Animais Domésticos. 2ed. Rio de Janeiro: Guanabara Koogan; 2002. p.117-133.

16. Chen KTK. Pleomorphic adenoma of the breast. Am J Clin Pathol. 1990; 93: 792-4.
17. Cho KJ, Lee YS. Proliferating cell nuclear antigen and c-*erb*B-2 oncoprotein expression in adenoid cystic carcinomas of the salivary glands. Head Neck. 1999; 21(5): 414-9.
18. Clark JW, Sell L, Shiu RP, Orr FW, Maitre N, Vary CP, Cole DJ, Watson PH. The potential role for prolactin-inducible protein (PIP) as a marker of human breast cancer micrometastasis. Br J Cancer. 1999; 81(6): 1002-8.
19. Cohen D, Reif J, Brooy RS; Keiser H. Epidemiological analysis of the most prevalent sites and types of canine neoplasia observed in a veterinary hospital. Cancer Res. 1974; 34:2859-68.
20. Damiani S, Pasquinelli G, Lamovec J, Peterse JL, Eusebi V. Acinic cell carcinoma of the breast: an immunohistochemical and ultrastructural study. Virch Arch. 2000; 43(1): 74-81.
21. Dardick I, Van Nostrand AW, Phillips MJ. Histogenesis of salivary gland pleomorphic adenoma (mixed tumor) with an evaluation of the role of the myoepithelial cell. Hum Pathol. 1982; 13(1): 62-75.
22. Dimery IW, Jones LA, Verjan RP, Raymond AK, Goepfert H, Hong WK. Estrogen receptors in normal salivary gland and salivary gland carcinoma. Arch Otolaryngol Head Neck Surg. 1987; 113(10):1082-5.
23. Dori S, Trougouboff P, David R, Buchner A. Immunohistochemical evaluation of estrogen and progesterone receptors in adenoid cystic carcinoma of salivary gland origin. Oral Oncol. 2000; 36(5):450-53.

24. Dunn J, Bragg K, Sautter C Gardipee C. Breast Cancer risk following a major salivary gland carcinoma. Cancer 1972; 29:1343-6.

25. Duprey P, Paulin D. What can be learned from intermediate filament gene regulation in the mouse embryo. Review. Int J Dev Biol. 1995; 39(3): 443-57.

26. Economopoulou P, Hamby A, Odell EW. Expression of E-cadherin, cellular differentiation and polarity in epithelial salivary neoplasms. Oral Oncol. 2000; 36(6): 15-8.

27. Edwards PC, Bhuiya T, Kelsch RD. Assessment of p63 expression in the salivary gland neoplasms adenoid cystic carcinoma, polymorphous low-grade adenocarcinoma, and basal cell and canalicular adenomas. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004; 97(5):613-9.

28. Elling H, Ungemach FR. Simultaneous occurrence of receptors for estradiol, progesterone and dihidrotestosterone in canine mammary tumors. J. Cancer Res. Clin. Oncol. 1983;105:231-37.

29. Evans CR, Pierrepoint CG. Tissue-steroid interactions in canine hormone-dependent tumors. Vet. Rec. 1975; 13:464-467.

30. Fernandes MMG. Estudo da patologia dos tumores mamários caninos através de técnicas de imuno-histoquímica e citometria estática. Faculdade de Medicina da Universidade do Porto, 1996, 93p. Dissertação (Mestrado em Oncobiologia).

31. Flores Alés AJ. Factores epidemiológicos de interés pronóstico en los tumores de mama en la perra. 1997; 14(5): 273-80.

32. Foschini MP, Scarpellini F, Gown AM, Eusebi V. Differential Expression of Myoepithelial Markers in Salivary, Sweat and Mammary Glands. Int J Surg Pathol. 2000; 8(1): 29-37.

33. Freedman PD, Lumerman H. Lobular carcinoma of intraoral minor salivary gland origin. Report of twelve cases. Oral Surg Oral Med Oral Pathol. 1983; 56(2): 157-66.

34. Furuse c, Souza SO, Nunes FD, Magalhães MH, Araújo VC. Myoepithelial cells in salivary gland neoplasms. Int J Surg Pathol. 2005: 13(1): 57-65.

35. Gaffney EV, Pinkstone JA, Eidson JJ. Estrogen receptors in parotid tumors. Endocr Res. 1995; 21(3): 635-43.

36. Gama A, Alves A, Gärtner F, Schmitt F. p63: a novel myoepithelial cell marker in canine mammary tissues. Vet Pathol. 2003; 40(4): 412-20.

37. Gärtner F, Geraldes M, Cassali G, Rema A, Schmitt F. DNA measurement and immunohistochemical characterization of epithelial and mesenchymal cells in canine mixed mammary tumours: putative evidence for a common histogenesis. Vet J. 1999; 158(1): 39-47.

38. Geraldes M, Gärtner F, Schmitt F. An immunohistochemistry study of hormonal receptors and cell proliferation in normal canine mammary glands and spontaneous mammary tumors. Vet Rec. 2000; 146(14): 403-06.

39. Glas AS, Hollema H, Nap RE, Plukker JT. Expression of estrogen receptor, progesterone receptor, and insulin-like growth factor receptor-1 and MIB-1 in patients with recurrent pleomorphic adenoma of the parotid gland. Cancer 2002; 94(8): 2211-6.

40. Gnepp DR. Malignant mixed tumors of the salivary glands: a review. Pathol Annu. 1993; 28 Pt 1:279-328.

41. Gobbi H, Dupont WD, Parl FF, Schuyler PA, Plummer WD, Olson SJ, Page DL. Breast cancer risk associated with estrogen receptor expression in epithelial hyperplasia lacking atypia and adjacent lobular units. Int J Cancer. 2005; 113(5):857-9.

42. Gomes LR. Estudo imuno-histoquímico da expressão de E-caderina e P-caderina em tumores mamários caninos. Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, 2004, 95p. Dissertação (Mestrado em Patologia).

43. Graham JC, O'Keefe DA, Gelberg HB. Immunohistochemical assay for detecting estrogen receptors in canine mammary tumors. Am J Vet Res. 1999; 60(5): 627-30.

44. Hamilton JM, Else RW, Forshaw P. Oestrogen receptors in canine mammary tumors. Vet Rec. 1977; 101: 258-60.

45. Hampe JF, Misdorp W. Tumours and dysplasias of the mammary gland. Bull World Health Organ. 1974; 50(1-2): 111-33.

46. Harrington KJ, Syrigos KN. The role of E-cadherin-catenin complex: more than an intercellular glue? Ann Surg Oncol. 2000; 7(10):783-8.

47. Hayes MM, Lesack D, Girardet C, Del Vecchio C, Eusebi V. Carcinoma ex-pleomorphic adenoma of the breast. Report of three cases suggesting a relationship to metaplastic carcinoma of matrix-producing type. Virch Arch. 2005; 446(2): 142-9.

48. Hayward SW, Brody JR, Cunha GR. An edgewise look at basal epithelial cells; three-dimensional views of the rat prostate, mammary gland and salivary gland. Differentiation. 1996; 60: 219-27.

49. Hirokawa M, Sugihara K, Sai T, Monobe Y, Kudo H, Sano N, Sano T. Secretory carcinoma of the breast: a tumor analogous to salivary gland acinic cell carcinoma? Histopathology. 2002; 40(3):223-29.

50. Hoang MP, Callender DL, Sola Gallego JJ, Huang Z, Sneige N, Luna MA, Batsakis JG, El-Naggar AK. Molecular and biomarker analyses of salivary duct carcinomas: comparison with mammary duct carcinoma. Int. J. Oncol. 2001; 10(4): 865-871.

51. Jankowski JA, Bruton R, Shepherd N, Sanders DS. Cadherin and catenin biology represent a global mechanism for epithelial cancer progression. Mol Pathol. 1997; 50(6):289-90.

52. Jeannon JP, Soames JV, Bell H, Wilson JA. Immunohistochemical detection of oestrogen and progesterone receptors in salivary tumours. Clin Otolaryngol. 1999; 24(1):52-4.

53. Kalof AN, Tam D, Beatty B, Cooper K. Immunostaining of myoepithelial cells in breast lesions: a comparison of CD10 and smooth muscle myosin heavy chain. J Clin Pathol. 2004; 57:625-29.

54. Koga F, Kawakami S, Kumagai J Takizawa T, Ando N, Arai G, Kageyama Y, Kihara K. Impaired DeltaNp63 expression associates with reduced beta-catenin and aggressive phenotypes of urothelial neoplasms. Br J Cancer 2003; 88: 740-47.

55. Kumagami H, Onitsuka T. Estradiol and testosterone in minor salivary glands of Sjögren's syndrome. Auris Nasus Larynx. 1993; 20: 137-143.

56. Kumar V, Kingen MW. In: Kumar V, Abbas AK, Fausto N. Cabeça e Pescoço. Robbins & Cotran Patologia – Patológicas das Doenças. 7ed. Rio de Janeiro:

57. Lakhani SR, O'Hare MJ. The mammary myoepithelial cell – Cinderella or ugly sister? Breast Cancer Res. 2001; 3: 1-4.

58. Lamey PJ, Leake RE, Cowan SK, Soutar DS, McGregor JA, McGregor FM. Steroid hormone receptors in human salivary gland tumours. J Clin Pathol. 1987; 40(5): 532-4.

59. Lebeau A. L'âge du chien et celui de l'homme essai de statistique sur la mortalité canine. Bulletin de l'Académie Vétérinaire. 1953; 26:229-32.

60. Leimola-Virtanen R, Salo T, Toikkanen S, Pulkkinen J, Syrjänen S. Expression of estrogen receptor (ER) in oral mucosa and salivary glands. Maturitas. 2000; 36(2):131-7.

61. Leong ASY, Wright J. The contribution of immunohistochemical staining in tumour diagnosis. Histopathology 1987; 11(12):1295-305.

62. Little NA, Jochemsen AG. p63. Int J Biochem Cell Biol. 2002; 43:6-9.

63. MacEwen EG, Patnaik AK, Harvey HJ, Panko WB. Estrogen receptors in canine mammary tumors. Cancer Res. 1982; 42(6): 2255-9.

64. Machado GF, Figueiredo F. Revisão: Filamentos intermediários. Medicina, Ribeirão Preto; 1996; 29: 104-113.

65. Marchant J. Animal model for tumors of the female genital tract. In: Kurman RJ. Blaustein's pathology of female genital tract. 3ed. New York: Springer Verlag, 1987. p.899-924.

66. Margaritescu C, Florescu M, Raica M, Simionescu C, Mogoanta L, Preda E. The immunohistochemical profile of luminal epithelial neoplastic component from pleomorphic adenomas of salivary glands. Rom J Morphol Embryol. 1999-2004; 45: 97-118.
67. Martin PM, Cotard M, Mialot JP, Andre F, Raynaud JP. Animal model for hormone-dependent human breast cancer. Relationship between steroid receptor profiles in canine and feline mammary tumors and survival rate. Cancer Chemother Pharmacol. 1984; 12(1): 13-17.
68. Mialot M, Lagadic M. Epidémiologie des tumours du chien et du chat. Rec Méd Vét. 1990; 166(11): 937-47.
69. Miller AS, Hartman GG, Sow-Yeh C, Edmonds PR, Brightman SA, Harwick RD. Estrogen receptor assay in polymorphous low-grade adenocarcinoma and adenoid cystic carcinoma of salivary gland origin. An immunohistochemical study. Oral Surg Oral Med Oral Pathol. 1994; 77:36-40.
70. Miller WR. Oestrogens and breast cancer: biological considerations. Br Med Bull. 1991; 47:470-83.
71. Miller WR. Pathways of hormone metabolism in normal and nonneoplastic breast tissue. Ann N Y Acad Sci. 1990; 586:53-9.
72. Moran CA, Suster S, Carter D. Benign mixed tumors (pleomorphic adenomas of the breast. Am J Surg Pathol. 1990; 14(10): 913-21.
73. Mottolese M, Sciarretta F, Antonucci G, Natali PG. Spontaneous canine mammary tumors. A model for monoclonal antibody diagnosis and treatment of human breast cancer. Lab Invest. 1994; 71(2):182-87.

74. Moulton JE, Rosemblatt LS, Goldman M. Mammary tumors in a colony of beagle dogs. Vet. Pathol. 1996; 223:741-49.

75. Moulton JE, Taylor DON, Dorn CR, Andersen AC. Canine mammary tumors. Pathol Vet. 1970; 7(3): 289-320.

76. Moulton JE. Tumors of the mammary glands. In: Moulton JE. Tumors in domestic animals. 3ed. University of California Press, Berkeley, 1990. p.518-552.

77. Nagao T, Sugano I, Ishida Y, Tajima Y, Matsuzaki O, Cono A, Kondo Y, Nagao K. Salivary gland malignant myoepithelioma: a clinicopathologic and immunohistochemical study of ten cases. Cancer 1998; 83: 1292-99.

78. Nasser SM, Faquin WC, Dayal Y. Expression of androgen, estrogen, and progesterone receptors in salivary gland tumors. Frequent expression of androgen receptor in a subset of malignant salivary gland tumors. Am J Clin Pathol. 2003; 119(6): 801-6.

79. Nicol KK, Iskandar SS. Lobular carcinoma of the breast metastatic to the oral cavity mimicking polymorphous low-grade adenocarcinoma of the minor salivary glands. Arch Pathol Lab Med. 2000; 124(1): 157-9.

80. Nieto A, Peña L, Perez-Alenza MD, Sanchez MA, Flores JM, Castano M. Immunohistologic detection of estrogen receptor alpha in canine mammary tumors: clinical and pathologic associations and prognostic significance. Vet Pathol. 2000; 37(3): 239-47.

81. Nikitakis NG, Tosios KI, Papanikolaou VS, Rivera H, Papanikolaou SI, Ioffe OB. Immunohistochemical expression of cytokeratins 7 and 20 in malignant salivary glands tumors. Mod Pathol. 2004; 17(4): 407-15.

82. Patturajan M, Nomoto S, Sommer M, Fomenkov A, Hibi K, Zangen R, Poliak N, Califano J, Trink B, Ratovitski E, Sidransky D. DeltaNp63 induces beta-catenin nuclear accumulation and signaling. Cancer Cell. 2002 May;1(4):369-79.

83. Peleteiro MC. Tumores mamários na cadela e na gata. Revista Portuguesa de Ciências Veterinárias. 1994; 89(509):10-29.

84. Pia-Foschini M, Reis-Filho JS, Eusebi V, Lakhani S R. Salivary gland-like tumours of the breast: surgical and molecular pathology. J Clin Pathol. 2003; 56:497–506.

85. Prior P, Waterhouse JAH. Second primary cancers in patients with tumours of the salivary glands. Br J Cancer 1977; 36:362-7.

86. Reid-Nicholson M, Bleiweiss I, Pace B, Azueta V, Jaffer S. Pleomorphic adenoma of the breast. A case report and distinction from mucinous carcinoma. Arch Pathol Lab Med. 2003; 127(4): 474-7.

87. Reis-Filho JS, Schmitt FC. Taking advantage of basic research: p63 is a reliable myoepithelial and stem cell marker. Adv Anat Pathol. 2002a; 9(5): 280-9.

88. Reis-Filho JS, Simpson PT, Fulford LG, Martins A, Schmitt F. p63-driven nuclear accumulation of β-catenin is not a frequent event in human neoplasms. Pathol Res Pract. 2003; 199(12): 785-93.

89. Reis-Filho JS, Torio B, Albergaria A, Schmitt F. p63 expression in normal skin and usual cutaneous carcinomas. J Cutan Pathol. 2002b; 29:517-23.

90. Restucci B, Papparella S, De Vico, Maiolino P. E cadherin expression in normal and neoplastic canine mammary gland. J Comp Pathol. 1997;116(2):191-202.

91. Rosen PP, Oberman HA. Tumors of the mammary gland. Washington: Armed Forces Institute of Pathology, 1993, 390p.

92. Santos JA. Patologia Especial dos Animais Domésticos 2ed. Rio de Janeiro: Interamericana. 1979. p.150-60.

93. Sarli G, Preziosi R, De Tolla L, Brunetti B, Benazzi C. E-cadherin immunoreactivity in canine mammary tumors. J Vet Diagn Invest. 2004; 16(6):542-7.

94. Savera AT, Gown AM, Zarbo RJ. Immunolocalization of three novel smooth muscle-specific proteins in salivary pleomorphic adenoma: assessment of the morphogenetic role of myoepithelium. Mod Pathol. 1997; 10(11): 1093-100.

95. Savera AT, Zarbo RJ. Defining the role of myoepithelium in salivary gland neoplasia. Adv Anat Pathol. 2004; 11(2):69-85.

96. Schafer KA, Kelly G, Schrader R, Griffith WC, Muggenburg BA, Tierney LA et al. A canine model of familial mammary gland neoplasia. Vet Pathol. 1998; 35(3): 168-77.

97. Schneider R, Dorn CR, Taylor DO. Factors influencing canine mammary cancer development and post surgical survival. J Natl Cancer Inst. 1969; 43(6):1249-61.

98. Schneider R. Comparison of age, Sex and incidence rates in human and canine breast cancer. Cancer. 1970; 26(2):419-26.

99. Scholl S, Beuzeboc P, Pouillart P. Targenting HER2 in other tumor types. Ann Oncol. 2001; 12 Suppl1: S81-7.

100. Scott J. A morphometric study of age changes in the histology of the ducts of human submandibular salivary glands. Arch Oral Biol. 1977; 22(3): 221-7a.

101. Scott J. Quantitative age changes in the histological structure of human submandibular salivary glands. Arch Oral Biol. 1977; 22(4): 243-9b.

102. Seifert G. Are adenomyoepithelioma of the breast and myoepithelial-myoepithelial carcinoma of the salivary glands identical tumours? Virch Arch. 1998; 433:285-7.

103. Shick PC, Riordan GP, Foss RD. Estrogen and progesterone receptors in salivary gland adenoid cystic carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1995; 80(4): 440-4.

104. Shieh YS, Chang LC, Chiu KC, Wu CW, Lee HS. Cadherin and catenin expression in mucoepidermoid carcinoma: correlation with histopathologic grade, clinical stage, and patient outcome. J Oral Pathol Med. 2003; 32(5):297-304.

105. Shiozaki H, Oka H, Inoue M, Tamura S, Monden M. E-cadherin mediated adhesion system in cancer cells. Cancer. 1996; 77(8 Suppl): 1605-13.

106. Simpson RH, Cope N, Skalova A, Michal M. Malignant adenomyoepithelioma of the breast with mixed osteogenic, spindle cell, and carcinomatous differentiation. Am J Surg Pathol. 1998; 22(5): 631-6.

107. Skálová A, Stárek I, Kucerová V, Szépe P, Plank L. Salivary duct carcinoma: a highly aggressive salivary gland tumors with HER-2/neu oncoprotein overexpression. Pathol Res Pract. 2001; 197(9):621-26.

108. Sternlicht MD, Kadeshian P, Shao ZN, Safarians S, Barsky SH. The human myoepithelial cell is a natural tumor suppressor. Clin Cancer Res. 1997; 3:1949-58.

109. Strandberg JD, Goodman DG. Breast cancer – Animal model: canine mammary neoplasia. Am J Pathol. 1974; 75(1):225-28.

110. Sugano I, Nagao T, Tajima Y, Ishida Y, Nagao K, Ooeda Y, Takahashi T. Malignant adenomyoepithelioma of the breast: non-tubular and matrix-producing variant. Pathol. Int. 2001; 51(3): 193-9.

111. Szabó C, Wagner LA, Francisco LV, Roach JC, Argonza R, King MC, Ostrander EA. Human, canine and murine BRCA1 genes sequence comparison among species. Human Mol. Genet. 1996; 5(9):1289-98.

112. Taylor GN, Shabestari L, Williams J, Mays CW, Angus W, McFarland S. Mammary neoplasia in a closed beagle colony. Cancer Res. 1976; 36(8):2740-43.

113. Weber A, Langhanki L, Schütz A, Schütz A, Gerstner A, Bootz F, Wittekind C, Tannapfel A. Expression profiles of p53, p63, and p73 in benign salivary gland tumors. Virch Arch. 2002; 441(5):428-36.

114. Wenig BM, Gnepp DR. Polymorphous low-grade adenocarcinoma of minor salivary glands. In: Ellis GL, Auclair PL, Gnepp DR, editors. Surgical pathology of the salivary glands. Philadelphia: WB Saunders Co; 1991. p.390-411.

115. Werling RW, Hwang H, Yaziji H; Gown AM. Immunohistochemical distinction of invasive from noninvasive breast lesions: a comparative study of p63 versus calponin and smooth muscle myosin heavy chain. Am J Surg Pathol. 2003; 27(1): 82-90.

116. Wick MR, Ockner DM, Mills SE, Ritter JH, Swanson PE. Homologous carcinomas of the breast, skin, and salivary glands. A histologic and immunohistochemical comparison of ductal mammary carcinoma, ductal sweat gland carcinomas, and salivary duct carcinoma. Am J Clin Pathol. 1998; 109(1):75-84.

117. Yang A, Kaghad M, Wang Y, Gillett E, Fleming MD, Dötsch V et al. p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. Mol Cell. 1998; 2:305-16.

118. Yazigi H, Gown AM, Sneige N. Detection of stromal invasion in breast cancer: the myoepithelial markers. Adv Anat Pathol. 2000; 7(2): 100-9.

119. Zarbo RJ, Prasad AR, Regezi JA, Gown AM, Savera AT. Salivary gland basal cell and canalicular adenomas. Immunohistochemical demonstration of myoepithelial cell participation and morphogenetic considerations. Arch Pathol Lab med. 2000; 124:401-5.

120. Zhang, ZY, WU YQ, Zhang WG, Tian Z, Cao J. The expression of E-cadherin-catenin complex in adenoid cystic carcinoma of salivary glands. Chin J Dent Res. 2000 Nov;3(3):36-9.

121. Zhuang Z, Lininger RA, Man Y, Albuquerque A, Merino MJ, Tavassoli FA. Identical clonality of both components of mammary carcinosarcoma with differential loss heterozygosity. Mod. Pathol.1997; 10(4): 354-62.

ANEXOS

Anexo 1

UFMG

Universidade Federal de Minas Gerais
Comitê de Ética em Pesquisa da UFMG - COEP

Parecer nº. ETIC 193/04

Interessado: Prof. Dr. Giovani Dantas Cassali
Instituto de Ciências Biológicas – ICB/UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP, aprovou no dia 23 de junho de 2004 o projeto de pesquisa intitulado **« Tumores Mistos e Carcinomas Metaplásicos de Glândula Mamária da Cadela: Aspectos Comparativos com Tumores de Glândula Salivar da Espécie Humana. »** e o Termo de Consentimento Livre e Esclarecido do referido projeto.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

Profa. Dra. Maria Elena Lima Perez Garcia
Presidente do COEP/UFMG

Av. Presidente Antônio Carlos, 6627, Prédio da Reitoria – 7º andar sala: 7018 - 31.270-901– BH - MG
(31) 3499-4592 - FAX: (31) 3499-4027 - coep@prpq.ufmg.br

Anexo 2

Comunicações Científicas Relacionadas ao Trabalho de Tese

Apresentações Orais

- Apresentação Oral de Tema Livre: ***Tumores Mistos e Carcinomas Metaplásicos de Glândula Mamária da Cadela: Aspectos Comparativos com Tumores de Glândula Salivar da Espécie Humana.*** GENELHU, M.C.L.S.; GOBBI, H.; SOARES, F.A.; CAMPOS, A.H.J.M.; CASSALI, G.D. X Fórum de Pesquisa Básica e Clínica em Câncer de Cabeça e Pescoço. Centro de Tratamento e Pesquisa Hospital do Câncer, São Paulo, 26-28 de junho de 2003.

- Apresentação Oral de Tema Livre: ***Tumores mistos e carcinomas metaplásicos de glândula mamária da cadela: aspectos comparativos com tumores de glândula salivar da espécie humana.*** GENELHU MCLS, GOBBI H, SOARES FA, CAMPOS AJHFM, CASSALI GD. Simpósio de Pesquisa e Iniciação Científica da Universidade Vale do Rio Doce – UNIVALE, Governador Valadares, 01a 03 de outubro de 2003.

- Apresentação Oral de Tema Livre: ***Immunohistochemical expression of p63 in pleomorphic adenomas and carcinomas ex-pleomorphic adenomas of salivary glands.*** GENELHU MCLS, GOBBI H, SOARES FA, CAMPOS AHJFM, RIBEIRO CA, CASSALI GD. II Intercontinental Congress of Pathology, Foz do Iguaçu, Brasil, 09-13 de junho de 2004.

- Apresentação Oral de Tema Livre: ***Immunolocalization of β-catenin in pleomorphic adenomas and carcinomas ex-pleomorphic adenomas of salivary gland.*** GENELHU MCLS, GOBBI H, ARANTES, DCB, SOARES FA, CAMPOS AHJFM, RIBEIRO CA, CASSALI GD. XXV Congresso Brasileiro de Patologia, Natal, Brasil, 12 a 15 de outubro de 2005.

Apresentação de Pôsteres

- Apresentação do Pôster: ***Tumores mistos e carcinomas metaplásicos de glândula mamária da cadela: aspectos comparativos com tumores de glândula salivar da espécie humana.*** GENELHU MCLS, GOBBI H, SOARES FA, CAMPOS AJHFM, CASSALI GD. XVI Congresso Brasileiro de Cancerologia e XIII Congresso Brasileiro de Oncologia Clínica, 26 a 30 de novembro de 2003.

- Apresentação do Pôster: ***p63, um novo marcador de stem cells/células mioepiteliais de tumores mistos e carcinomas em tumores mistos da glândula mamária da cadela.*** COSTA FA, GENELHU MCLS, GOBBI H, CASSALI GD. XII Semana de Iniciação Científica da UFMG, 09 a 12 de dezembro de 2003.

- Apresentação do Pôster: ***Mixed tumors and metaplastic carcinomas of the mammary glands of the female dogs: comparative aspects with human salivary glands tumors. A new model of study.*** GENELHU MCLS, GOBBI H, SOARES FA, RIBEIRO, CA, CASSALI GD. 12th Annual Meeting of the Portuguese Society of Animal Pathology jointly with 16th Annual Meeting of the Spanish Society of Veterinary Pathology, Vila Nova de Famalicão, Portugal, 2 a 4 junho de 2004.

- Apresentação do Pôster: ***p63, um novo marcador de stem cells/células mioepiteliais de tumores mistos e carcinomas em tumores mistos da glândula mamária da cadela.*** COSTA FA, GENELHU MCLS, GOBBI H, CASSALI GD. 12th Annual Meeting of the Portuguese Society of Animal Pathology jointly with 16th Annual Meeting of the Spanish Society of Veterinary Pathology, Vila Nova de Famalicão, Portugal, 2 a 4 junho de 2004.

- Apresentação do Pôster: ***Tumores mistos e carcinomas metaplásicos de glândula mamária da cadela: aspectos comparativos com tumores de glândula salivar da espécie humana. Um novo modelo de estudo.*** GENELHU MCLS, GOBBI H, SOARES FA, CAMPOS AJHFM, CASSALI GD. VI Simpósio Mineiro de Oncologia, Belo Horizonte, Brasil, 14 a 17 de abril de 2004.

- Apresentação do Pôster: ***p63, um novo marcador de stem cells/células mioepiteliais de tumores mistos e carcinomas em tumores mistos da glândula mamária da cadela.*** COSTA FA, GENELHU MCLS, GOBBI H, CASSALI GD. VI Simpósio Mineiro de Oncologia, Belo Horizonte, Brasil, 14 a 17 de abril de 2004.

- Apresentação do Pôster: ***Immunohistochemical expression of p63 expression in pleomorphic adenomas and carcinomas ex-pleomorphic adenomas of salivary glands.*** GENELHU MCLS, GOBBI H, SOARES FA, CAMPOS AHJFM, RIBEIRO CA, CASSALI GD. Reunião Anual da Sociedade Brasileira de Pesquisa Odontológica – SBPqO, Águas de Lindóia, Brasil, 8 a 12 de setembro de 2004.

Resumos publicados em revistas indexadas:

- ***Tumores mistos e carcinomas metaplásicos de glândula mamária da cadela: aspectos comparativos com tumores de glândula salivar da espécie humana.*** GENELHU MCLS, GOBBI H, SOARES FA, CAMPOS AJHFM, CASSALI GD. *Revista Prática Hospitalar* (ISSN 1679-5512), Suplemento, abril de 2004, p.78.

- ***p63, um novo marcador de stem cells/células mioepiteliais de tumores mistos e carcinomas em tumores mistos da glândula mamária da cadela.*** COSTA FA, GENELHU MCLS, GOBBI H, CASSALI GD. *Revista Prática Hospitalar* (ISSN 1679-5512), Suplemento, abril de 2004, p.79.

- ***Immunohistochemical expression of p63 expression in pleomorphic adenomas and carcinomas ex-pleomorphic adenomas of salivary glands***. GENELHU MCLS, GOBBI H, SOARES FA, CAMPOS AHJFM, RIBEIRO CA, CASSALI GD. *Revista Médica do Paraná* (ISSN 0100-073-X), Curitiba, 2004; v.62 (n.esp.), p.63.

- ***Mixed tumors and metaplastic carcinomas of the mammary glands of the female dogs: comparative aspects with human salivary glands tumors. A new model of study.*** GENELHU MCLS, GOBBI H, SOARES FA, CAMPOS AHJFM, RIBEIRO, CA, CASSALI GD. *Revista Médica do Paraná* (ISSN 0100-073-X), Curitiba, 2004; v.62 (n.esp.): 37.

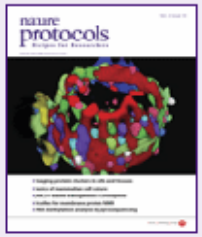
- ***Immunolocalization of β-catenin expression in pleomorphic adenomas and carcinomas ex-pleomorphic adenomas of salivary glands.*** GENELHU MCLS, GOBBI H, SOARES FA, CAMPOS AHJFM, BARRETO DC, RIBEIRO CA, CASSALI GD. *Jornal Brasileiro de Patologia e Medicina Laboratorial* (ISSN 1676-2444), Rio de Janeiro, 2005; v.41, n.3 (supl): 92.

Imagem publicada em *web site*

- ***Hair follicles in derme (área of the parotid gland) in two serial histological cuts: immunohistochemical staining by AE1/AE3 and p63.*** GENELHU M. *Nature Protocols*, 2007.

Nature Protocols
Home
Current issue
Archive
Sample issue
Discussion Forum
Tools & Reagents
Featured Movies

Welcome to *Nature Protocols*



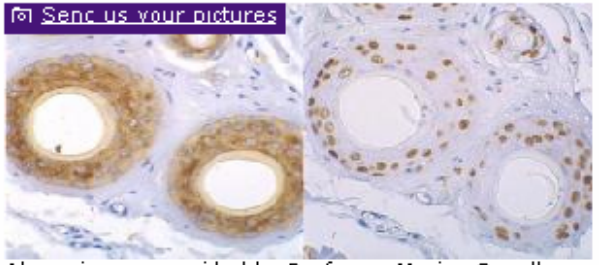
Nature Protocols is an online resource for protocols, including authoritative, peer reviewed 'Nature Protocols' and an interactive 'Protocols Network'. The two create a dynamic forum for scientists to upload and comment on protocols.

Sign up for e-alerts
Recommend to your library
Web feeds (RSS)

Browse Categories

Search Protocols Go

Send us your pictures



Above image provided by Professor Marisa Genelhu

Hair follicles in derme (area of the parotid gland) in two serial histological cuts. Immunuhistochemical staining by AE1/AE3 (A) and p63 (B).