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DETECÇÃO DE GALECTINA-3 POR IMUNOQUIMILUMINESCÊNCIA EM TECIDOS TUMORAIS DE PRÓSTATA E TIREÓIDE

TESE DE DOUTORADO

Recife, 2011

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Tese de doutorado apresentada ao Programa de Pós-Graduação em Ciências Biológicas, do Centro de Ciências Biológicas da Universidade Federal de Pernambuco, como requisito para a obtenção do título de Doutor em Ciências Biológicas. Área de Concentração: Biotecnologia.

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Tese de Doutorado

Título: **DETECÇÃO IMUNOQUIMILUMINESCENTE DE GALECTINA-3 EM LESÕES TUMORAIS GLANDULARES**

Doutorando: JORGE LUIZ SILVA ARAÚJO FILHO

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RESUMO

Embora o estudo imunoistoquímico seja um método bastante utilizado na rotina do diagnóstico anatomopatológico, este teste ainda apresenta importantes limitações principalmente quanto a incapacidade de distinção entre alguns tipos de lesões tumorais. Dessa maneira, outras ferramentas vêm sendo desenvolvidas a fim de aumentar a sensibilidade e especificidade da identificação antígeno-anticorpo. Dentre estas, os ensaios quimiluminescentes vem sendo cada vez mais utilizados em estudos para o mapeamento de amostras biológicas para fins diagnósticos. Dentre os inúmeros benefícios da utilização dos métodos quimiluminescentes, podemos citar o limite de detecção ultra-sensível, testes rápidos e um amplo campo de aplicações. O presente estudo buscou elaborar um protocolo para detecção da proteína galectina-3 em tecidos tumorais da tireóide e próstata a partir de anticorpo conjugado ao éster de acridina realizando ensaios imunoquimiluminescentes e compará-los aos resultados da imunoistoquímica convencional. O anticorpo monoclonal anti-galectina-3 foi conjugado ao éster de acridina e esse conjugado foi submetido à ensaios imunoquimiluminescentes em tecidos de próstata e tireóide além da imunohistoquímica convencional. Nossos resultados demonstram a eficiência na conjugação da anti-galectina-3 ao éster de acridina, um *shelf-life* do conjugado anticorpo/éster de acridina (AC-EA) com aproveitamento de 96,51% após 12 meses e a aplicação desse conjugado em ensaios imunoquimiluminescentes nos tecidos tireoidianos e prostáticos demonstraram padrões diferenciais entre as lesões estudadas e sua contraparte normal. Concluiu-se então, que o ensaio imunoquimiluminescente proposto pode ser utilizado como método diagnóstico alternativo na identificação de antígenos principalmente em amostras teciduais pequenas.

Palavras-chave: imunoquimiluminescência, tumores, éster de acridina, galectina-3, próstata, tireóide.

ABSTRACT

Although immunohistochemistry is a method widely used in routine pathological diagnosis, this test also shows significant limitations especially regarding the inability to distinguish between some types of tumors. Thus, other tools have been developed to increase the sensitivity and specificity of antigen-antibody identification. Among these methods, chemiluminescent assays are being increasingly used in studies for the mapping of biological samples for diagnostic purposes. There are numerous benefits related to chemiluminescent methods, like a limit of ultra-sensitive detection, rapid testing and a wide range of applications. This study aims to develop a protocol for detection of protein galectin-3 in tumor tissues of the thyroid and prostate using antibody conjugated to acridinium ester (AB-AE) for chemiluminescent immunoassay tests and compared them with results of conventional immunohistochemistry. The monoclonal anti-galectin-3 was conjugated to acridinium ester and this conjugate was subjected to chemiluminescent immunoassay in tissues of prostate and thyroid in addition to conventional immunohistochemistry. Our results demonstrate the efficiency of conjugation of anti-galectin-3 to the acridine ester, a shelf-life of the conjugate AB-AE with utilization of 96.51% after 12 months and the application of combined AB-AE tests in thyroid and prostate tissues. Chemiluminescent immunoassay showed different patterns between the studied tumoral lesions and their normal counterpart. It was concluded that the proposed chemiluminescent immunoassay test can be used as an alternative diagnostic method for identifying antigens mainly in small tissue samples.

Keywords: chemiluminescent immunoassay, tumors, acridinium ester, galectin-3, prostate, thyroid

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LISTA DE SIGLAS E ABREVIATURAS

ALP - Alkaline Phosphatase
BCL - Biochemiluminescence
Bcl-2 - B-cell lymphoma 2
CAT - carcinoma anaplásico da tireóide
CCH - Carcinoma Celular de Hurthle
CEA - Carcinoembryonic Antigen
CF - Carcinoma Folicular
CL - Chemiluminescence
CLEIA - Chemiluminescence Enzyme Immunoassay
CLIA - Chemiluminescence Immunoassay
CP - Carcinoma Papilar
CRD – Carbohydrate Recognition Domais
DAB - Diaminobenzidina
EA - Éster de Acridina
ELISA - Enzyme-Linked Immunosorbent Assays
FITC - Fluorescein Isothiocyanate
HCC - Hepatocellular carcinoma
HE - hematoxilina e eosina
HRP - Horseradish Peroxidase
IARC – International Agency for Research of Cancer
IgG - Immunoglobulin G
INCA - Instituto Nacional do Câncer
ISH - In Situ Hybridization
kDa - Kilodalton
LOD - Low Limits of Detection
OMS - Organização Mundial da Saúde
PAAF - Punção Aspirativa por Agulha Fina
PBS - Phosphate Buffered Saline
PCNA – Proliferating Cell Nuclear Antigen
PECAM-1 - Platelet Endothelial Cell Adhesion Molecule
pH – Potencial Hidrogêniônico
PSA - Prostate-Specific Antigen
RB - Retinoblastoma
RFC - Replication Factor
RLU - Relative Light Unit
SERS - Surface-Enhanced Raman Scattering
T3 – triiodotironina
T4 – tiroxina

SUMÁRIO

1. INTRODUÇÃO

Na avaliação prognóstica, no diagnóstico ou acompanhamento terapêutico é fato que a técnica histopatológica atualmente é considerada “padrão ouro” no estudo dos mais diferentes tipos de neoplasias existentes, apresentando grande relevância clínica. Exames histológicos e sorológicos descrevem aspectos muito importantes, possibilitando o monitoramento da evolução da doença (Wawroschek *et al*., 2003; Akimoto *et al*., 2006).

Porém, como todo método diagnóstico, também apresenta limitações como a incapacidade de distinção entre algumas lesões. Dessa maneira, marcadores específicos vêm sendo pesquisados como coadjuvantes nessa investigação (Torregrossa *et al*., 2007; Inohara *et al*., 2008).

Estudos relatam que alterações na expressão de glicoconjugados são freqüentemente observadas em tumores, dentre os diversos distúrbios celulares que resultam em neoplasias (Krzeslaka *et al.,* 2003), diante disso diversos trabalhos têm descrito o comportamento de determinadas proteínas relacionadas ao surgimento dessas alterações (Faggiano *et al.,* 2003; Fanggui *et al.,* 2010).

O estudo de proteínas relacionadas ao processo da carcinogênese tem grande importância acadêmica na investigação da biologia tumoral, bem como na prática clínica, auxiliando no diagnóstico, funcionando como marcadores moleculares específicos (Araújo-Filho *et al.,* 2006). Como exemplo, podemos citar galectinas, proteínas S100, CD31 (PECAM-1), todas proteínas da superfície celular, já testadas em diferentes tipos de neoplasias (Jiang *et al.,* 2005; Dey *et al.,* 2010).

Dentre essas proteínas citadas, as galectinas vêm sendo largamente utilizada em reações para detectar alterações na superfície das células tumorais e são associadas a diversos eventos como, por exemplo: no crescimento das células tumorais, envolvidas no mecanismo da indução da apoptose, no processo de metástase entre outros mecanismos (Elola *et al.,* 2005; Dumic, 2006).

Existem atualmente diversas técnicas diagnósticas que auxiliam no tratamento das neoplasias, dentre elas destacam-se a imunohistoquímica de biópsias teciduais bem como diferentes tipos de imunoensaios enzimáticos ou

quimiluminescentes, método este de grande auxilio na discriminação de tumores assemelhados e, muitas vezes, de difícil interpretação pelos métodos histológicos de rotina (Novellino, *et al.,* 2003; Pavelic, 2006, Zhao *et al.,* 2009).

Com o objetivo de fornecer um diagnóstico mais eficiente, análises laboratoriais baseadas nos princípios quimiluminescentes (QL) têm sido desenvolvidas para dosagens que requerem uma alta sensibilidade, como a determinação de citocinas, fatores de crescimento e proteínas relacionadas a processos patológicos específicos como o câncer.

Dentre a grande variedade de testes já disponíveis comercialmente estão os utilizados na avaliação da função tireoidiana, dosagem do antígeno prostático específico (PSA), fertilidade, marcadores tumorais, monitoramento de drogas terapêuticas, hepatite, proteínas específicas e esteróides (Kumar *et al.,* 2009).

Diversas são as substâncias quimiluminescentes utilizadas nesses ensaios, e elas podem ser detectadas na faixa de fentomoles ou atomoles (10^{-15} a 10^{-18} mol), com sensibilidade superior aos ensaios espectofotométricos (10^{-6} a 10^{-9} mol) e fluorimétricos (10^{-9} a 10^{-12} mol) (Campbell *et al.,* 1985). Vários compostos orgânicos exibem quimiluminescência em condições apropriadas, dentre os quais se destacam o luminol, isoluminol, éster de acridina ou seus derivados como alguns dos marcadores utilizados em imunoensaios quimiluminescentes (Roda *et al.,* 2000).

O éster de acridina foi introduzido em imunoensaios a partir da necessidade de substituir os marcadores radioisotópicos que estavam se tornando poucos populares devido à sua curta meia vida, ao perigo potencial à saúde e aos problemas quanto aos dejetos gerados (Weeks *et al.,* 1986). Desde então o éster de acridina tem sido foco de pesquisas para ampliar sua aplicação nas mais diversas áreas de Biotecnologia (Adamezyk *et al.,* 2001).

Apesar de todas as inter-relações feitas entre ensaios quimiluminescentes com lectinas (Campos *et al.,* 2006) e com anticorpos (Dreveny *et al.,* 1999; Yang *et al.,* 2002; Zhao *et al.,* 2009) não foram encontrados trabalhos demonstrando a aplicabilidade do emprego desta técnica na marcação de tecidos de lesões tumorais benignas ou malignas da tireóide ou próstata. E neste contexto, parece haver ainda muitas aplicações a serem exploradas neste promissor método de investigação.

2. REVISÃO DA LITERATURA

2.1 Câncer, uma visão geral

O termo câncer refere-se a alterações tumorais malignas que podem ocorrer na maioria dos tecidos do corpo humano e, alguns, podem apresentar diferentes tipos dessas alterações. No entanto, os processos básicos de caracterização parecem similares (Hanson & Hodgson, 2010).

Segundo relatório da Agência Internacional para Pesquisa em Câncer (IARC)/OMS, o impacto global do câncer dobrou em 30 anos. Estimou-se que, no ano de 2008, ocorreriam cerca de 12 milhões de novos casos de câncer e 7 milhões de óbitos, e que 84 milhões é o número de vítimas fatais do câncer no período de 2005 à 2015 (World Cancer Report, 2008).

No Brasil, as estimativas para o ano de 2011 apontam que ocorrerão 489.270 casos novos de câncer (INCA - Instituto Nacional do Câncer, 2010).

São várias as causas que podem originar o câncer, podendo ser endógenas ou exógenas ao organismo, estando ambas inter-relacionadas. As causas endógenas são, na maioria das vezes, geneticamente pré-determinadas provocando mutações genômicas, resultando na ativação de oncogenes, responsáveis pela proliferação celular e regulação da apoptose, além de ocorrer à inativação dos genes supressores. Enquanto que as causas exógenas estão relacionadas ao estilo de vida, hábitos alimentares e culturais, esses fatores causais podem interagir de várias formas, aumentando a probabilidade de transformações e alterações malignas nas células normais (Gua *et al*. 2006).

O desenvolvimento do câncer segue um processo de múltiplos estágios, a partir da célula normal e é caracterizado pelo acúmulo de alterações genéticas que interferem no controle normal do crescimento e diferenciação celular (Figura 1) (Peterson, 2005).

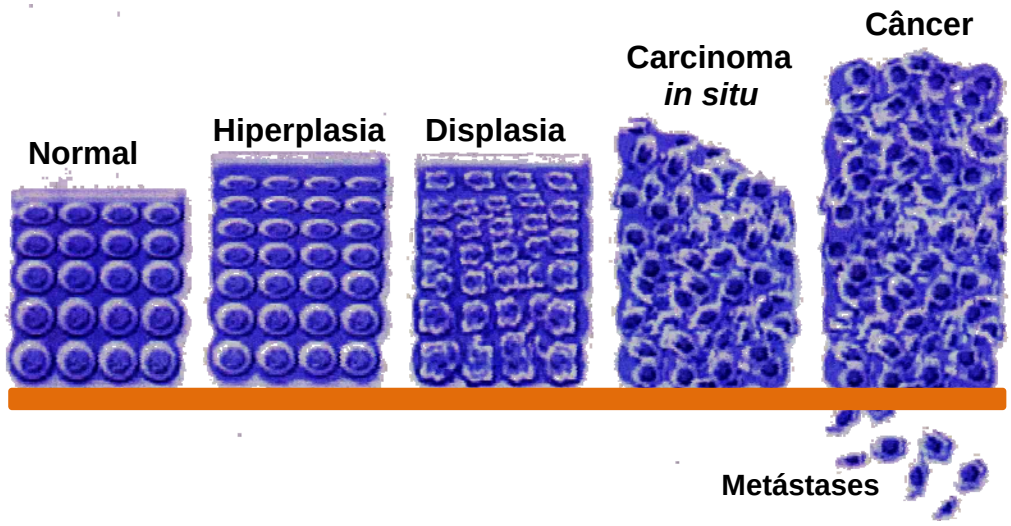

Figura 1. Tipos de crescimento celular anormal (fonte: Peterson, 2005).

Pelo menos no último século, todos os esforços intelectuais e tecnológicos empreendidos na cura dessa doença, contribuíram para revelar uma complexa série de mecanismos de comunicação molecular, intra e intercelular que, em condições fisiológicas, atua na manutenção dos sistemas vivos. Essa complexa rede de sinalização é constituída de vias metabólicas de manutenção e transmissão da informação genética e da síntese e regulação de macromoléculas, como as proteínas, contendo inúmeros pontos de regulação, sinais divergentes e locais de cruzamento com outras vias e cascatas de sinalização, exercendo mútuas influências (Taniguchi *et al.,* 2006).

No que se referem às funções bioquímicas, as moléculas de adesão e as vias de transdução dos sinais para o crescimento tumoral, esses são bastante alterados durante a progressão da alteração. Assim como as proteínas normais codificadas pelos proto-oncogenes (genes que regulam a proliferação, divisão e diferenciação celular) desempenham funções iniciais nas células durante o desenvolvimento das neoplasias. Dentre estas moléculas, destacam-se os fatores de crescimento, proteínas envolvidas na recepção e tradução de sinais localizados na superfície celular (Goo *et al.,* 2010).

O mecanismo de reconhecimento molecular na superfície da célula é afetado quando células normais sofrem o processo de neoplasia. O resultado é um crescimento e divisões descontroladas, devido às alterações nos

mecanismos de reconhecimento que agem na membrana celular (Shekhar *et al.,* 2004).

Nesse contexto estudo histoquímico do tecido obtido a partir de uma amostra do tumor (biópsia incisional) ou da peça completa (biópsia excisional) dos tumores assume grande importância prática no momento de decidir sobre o tratamento de cada paciente. Embora um câncer possa ser fortemente sugerido clínica e radiologicamente, seu diagnóstico não pode ser definido até que se obtenha confirmação citológica ou histológica da lesão. Sem a prova anatomopatológica da malignidade, o tratamento não pode ser adequadamente planejado, procedimentos terapêuticos não podem ser comparados, nem qualquer prognóstico pode ser feito de maneira precisa e eficiente (Faria, 1999; Akimoto *et al.* 2006).

Grande parte dos tumores ainda apresentam algumas das características estruturais das células das quais se originaram e isto permite ao patologista estabelecer, a grosso modo, o grau de malignidade pela extensão com que a histologia do tumor se diferencia da histologia normal (diferenciação). Mas ainda há problemas, pois alguns podem ser tão indiferenciados que não mais apresentam qualquer estrutura indicativa do tecido original (Montenegro & Franco, 1999).

Estudos com extratos de tumores realizados na década de 30 foram os pioneiros em demonstrar alterações bioquímicas nas células tumorais, o que foi posteriormente confirmado por estudos que demonstraram padrões aberrantes de glicosilação associados ao tumor (Dabelsteen *et al.,* 1992, Melo-Junior *et al.,* 2008).

Alterações na glicosilação são agora modelos universais em células cancerosas e certamente estruturas formadas por carboidratos são marcadores bem conhecidos na progressão de tumores. Como as células normais durante a embriogênese, as células tumorais também sofrem ativação e rápido crescimento, aderem a uma variedade de outros tipos celulares e invadem tecidos. O desenvolvimento embrionário e a ativação celular são acompanhados por mudanças no perfil de glicosilação celular (Veiga *et al.,* 2009).

Transformações celulares são freqüentemente acompanhadas por um aumento geral no tamanho dos glicolipídeos do metabolismo. Com o advento da

tecnologia de anticorpos monoclonais, descobriu-se que muitos dos anticorpos "tumor-específicos" reconhecem os epítopos carboidratados especialmente em glico-esfingolipídeos. Além do que, correlações significativas entre certos tipos de glicosilação alterada e o atual prognóstico de tumores referidos de animais experimentais ou humanos aumentam o interesse sobre essas mudanças bioquímicas específicas (Veiga *et al.,* 2009).

2.2 Lesões tumorais glandulares

As células que apresentam algum tipo de lesão tumoral carregam uma capacidade proliferativa indefinida, sendo capazes de mascarar o compromisso de diferenciação terminal e a quietude pós-mitótica que regulam normalmente a homeostase do tecido em um organismo. Essa proliferação descontrolada das células é também, freqüentemente, conseguida por uma desregulação direta do controle do ciclo celular (Herzig e Christofori, 2002; Duffy *et al.,* 2010).

As glândulas possuem a função de produzir e secretar importantes substâncias, muitas vezes que apresentam funções pré determinadas no organismo. Estas substâncias podem ser secretadas no interior do sistema circulatório ou fora dele (Ross, 2008).

Devido ao aumento nos casos de lesões tumorais em glândulas estudos vêm tendo esse tipo de tecido como alvo (Araújo-Filho *et al*., 2006; Guarino *et al.,* 2010). Entre as glândulas com aumento de casos de incidência e mortalidade relacionado as alterações tumorais elencam a próstata e tireóide (INCA, 2010).

2.2.1 Doenças tumorais da tireóide

A tireóide é constituída por dois tipos distintos de células produtoras de hormônio, as foliculares de origem epitelial, que produzem os hormônios tiroidianos triiodotironina (T3) e tiroxina (T4); e as células C oriundas da crista neural, responsáveis pela produção do hormônio calcitonina (Kondo *et al.,* 2006).

Nódulos tiroidianos palpáveis estão presentes em 4% a 7% da população adulta, no entanto, apenas uma pequena fração destas lesões representa câncer (Hegedus, 2004).

Estudos estão sendo realizados em nível de genômica, transcriptômica e proteômica em nodulos foliculares benignos e malignos da tireóide que poderão fornecer novos métodos diagnósticos no futuro (Durand *et al.,* 2008).

Cerca de 90 a 95% de todos os nódulos da tireóide são provocados por alterações benignas. O nódulo adenomatoso constitui área de hiperplasia, que pode apresentar padrão de arquitetura variado onde o padrão mais comum é o folicular, é geralmente encapsulado ou apresenta cápsula descontínua. Já o adenoma folicular representa um nódulo encapsulado, constituído de folículos tireoideanos bem diferenciados num padrão homogêneo não apresentando características invasoras, como invasão de cápsula ou vasos (Maitra & Abbas, 2005; Basílio-De-Oliveira & Barretto Netto, 2006).

O câncer de tiróide (figura 2) se apresenta como nodulação no parênquima tireoidiano, sendo sua principal característica a presença de um nódulo único na maioria dos casos. Porém, outras condições patológicas podem, também, se manifestar através de nódulos, sendo necessária uma abordagem sistemática dos pacientes (Guarino *et al.,* 2010).

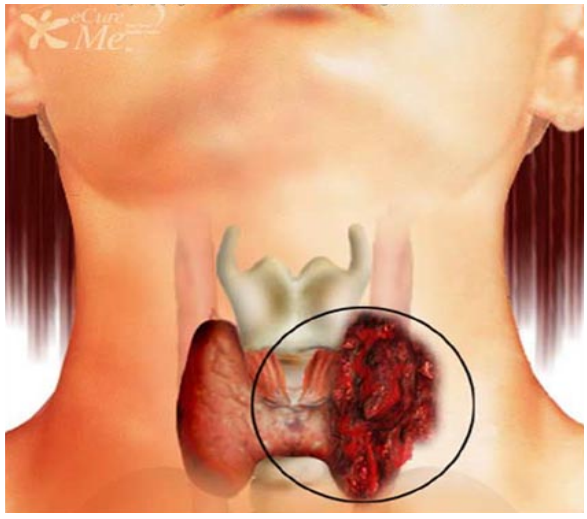

Figura 2: Ilustração do aspecto macroscópico do câncer da tireóide
(fonte: www.ecureme.com).

Os carcinomas tireoidianos são classificados através de parâmetros clínicos e histológicos e incluem os subtipos: papilar, folicular, indiferenciado e medular (Segev *et al.,* 2003).

A Organização Mundial da Saúde (OMS) define o carcinoma papilar de tireóide como sendo um tumor epitelial que apresenta evidência de diferenciação para célula folicular e características nucleares distintas (Livolsi *et al.,* 2004). É responsável por cerca de 85% das neoplasias malignas da tireóide, acometendo principalmente indivíduos entre os 30 e 50 anos de vida, sendo mais freqüente em mulheres (Thompson, 2006; Kondo *et al.,* 2006). Sua prevalência é mais comum em populações com dieta insuficiente de iodo (Gimm, 2001).

Macroscopicamente, o carcinoma papilar apresenta característica sólida, firme, de coloração branco-amarelada, com bordas irregulares e infiltradas. O tumor pode apresentar-se encapsulado e, em alguns casos, associado a calcificações. O diagnóstico histopatológico do carcinoma papilar é baseado em suas características citológicas e arquiteturais, não havendo uma única alteração isolada que seja diagnóstica (Thompson, 2006).

Dentre os carcinomas tireoidianos, o carcinoma papilar (figura 3) é o que apresenta uma menor dificuldade no seu diagnóstico citopatológico. Com uma amostragem adequada, a eficiência diagnóstica fica em torno de 90% devido às marcantes características nucleares do carcinoma papilar, facilmente avaliadas pelo exame patológico (Inohara *et al*., 2008).

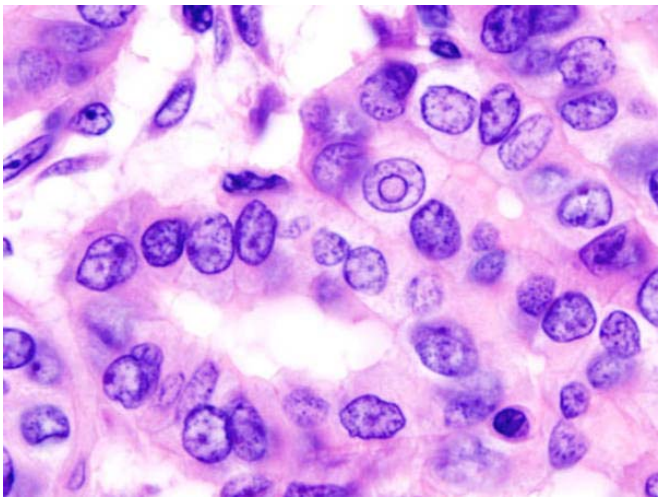

Figura 3: Fotomicrografia de amostra diagnosticada como carcinoma papilar da tireóide (Fonte: http://news.softpedia.com/newsImage/Endoscopic-Robot-Treats-Thyroid-Cancer-2.jpg/).

O carcinoma folicular é definido pela OMS como uma neoplasia epitelial maligna com diferenciação para células foliculares na qual faltam as

características nucleares do carcinoma papilar (Sobrinho Simões *et al.,* 2004). Representa de a 10 a 20% das neoplasias primárias da tireóide, mas em regiões deficientes de iodo apresentam incidência aumentada. Acomete principalmente mulheres de meia idade e raramente crianças. Em casos mais raros, a manifestação inicial pode ser de metástases à distância para pulmão, cérebro ou osso (Chan, 2003).

Do ponto de vista macroscópico o carcinoma folicular tende a ser solitário, unifocal e encapsulado. A superfície de corte tem coloração amarelada e as formas amplamente invasivas apresentando hemorragia, necrose e ainda invasão clara do parênquima tireoidiano. A característica diagnóstica que define o carcinoma folicular e o distingue dos adenomas e hiperplasias nodulares é a invasão capsular e/ou vascular. Isto significa que a cápsula desses tumores deve ser bastante estudada (Chan, 2003).

O carcinoma indiferenciado de tireóide representa de 3 a 5% dos carcinomas da tireóide, ocorre principalmente em mulheres idosas. Clinicamente, o carcinoma indiferenciado se apresenta como uma massa cervical de crescimento rápido associado aos sintomas de disfagia e rouquidão, dispnéia e linfadenopatia. Trata-se de uma patologia potencialmente letal, cujo diagnóstico geralmente é tardio resultando em um tratamento ineficaz e um mal prognóstico. Na metade dos casos a lesão é inoperável, apesar do tratamento a maioria dos pacientes morre em até 1 ano após o diagnóstico, com sobrevida entre 3 a 4 meses (Maia *et al.,* 2007).

O carcinoma medular da tireóide é um tumor maligno com diferenciação para a célula parafolicular, é mais comum sua ocorrência na forma esporádica, porém a forma familiar pode representar mais de 20% dos casos (Kebebew *et al.,* 2000). A sobrevida em 5, 10 e 15 anos é respectivamente de 65 a 87%, 51 a 78% e 65%. Histologicamente caracteriza-se por uma variedade de padrões: ninhos, cordões, placas e trabéculas com células variando de poligonais a fusocelulares. Os núcleos dessas células são redondos a ovais e apresentam uma cromatina fina granular (Chan, 2003).

Na propedêutica de investigação de um nódulo tireoidiano existem alguns fatores relacionados ao risco de malignidade, por exemplo, pacientes do sexo masculino, com nódulo único e hipocaptante à cintilografia são considerados de

alta probabilidade de serem portadores do câncer, enquanto que nas mulheres, tireóides multinodulares e hipercaptantes existe uma maior probabilidade de ser uma doença benigna, todavia nenhuma dessas características é definitiva (Amrikachi *et al.,* 2001).

A punção asporativa por agulha fina (PAAF) da tireóide vem sendo utilizada com grande freqüência na investigação de lesões e é considerada o teste de maior acurácia para a distinção entre nódulos benignos dos malignos (Amrikachi *et al.,* 2001; Sclabas *et al.,* 2003; Carpi *et al.,* 2005).

Porém, como todo método diagnóstico, também apresenta limitações como tamanho das amostras e mesmo incapacidade de distinção entre hiperplasias, adenoma folicular e o carcinoma folicular. Dessa maneira, outras ferramentas vêm sendo pesquisadas como coadjuvantes nessa investigação. A galectina-3, uma molécula envolvida com a carcinogênese das células foliculares, é apontada pela literatura como um desses potenciais marcadores de malignidade (Bartolazzi *et al.,* 2001; Torregrossa *et al.,* 2007; Inohara *et al.,* 2008).

2.2.2 Alterações tumorais da Próstata

O mecanismo de controle da divisão e diferenciação celular é realizado por um sistema integrado e complexo, que possibilita a manutenção de população celular nos parâmetros fisiológicos. Qualquer alteração nesse sistema resulta nos distúrbios tanto do crescimento como da diferenciação ou ambos simultaneamente, nesse contexto, a próstata pode ser acometida por dois principais distúrbios tumorais: hiperplasia prostática benigna (HPB) e câncer de próstata (Robbins *et al.,* 2005).

A Hiperplasia prostática benigna (HPB), conhecida como o mais comum entre as lesões tumorais da próstata, é também, a associada a maioria dos sintomas urinários que afetam homens com idade superior a 50 anos e por 20 a 30% das prostatectomias radicais nos indivíduos que vivem até 80 anos (Phipps *et al.,* 2005).

As alterações iniciais ocorrem por volta dos 35 anos de idade e consistem em nódulos microscópicos no estroma que surgem ao redor das glândulas periuretrais. Esses nódulos podem ser compostos tanto de elementos glandulares quanto fibromusculares ou podem ser mistos (Narayan *et al.,* 1994).

Relativamente incomum abaixo de 50 anos (20%), muito comum entre os 50 e os 60 anos de idade (70%), e quase a regra entre os 70 e os 80 anos (90%), o HPB é uma doença característica da glândula interna: 95% originando-se na zona de transição e 5% a partir do tecido glandular periuretral, ela pode ocorrer em grau leve (Figura 4a), moderado (Figura 4b) ou severo (Figura 4c).

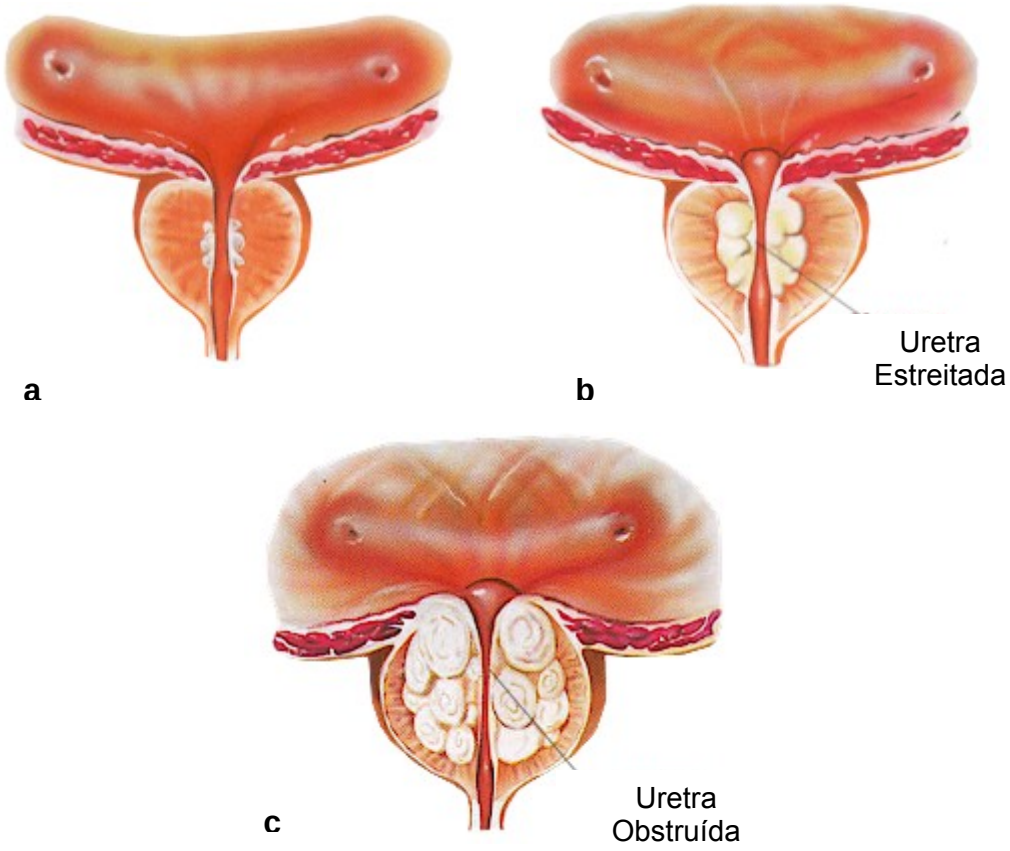

Figura 4. Próstata com HPB. (a) HPB em grau leve causando pouca obstrução da uretra; (b) HPB em grau moderado causando estreitamento na uretra; (c) HPB em grau severo resultando numa obstrução da uretra (Fonte: www.medicaangelus.com.mx).

A hiperplasia do tecido glandular periuretral estende-se em direção cranial, fazendo compressão sobre o assoalho vesical. Essa compressão era

designada anteriormente como “hipertrofia do lobo mediano”. A maioria dos aumentos da zona de transição é arredondada e simétrica, mas ocasionalmente pode haver nódulos hiperplásicos exofíticos crescendo em direção à zona periférica. À macroscopia os nódulos hiperplásicos são facilmente visualizados, pois comprimem o restante do tecido prostático. O HPB pode ocorrer sob três formas histopatológicas distintas, a 1ª: glandular (podendo causar aumento do PSA), 2ª: intersticial, e 3ª: focal (adenoma) (Djavan *et al*., 2009).

O número de casos novos de câncer de próstata estimado para o Brasil no ano e 2010 foi de 52.350. Estes valores correspondem a um risco estimado de 54 casos novos a cada 100 mil homens. Na Região Centro-Oeste (48/100.000) o câncer de próstata é o mais incidente entre os homens. Sem considerar os tumores de pele não melanoma, é o mais freqüente nas regiões Sul (69/100.000), Sudeste (62/100.000), Nordeste (44/100.000) e Norte (24/100.000) (INCA, 2010)

O aumento acentuado na incidência tem sido justificado pelo diagnóstico de casos latentes em indivíduos assintomáticos, após maior utilização da dosagem do antígeno prostático específico (PSA) (INCA, 2010). Esse câncer, também, é considerado a neoplasia maligna mais comum em homens idosos e é freqüentemente complicado por metástase óssea (Garnero, 2001; Scott *et al.,* 2001).

Como a maioria dos cânceres comuns, os fatores etiológicos associados com o câncer da próstata são variados, abrangendo influências genéticas e ambientais. Os fatores ambientais são indicados claramente por estudos da migração. Outros fatores etiológicos incluem o envelhecimento (idade maior ou igual a 50 anos), história familiar de pai ou irmão com câncer da próstata antes dos 60 anos de idade, raça, influência hormonal, dieta (fatores indutivos e preventivos) e o estilo de vida (Isaacs *et al.,* 2002; Stavridi *et al.,* 2010).

O câncer da próstata em sua fase inicial tem uma evolução silenciosa. Muitos pacientes não apresentam nenhum sintoma ou, quando apresentam, são semelhantes ao crescimento benigno da próstata (dificuldade miccional, freqüência urinária aumentada durante o dia ou à noite). Uma fase avançada da doença pode ser caracterizada por um quadro de dor óssea, sintomas urinários

ou, quando mais grave, como infecções generalizadas ou insuficiência renal (INCA, 2010).

Um tipo de câncer de próstata de alta prevalência é o adenocarcinoma. O exame histológico revela este câncer, em geral, constituído, por pequenos ácinos que infiltram difusamente o estroma. Um dos aspectos peculiares é a formação de glândulas no interior de glândulas, conferindo aspecto crivado. No estudo morfológico, entre os vários sistemas de graduação existentes, o de Gleason é o mais utilizado e se baseia na diferenciação glandular e no padrão de crescimento em relação ao estroma (Brasileiro Filho, 2000).

Este sistema possui 5 graus de diferenciação (Figura 5): em 1 e 2, a neoplasia mostra apenas desarranjo arquitetural, as glândulas crescem proximamente juntas; em 3, 4 e 5, há infiltração do estroma e em 5 o arranjo é sólido bem indiferenciado (Wetzel e Becich, 2000). A classificação final é dada pela combinação de dois graus de diferenciacão diferentes. O primeiro chamado de grau primário, representa a maior parte do tumor (deve ser maior que 50% do padrão total observado). O segundo, grau secundário, está relacionado com a minoria do tumor (deve ser menos que 50%, mas no mínimo 5% do padrão total do câncer observado). Estes graus são então somados para se obter o padrão de Gleason (Gleason, 1977).

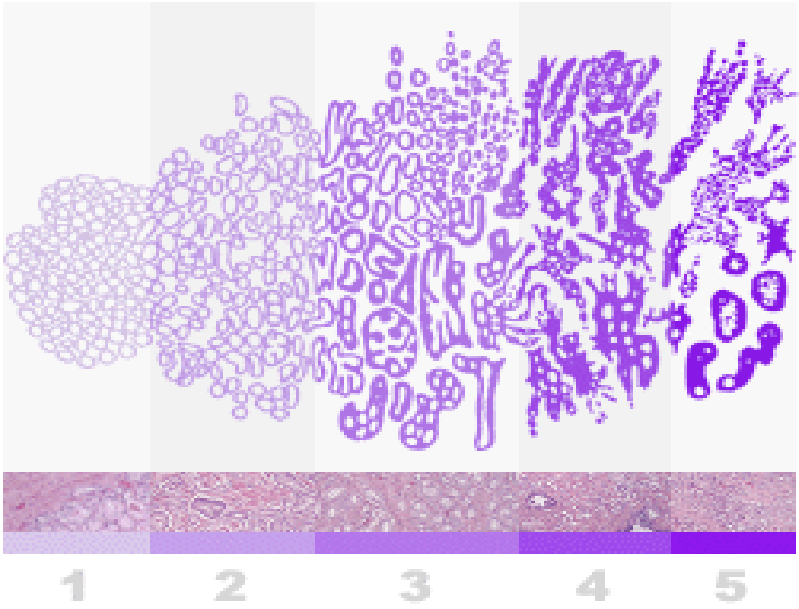

Figura 5. Classificação dos graus histológicos do carcinoma prostático, de acordo com a escala de Gleason (fonte: Lippincott Williams & Wilkins).

Dentre os diversos distúrbios celulares que resultam em neoplasias, alterações na expressão de glicoconjugados são freqüentemente observadas em tumores. Apesar disso, estudos a cerca das mudanças na glicosilação celular em determinados estágios da carcinogênese prostática são escassos (Chan *et al.,* 2001). Desta forma, conhecer a regulação da proliferação celular através de alterações bioquímicas específicas ao câncer de próstata é de grande importância para o desenvolvimento de novas soluções (Matera 2010).

2.3 Galectinas, definições e aplicações

As galectinas são proteínas da crescente família das lectinas animais (lectinas endógenas), envolvidas em diversos processos biológicos, tais como interações com glicoconjugados contendo galactose, controle do ciclo celular, proliferação celular, resposta imune, apoptose e em processos neoplásicos. (Liu *et al.,* 2002).

Membros da família das galectinas são encontradas em esponjas, fungos, nematóides, insetos, vertebrados incluindo os mamíferos, e algumas galectinas virais foram identificadas (Cooper *et al.,* 2002). A estrutura molecular das galectinas geralmente consiste de cerca de 130 aminoácidos, altamente conservados, bem como uma região de reconhecimento a carboidratos (CRD – carbohydrate recognition domains) e por reconhecer estruturas de β-galactosídeos (Barondes *et al.,* 1994; Lahm *et al.,* 2004) (Figura 6).

Figura 6. Estrutura molecular da galectina-3 complexada ao carboidrato.
(fonte: http://web.mit.edu/glycomics/moleculepages/cbp/galectins/gal3_human/)

Até o momento foram descritas 15 galectinas oriundas de mamíferos e essa família de proteínas é subdividida em 3 categorias com base na homologia das seqüências de aminoácidos: I) “prototypical”, são aquelas que contem um único CRD e formam homodímeros não-covalentes; II) “Chimeric”, apresentam um CRD e um domínio amino-terminal rico em resíduos de prolina, glicina e tirosina; o qual é sensível a metaloproteinases e contribui para a oligomerização dessas lectinas; III) “Tandem-Repeat”, polipeptídeos únicos compostos por dois CRDs distintos conectados por um peptídeo ligador de 5 a 50 resíduos de aminoácidos (Cummings & Liu, 2009).

Além das galectinas dos mamíferos, outras são descritas provenientes de outras espécies animais (Barondes *et al.,* 1994a; Cooper *et al.,* 1997; Kasai *et al.,* 1996; Muller *et al.,* 1997; Leffler *et al.,* 1997; Cooper *et al.,* 2002). Galectinas são pequenas proteínas solúveis, com peso molecular variando entre 14 a 36 kDa. Elas são sintetizadas no citosol onde permanecem, podendo ser exportadas (Barondes *et al.,* 1994a; Hughes *et al.,* 1997; Hughes *et al.,* 1999) ou translocadas para o núcleo (Wang *et al.,* 2009). Desta forma, As galectinas dos vertebrados são encontradas no citoplasma e no núcleo, na superfície celular e no espaço extracelular (Hughes *et al.,* 1999).

Dentre as galectinas identificados em mamíferos, a galectina-3, é uma das mais estudadas e é uma proteína monomérica de 30 Kda indicada como um potencial marcador tumoral de malignidade nas neoplasias prostáticas (Califice *et al.,* 2004a). A Galectina-3 faz parte das lectinas com afinidade por beta-galactosideo, sua expressão vem sendo relacionada ao surgimento de tumores e metástases (Christoph *et al.,* 2004; Alves *et al.,* 2010).

Sua estrutura parece ser única quando comparada com as demais galectinas de vertebrados (Houzelstein *et al.,* 2004). Ela foi inicialmente identificada como marcador de diferenciação de macrófagos (Ho *et al.,* 1982).

Cada galectina apresenta um perfil específico e sua expressão é normalmente regulada durante o desenvolvimento das diversas células e tecidos. Na maioria das vezes são encontradas no citoplasma celular, algumas são secretadas pelas células e interagem com proteínas da superfície celular regulando a adesão entre as células e destas entre a matriz extracelular (Hughes, 2001; Martinez *et al.,* 2004).

As galectinas vêm sendo largamente utilizadas como ferramenta na imunohistoquímica. Alterações na expressão das galectinas são freqüentemente observadas em várias células tumorais durante seu desenvolvimento, crescimento, diferenciação, metástase, apoptose, “splicing” do RNA e funções imunoreguladoras (Rabnovich *et al.,* 2002; Elola *et al.,* 2005).

Uma série de evidências experimentais e clinicas vêm reforçado as correlações entre sua expressão e as neoplasias. Estudos demonstram que a presença de galectinas é relevante na progressão de neoplasias em certos tumores malignos, indicando estas como potenciais marcadores tumorais (Pacis *et al.,* 2000; Califice *et al.,* 2004).

Desde os trabalhos iniciais que demonstravam que mais de 90% de lesões tireoidianas malignas expressavam galectina-3, principalmente em carcinomas folicular e papilífero, surgiram fortes evidências de que essa proteína poderia ser um marcador de malignidade neste tipo de neoplasia (Xu *et al.,* 1995; Faggiano *et al.,* 2003).

Ela é encontrada em diversos tecidos normais, e sua expressão em adultos é similar à expressão durante a embriogênese, isso foi relatado para células epiteliais e mielóides. Galectina-3 foi encontrada nas células do epitélio intestinal (Tabela 1).

Tabela 1. Tecidos e células humanas que expressam galectina-3.

Local	Referência
Células do epitélio intestinal	Lotz *et al.,* 1993
Epitélio do cólon	Lotz *et al.,* 1993
Epitélio córneo	Gupta *et al.,* 1997; Cao *et al.,* 2002
Epitélio conjuntivo	Hrdlickova *et al.,* 2001
Epitélio olfatório	Heilmann *et al.,* 2000
Células epiteliais do rim	Sasaki *et al.,* 1999
Pulmão	Kasper *et al.,* 1996
Timo	Villa-Verde *et al.,* 2002
Mama	Castronovo *et al.,* 1996
Tireóide	Saggiorato *et al.,* 2004
Próstata	Pacis *et al.,* 2000
Células ductais das glândulas salivarias	Xu *et al.,* 2000
Pâncreas	Schaffert *et al.,* 1998
Rim	Sasaki *et al.,* 1999

Olho	Fautsch *et al.,* 2003
Ductos biliares	Shimonishi *et al.,* 2001
Fibroblastos	Moutsatsos *et al.,* 1987
Condrócitos	Colnot *et al.,* 1999
Osteoblastos	Stock *et al.,* 2003
Osteoclastos	Niida *et al.,* 1994
Ceracinócitos	Wollenberg, 1993; Konstantinov, 1994
Células de Schwann	Reichert *et al.,* 1994
Mucosa gástrica	Lotan *et al.,* 1994a
Células endoteliais	Lotan *et al.,* 1994b
Neutrófilos	Truong *et al.,* 1993a
Eosinófilos	Truong *et al.,* 1993b
Basófilos	Frigeri *et al.,* 1993
Mastócitos	Craig *et al.,* 1995
Células de Langerhans	Wollenberg 1993; Smetana *et al.,* 1999
Células denditricas	Flotte *et al.,* 1983; Dietz *et al.,* 2000
Monócitos	Li *et al.,* 1995
Macrófagos	Flotte *et al.,* 1983; Kasper *et al.,* 1996; Saada *et al.,* 1996; Maeda *et al.,* 2003

Em outros tipos celulares, como os linfócitos, a galectina-3 não é normalmente expressa, porém sua expressão pode ser observada após estimulação.

Alterações no perfil de expressão da galectina-3 vêm sendo observado na progressão maligna de diversos tipos de câncer como no de cólon, tireóide, mama, estômago (Miyazaki *et al.,* 2002), próstata e em metástases do fígado (Inufusa *et al.,* 2001) (Tabela 2).

Tabela 2. Padrão de marcação através de imunohistoquímica para galectina-3 em lesões tumorais:

Tecido/Órgão	Perfil de expressão	Referência
Estômago	Aumentado	Miyazaki *et al.,* 2002
Linfonodos	Aumentado	Miyazaki *et al.,* 2002
Cabeça e Pescoço	Aumentado	Gillenwater *et al.,* 1996
Língua	Aumentado	Honjo *et al.,* 2000
Hepatocelular	Aumentado	Hsu *et al.,* 1999; Chung *et al.,* 2002
Cólon	Aumentado	Irimura *et al.,* 1991; Lotan *et al.,* 1991
Tireóide	Aumentado	Bartolazi *et al.,* 2008, Inohara *et al.,* 2008
Glândulas salivares	Reduzido	Xu *et al.,* 2000
Mama	Reduzido	Stannard *et al.,* 2010
Ovário	Reduzido	Van den Brule *et al.,* 1994
Próstata	Reduzido	Araújo-Filho *et al.,* 2002 Melo-Junior *et al.,* 2010
Útero	Reduzido	Van den Brule *et al.,* 1996
Células basais de pele	Reduzido	Castronovo *et al.,* 1991
Pele	Reduzido	Mollenhauer *et al.,* 2003
Metástases do fígado	Reduzido	Inufusa *et al.,* 2001
Pâncreas	Reducido	Schaffert *et al.,* 1998
Adenocarcinoma	Reduzido	Inufusa *et al.,* 2001

Como se pode constatar a galectina-3 é expressa em uma grande variedade de tumores, e a expressão depende do estadiamento tumoral, invasividade e potencialidade de metástases (Van den Brule *et al.,* 2002; Danguy *et al.,* 2002; Dumic *et al.,* 2006).

Após sintetizada, a galectina-3 localiza-se predominantemente no citoplasma podendo ser encontrada também no núcleo, sugerindo que esta proteína fica alternando entre núcleo e citoplasma (Davidson *et al.,* 2002). A localização intracelular da galectina-3 no núcleo e/ou no citoplasma é dependente de inúmeros fatores como o tipo, nível de proliferação celular e a progressão neoplásica (Moutsatsos *et al.,* 1987; Openo *et al.,* 2000).

A localização intracelular da galectina-3 desempenha papel importante na sua função antiapoptótica. Nos carcinomas de língua, os níveis da expressão nuclear dessa proteína diminuíram durante a progressão do tumor quando comparados com a mucosa normal, enquanto que a expressão citoplasmática aumentou mostrando que a translocação da galectina-3 do núcleo para o citoplasma durante a progressão neoplásica pode ser utilizado como fator prognóstico em pacientes com carcinoma de língua (Honjo *et al.,* 2000). Esses resultados juntos sugerem que a galectina-3 parece ter uma atividade anti-tumoral quando está presente no núcleo e favorecer a progressão tumoral quando expressa no citoplasma.

Alguns trabalhos sugerem que a galectina-3, quando localizada no compartimento extracelular exerce uma função anti-apoptótica. A galectina-3 extracelular pode não ser uma molécula anti-apoptótica por si só, mas existe a possibilidade de que a sua interação com alguma proteína na superfície celular possa exercer essa atividade anti-apoptótica em algumas células, contribuindo dessa forma para a sobrevivência celular (Nakahara *et al.,* 2005).

Outros trabalhos também demonstraram seu envolvimento na inibição da apoptose. Células que apresentaram superexpressão dessa proteína apresentaram também um aumento da resistência à estimulação apoptótica quando induzidas por óxido nítrico e radiação (Yang *et al.,* 1996; Moon *et al.,* 2001).

Por outro lado, também foi descrita uma função pró-apoptótica para a galectina-3, Takenaka e colaboradores (2004) relataram que a galectina-3 extracelular secretada por células tumorais pode exercer uma função pró-apoptótica nos linfócitos T humanos, contribuindo no mecanismo de “escape imunológico” das células tumorais, e favorecendo indiretamente a progressão tumoral.

O mecanismo molecular de regulação da apoptose ainda não está bem esclarecido, contudo, é possível que ela possa mimetizar a Bcl-2, uma proteína mitocondrial bem conhecida como supressora da apoptose (Yang *et al.,* 1996; Moon *et al.,* 2001). Apesar de existirem inúmeros trabalhos na literatura relacionando a expressão da galectina-3 à invasão tumoral e metástases em diversas neoplasias como neoplasias da tireóide, gástricas e da região da

cabeça e pescoço, alguns dados são conflitantes e inconsistentes e não há um consenso geral na literatura a respeito do papel dessa proteína na progressão tumoral (Dumic *et al.,* 2006).

Por outro lado, em algumas lesões como tumores de mama, próstata e ovário, a expressão da galectina-3 é inversamente proporcional ao potencial metastático da lesão (Castronovo *et al.,* 1996; Krzeslak & Pacis *et al.,* 2000; Lipinska, 2004).

Em tumores malignos epiteliais da região da cabeça e do pescoço alguns estudos verificaram que a galectina-3 está localizada na superfície da célula, onde pode estar associada às interações celulares. Seu padrão de expressão parece estar associado à diminuição do padrão de diferenciação do tumor, sugerindo que a galectina-3 pode ser utilizada como um marcador biológico e de diferenciação nesse tipo de lesão (Gillenwater *et al.,* 1996; Plzak *et al.,* 2004).

A expressão da galectina-3 em neoplasias das glândulas salivares foi estudada inicialmente por Xu e colaboradores (2000). Os autores observaram marcação da proteína em 3 dos 14 casos de carcinomas adenóides císticos estudados, em 8 dos 9 casos de adenocarcinomas polimorfos de baixo grau e em 8 dos 9 casos de carcinomas ex-adenomas pleomórficos. Diante desse resultado os autores concluíram que a expressão da galectina-3 pode estar associada à diferenciação celular.

Teymoortash e colaboradores (2006) estudaram 35 casos de carcinoma adenóide cístico de cabeça e pescoço e observaram que todos os casos que apresentaram metástases à distância eram positivos para galectina-3 e todos os casos onde não ocorreram metástases à distância eram negativos para galectina-3. Da mesma forma, todos os pacientes que apresentaram metástases regionais também eram positivos para a galectina-3, com uma marcação predominantemente citoplasmática. Diante disso concluíram que a expressão da galectina-3 está associada ao acréscimo da incidência de metástases regionais e à distância nos carcinomas adenóides císticos de cabeça e pescoço.

A expressão imunohistoquímica da proteína galectina-3 em carcinomas adenóides císticos e adenocarcinomas polimorfos de baixo grau de malignidade foi estudada e observou-se que a marcação da galectina-3 foi predominantemente nuclear nos carcinomas adenóides císticos estudados e

citoplasmática nos adenocarcinomas polimorfos de baixo grau de malignidade. Além disso, de forma geral, a marcação imunohistoquímica da proteína galectina-3 nessas lesões manteve o mesmo padrão de marcação das citoqueratinas observado nas glândulas salivares normais, porém, com intensidade e padrões de expressão distintos, sugerindo a participação dessa proteína na diferenciação celular (Ferrazzo *et al.,* 2007).

Os resultados de estudos demonstram que, embora a galectina-3 esteja localizada freqüentemente no citoplasma, ela também pode ser observada no núcleo, na superfície celular ou no meio extracelular, o que indica uma multifuncionalidade dessa molécula (Hughes, 1999). Os níveis de expressão da galectina-3 dependem do organismo ou tecido avaliado, sugerindo que fatores específicos do tumor ou do tecido podem modular a expressividade da galectina-3 (Takenaka *et al.,* 2004).

Weinberger e colaboradores (2007) estudaram a marcação nuclear e citoplasmática da galectina-3, associando à expressão da β-catenina e da ciclina D1 em carcinomas da tireóide. Os autores apontam que a expressão da galectina-3 parece estar fortemente associada ao aumento da expressividade da β-catenina e da ciclina D1 nos carcinomas bem diferenciados de tireóide.

Estudos in vitro demonstraram a existência de uma relação entre a galectina-3 e a alteração maligna das células epiteliais da tireóide. Foi inibida a expressão da galectina-3 em células de carcinoma papilar, e houve uma redução no fenótipo maligno (Yoshii *et al.,* 2001). Em outro trabalho, a transfecção do cDNA da galectina-3 a tireócitos normais induziu uma transformação para o fenótipo maligno (Yang & Liu, 2003). Demonstrando assim que a galectina-3 exerce influência na manutenção do fenótipo maligno, para as células foliculares.

Com a evidência da relação entre a galectina-3 e as neoplasias malignas de tireóide, observou-se a possibilidade dessa molécula ser um marcador de malignidade para lesões proliferativas. Takenaka e colaboradores (2003) demonstraram que a galectina-3 é expressa em altos níveis pelos carcinomas foliculares e papilares, e em níveis variados por carcinoma medular, não sendo expressada pelos adenomas (Takenaka *et al.,* 2003).

Essa constatação originou inúmeras publicações sobre o uso dessa molécula, confirmando os achados anteriores (Gasbarri *et al.,* 1999; Orlandi *et*

al., 1998; Kawachi *et al.,* 2000; Herrmann *et al.,* 2002; Bartolazzi *et al.,* 2008; Lin *et al.,* 2009). Dentre os trabalhos desenvolvidos, foi realizado um onde seis diferentes centros de pesquisa utilizaram o mesmo anticorpo monoclonal contra a galectina-3 e a conclusão dos autores definiu que amostras de PAAF de tireóide, que apresentem marcação positiva para a galectina-3, sejam consideradas malignas e devem ser submetidas à tireoidectomia. Enquanto que as amostras negativas seriam definidas como alterações hiperplásicas ou adenomatosas, devendo ser acompanhadas clinicamente (Bartolazzi *et al.,* 2001).

Enquanto isso a expressão da galectina-3 em tumores prostáticos se comporta de forma oposta aos dados observados na tireóide, quando a sua expressão é diminuída em lesões tumorais (Ahmed *et al.,* 2007).

2.4 Aplicações da quimiluminescência na saúde

Por definição a luminescência pode ser considerada a emissão de luz através da alteração do nível energético de átomos ou moléculas provocadas por reações químicas, radiação ou interações biológicas (Van Dyke, 1985). Inúmeros compostos orgânicos exibem luminescência em condições apropriadas, e a maioria dos imunoensaios luminescentes realizados, tem como marcadores: luminol, isoluminol, éster de acridina ou seus derivados (Campbell *et al.,* 1985; Li *et al.,* 2008; Wang *et al.,* 2008).

A quimiluminescência (QL) é definida como o processo no qual moléculas ou átomos excitados através de reações químicas liberam o excesso de energia na forma de luz. Este processo envolve a absorção, pelos reagentes, de energia suficiente para a geração de um novo complexo, eletronicamente excitado. Se a espécie excitada for emissiva, esta produzirá a radiação diretamente, caso contrário, pode ocorrer transferência de energia do estado excitado formado por uma molécula aceptora apropriada, resultando na emissão indireta da radiação (Albertin *et al.,* 1998; Yang *et al*., 2008).

Este fenômeno possui aplicações diversas em química analítica, principalmente, na área da análise por injeção em fluxo, cromatografia de coluna

líquida e sistemas de separação por eletroforese capilar, bem como em imunoensaios (Garcia-Campaña *et al.,* 2003).

Dentre os inúmeros benefícios da utilização dos métodos luminescentes podemos citar: limite de detecção ultra-sensível, testes rápidos e um amplo campo de aplicações analíticas, toxicidade inferior comparada à oferecida por radioisótopos, não agressão ao meio ambiente e especificidade (Ferreira & Rossi, 2002). A quimioluminescência tem também como grande vantagem ser um sistema frio uma vez que a energia na forma de luz é produzida diretamente de uma reação química, sem passar por um estágio intermediário envolvendo calor (Catalani, 1996).

A aplicação da QL como instrumento analítico depende da acoplagem da substância de interesse a um dos elementos participantes da reação quimiluminescente ou ao produto no estado excitado. A concentração da amostra desconhecida será proporcional à produção de luz total emitida ou a um parâmetro físico associado à luminescência, tal como cor ou polarização da luz emitida (Campbell *et al.,* 1985).

Algumas análises laboratoriais baseadas em princípios quimiluminescentes têm sido desenvolvidas para dosagens que requerem uma alta sensibilidade, como a determinação de citocinas, fator de crescimento epidérmico, e de crescimento endotelial vascular. Dentre a grande variedade de testes já disponíveis comercialmente estão aqueles utilizados na avaliação de função tireoidiana, dosagem de PSA, fertilidade, marcadores tumorais, monitoramento de drogas terapêuticas, hepatite, proteínas específicas e esteróides (Kricka 2003).

As substâncias quimiluminescentes podem ser detectadas na faixa de fentomoles ou atomoles (10^{-15} a 10^{-18} mol), com sensibilidade superior aos ensaios espectofotométricos (10^{-6} a 10^{-9} mol) e fluorimétricos (10^{-9} a 10^{-12} mol) (Campbell *et al.,* 1985). Vários compostos orgânicos exibem QL em condições apropriadas, dentre os quais destacam-se o luminol, isoluminol, éster de acridina ou seus derivados como alguns dos marcadores utilizados em imunoensaios quimiluminescentes (Roda *et al.,* 2000).

Devido à necessidade de substituir os marcadores radioisotópicos que estavam se tornando poucos populares devido à sua curta meia vida, ao perigo

potencial à saúde e aos problemas quanto aos dejetos gerados, o éster de acridina foi introduzido em imunoensaios (Weeks *et al.,* 1986). Desde então o éster de acridina tem sido foco de pesquisas para ampliar sua aplicação nas mais diversas áreas de Biotecnologia. (Adamezyk *et al.,* 2001).

A acridina é um composto fluorescente cujos sais podem ser estimulados para produzir luz na presença de peróxido de hidrogênio em meio alcalino diluído (Figura 7) na ausência de um catalizador (Weeks *et al*., 1983). Essa propriedade se deve a presença de um nitrogênio quaternário central e a derivatização no carbono 9 que possui um Ester de fenil marcado com um radical. Dentre os derivados de acridina, o ester de acridina recebeu um destaque especial pela fácil oxidação com o peróxido de hidrogênio e a formação de um produto excitado (N-metilacridona), o qual irá emitir luz (Weeks *et al.,* 1983; Yang *et al.,* 2002).

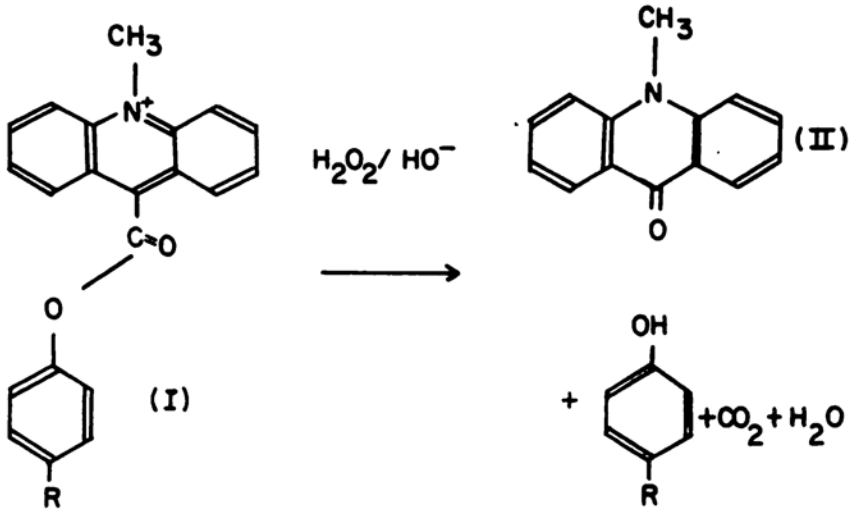

Figura 7. Reação Quimiluminescente do Ester de Acridina
(Fonte: Weeks *et al.,* 1983).

A utilização dos ésteres de acridina em imunoensaios quimiluminecentes foi promovida pela primeira vez por Weeks e colaboradores (1983) na preparação de anticorpos mono- ou policlonal marcado contra a alfa 1-fetoproteina, onde foi demonstrado que este composto possuía uma alta sensibilidade de detecção, igual ou superior ao I^{125}.

Após conjugado a diversas moléculas, o éster de acridina tem sido empregado na preparação de sondas citoquímicas e histoquímicas. Assim como

o éster de acridina, outros compostos, como o luminol e isoluminol, também são utilizados como marcadores em imunoensaios quimiluminescentes (Kricka, 2003).

Em nosso laboratório, foi testada a aplicação dos ensaios quimiluminescentes utilizando o éster de acridina conjugado à lectina concanavalina-A em lesões mamárias (Campos et al., 2006), e recentemente também estão sendo testados ensaios imunoquimiluminescentes em lesões de mama e pele.

3. OBJETIVOS

3.1 Objetivo geral

Estabelecer um protocolo para detecção de galectina-3 a partir de anticorpo conjugado ao Éster de Acridina em tecidos tumorais da tireóide e próstata, realizando ensaios imunoquimiluminescentes e compará-los aos resultados com imunohistoquímica convencional.

3.2 Objetivos específicos

- Aprimorar um método de conjugação do éster de acridina ao anticorpo galectina-3;
- Empregar quimiluminescência para quantificação de fótons (URL) através da hidrólise do éster de acridina conjugado aos anticorpos;
- Estabelecer o shelf-life do conjugado Éster de acridina-AntiGalectina3;
- Identificar o perfil de expressão imunohistoquímico da galectina-3 entre os tumores tireoidianos benignos (adenoma folicular), malignos (carcinoma papilar e carcinoma folicular) e tecido normal;
- Obter a imunomarcação através de imunoistoquímica para galectina-3 em lesões tumorais da próstata (hiperplasia prostática benigna, adenocarcinoma prostático e tecido normal da próstata);
- Comparar os resultados da expressão tecidual da galectina-3 para a imunohistoquímica através de reação enzimática (peroxidase) e reações de imunoquimiluminescência empregando conjugados de éster de acridina e avaliar seu potencial como ferramenta diagnóstica.

4. RESULTADOS

CAPÍTULO I

POTENTIAL APPLICATIONS OF THE CHEMILUMINESCENT METHODS IN TUMORAL DISEASES INVESTIGATION

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REVIEW ARTICLE

BIO TECHNOLOGY

POTENTIAL APPLICATIONS OF THE CHEMILUMINESCENT METHODS IN TUMORAL DISEASES INVESTIGATION

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ABSTRACT

Nowadays, in the clinic routine, protein biomarkers of efficacy, or mode of action, are used to better monitor and predict patient responses to drug treatment and diagnosis. Currently, the chemiluminescence biomarkers are applied for validation methods and screening. Here, was revised the development of the new analytical tool through immunological (antibody) and chemiluminescent methods combination, named chemiluminescence immunoassay (CLIA). In the tumoral process, increased expression of specific antigens is associated in patients with certain tumors. Thus, in our laboratories CLIA has been tested to study different tumoral lesions from glandular tissues, like prostate and thyroid. In conclusion, there is a great opportunity for introducing the adaptive chemiluminescence devices, since clinical diagnostics represents a huge, well-established and important analytical field.

KEY WORDS
Chemiluminescence, tumoral diseases, antibody, acridinium ester

INTRODUCTION:

In the last 15 years, the utility of luminescent labels has steadily increased. The phenomenon has been applied to biomedical science in immunoassays, DNA probe assays, and measurement of important enzymes and metabolites.

By developing chemiluminescent (CL) compounds that can be used as substrates for enzyme labels it is possible to increase the speed of ultrasensitive CL immunoassays without impairing the sensitivity.

The term "chemiluminescence" was first coined by Eilhardt Weideman in 1888. This reaction refers to the emission of light from a chemical transformation. Chemiluminescent reactions emit light of varying degrees of intensity and lifetime, with colors that span the visible spectrum[1,2].

In 1998 Robert Wilson of Liverpool University Chemistry Department was looking at similarities between simple iron containing compounds and enzymes such as horseradish peroxidase (HRP). During this work he acquired a group of compounds called acridan esters from the US company Lumigen. Works had shown that acridan esters could be used as a chemiluminescence substrate for HRP, and Wilson demonstrated that light-emitting reactions could also be triggered electrochemically. Acridan esters are oxidized to more familiar acridinium esters that have been used as labels in ultrasensitive chemiluminescence immunoassays and nucleotide assays for many years[3,4].

Chemiluminescent reactions occur in the gas, solid and liquid state. In its simplest it can be represented as:

$$\mathbf{A + B \longrightarrow [\ I\ ] \longrightarrow\ products\ +\ light}$$

where [I] is a highly energetic intermediate compound produced from a chemical activation reaction when two reagents (A and B) are mixed. The [I] is short-lived and returns to a lower energy state by emitting visible light[1,5].

Quantitative analysis of chemiluminescence images, performed by defining suitable areas and evaluating the total number of photons that fluxes from whitin those areas, permits the quantitative assessment of distribution of light emission. The luminescence intensity is usually expressed as photons per second and surface area or in arbitrary units (often called relative light units, RLU), depending on the instrument used. Nevertheless, absolute quantitative analysis is hampered by several factors, including the

lack of suitable calibration systems, the need for standardized and reproducible experimental conditions, and the influence of the sample properties on the emission intensity[6].

There are many systems of chemiluminescence of which the two most widely used are the luminal based and the peroxy-oxalate based systems[7].

In general, the luminol or acridinium ester is a major chemiluminescent analytical technique is used in clinical analysis[8]. As effective CL labels for biomolecules, acridinium esters have received great interest for immumoassay and DNA assay[9].

In the ours laboratories we labeling acridinium ester molecule with Concanavalin A (ConA), to be used as auxiliary histochemistry tool to help the clinical-pathological evaluation of infiltrating duct carcinoma, a human mammary tumour of high incidence in the State of Pernambuco-Brazil[10].

The attractiveness of chemiluminescence as an analytical tool is the simplicity of detection. The fact that a chemiluminescent process is, by definition, its own light source means that assay methods and the instruments used to perform them need only provide a way to detect light and record the result. Luminometers need consist of only a light-tight sample housing and some type of photodetector. Taken to the extremes of simplicity, photographic or x-ray film or even visual detection can be used[11,12].

Chemiluminescence (CL) has been known to be a powerful analytical technique that exhibits high sensitivity and selectivity, and the simple requirements of chemiluminescent methods make them robust and easy to use[9]. The recent availability of high sensitivity and high-resolution light imaging devices at reasonable cost has led to an increased diffusion of CL imaging analytical techniques. This techniques take advantage of detection in comparison with other spectroscopic methods (high sensitivity and specificity, low background signal, easy quantitative analysis and wide dynamic range) and the possibility to localize and quantify the light emission on a sample down to the single-photon level[13,14].

The efficiency of a chemiluminescent reaction is given by the quantum yield, which is a measure of the fraction of reacting molecules that actually produce light. For analytical chemists the main attraction of CL is the opportunity to carry out sensitive assays over a wide range of concentrations using relatively inexpensive equipment. In practice it is usually combined with a complementary technique that confers specificity on the CL reaction. The most widely used complementary technique is CL immunoassay

where antibody labels such as acridinium esters have been used to detect analytes at picomolar concentrations[3].

LUMINESCENCE METHODS:

Chemiluminescence immunoassay (CLIA) methods:

Immunoassays based on chemiluminescence have substantially greater sensitivity and dynamic range than those based on earlier-generation detection techniques. Efficient light emission with low background is coupled with the high sensitivity and broad range of the photomultiplier detector. For every photon of light striking the surface of the photomultiplier, there is a 10^6 fold electronic amplification of the signal. Photomultipliers have very low background noise and inherent dynamic ranges of 5 to 6 orders of magnitude[15].

Chemiluminescence enzyme immunoassay (CLEIA), which integrates the advantages of immunoassay and chemiluminescence determination such as high specificity and throughput, rapidity and convenience in operation and relatively simple and inexpensive instrumentation[16,17].

The chemiluminescence imaging have also been extensively applied for the evaluation of the spatial distribution of a given target molecule, chemical or biochemical process on macro or microsamples associated with traditional methods, immunohistochemistry (IMH), in situ hybridization (ISH), enzyme or chemical reactions are used for the localization of antigens, gene sequences, enzymes or metabolites in cells and tissue sections[2].

Even though a wide range of different bio- and chemiluminescent systems have been applied in conventional chemiluminescence assays, only a small number of luminescent systems proved suitable for imaging applications. The main requirement, which is particularly crucial in imaging microscopy, is the localization of the luminescent signal in close proximity to the site where the luminescent reaction takes place[6].

For example, bio- and chemiluminescence imaging microscopy a target molecule is often detected through its binding to a biospecific probe labeled with an enzyme that

catalyzes a chemiluminescent reaction. Accurate localization of the target, down to the micrometer scale, requires that the light emission take place close to enzyme label[17].

IMH is based on the use of highly specific antibodies, able to bind to an endogenous and/or tumoral antigen (usually a protein), which are subsequently detected by means of class-specific antibodies conjugated to enzymes (Figure 1).

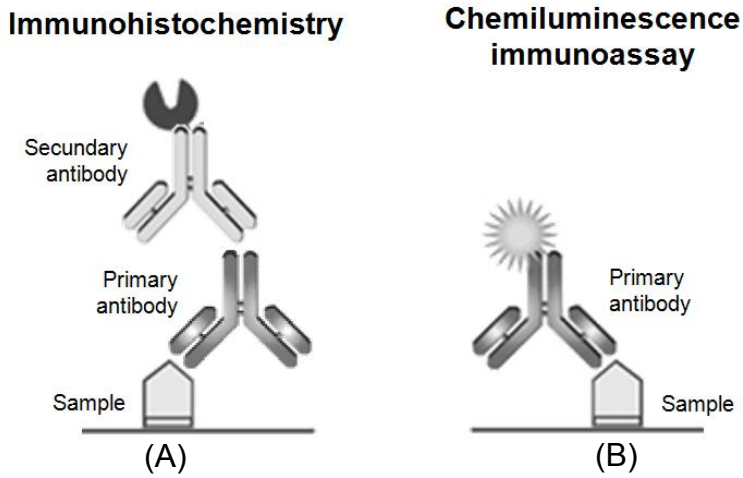

Figure 1. Two immunoassay techniques. (A) Illustration of classical Immunohistochemistry (based on enzymatic reactions) and (B) Chemiluminescence immunoassay method.

Now, The chemiluminescence immunoassay (CLIA) thus combine the specificity and sensitivity of labeled probes evidenced by chemiluminescence and spatial morphological resolution and localization of the signal of the IMH technique[18].

Among assay methods, chemiluminescence detection represents a versatile, ultrasensitive tool with a wide range of applications in biotechnology. It also gives a sensitive, rapid alternative to radioactivity as a detection principle in IA for the determination of molecules (e.g., proteins, hormones, drugs, nucleic acids and environmental pollutants). Chemiluminescence is now commonly used for immunoassay in the form of a CL label or as a CL detection reaction for an enzyme or a nanoparticle label. In recent years, CLIA has become very popular in clinical chemistry and environmental analysis, due to its high sensitivity, wide dynamic range and complete automation[19,20].

With the development and application of recombinant Ab (rAb) technology, markers and related techniques, solid-phase materials and improvements in automation, integration and miniaturization, CLIA has acquired an entirely new appearance[17].

Reagents required for reactions that produce CL may becoupled to Abs or antigens (Ags) and used as labels for immunoassay. Since the first report on CL labels in 1976, considerable efforts have been devoted to developing practical CL-labeling systems because of their low limits of detection (LODs) (Seitz, 1984). Luminol, isoluminol and its derivatives, acridinium ester, horseradish peroxidase (HRP) and alkaline phosphatase (ALP) have frequently been employed as CL labels in immunoassay for development and application of CLIA methods. Because the CL-detection methods have very low LODs, new CL labels and related substrates, new label technologies have been studied and obtained staggering results[12,22].

Scorilas and co-workers (2005)[23] synthesized two novel biotinylated acridinium derivatives, 9-(2-biotinyl-oxyethyl)-carboxylate-10-methyl-acridinium triflate (BOCMAT) and 9-(2-biotinyl-amidoethyl)-carboxylate-10-methyl-acridinium triflate (BACMAT), and described their luminescent properties and immunoassay applications.

Biochemiluminescence (BCL):

Owing to the light signal being generated by a chemical reaction in the dark, CL shows lower nonspecific signal and noninterference by light scattering. CL presents excellent performance in the mode of sandwich-type assays, the sensitivity of which is determined primarily according to the detection limit of the label. In the most format of CL immunoassay, the bound sample constituents are usually separated by an immobilized immunoreagent on a solid phase, for example, microwell plates, assay tubes and microparticles[24].

In the same way, the bound and free tracers can also be separatedand the separation step guarantees the low background signal of CL reaction.

LUMINESCENT TUMOR MARKERS:

Despite years of research and hundreds of reports on tumor markers in oncology, the number of markers that have emerged as clinically useful is pitifully small. Often, initially reported studies of a marker show great promise, but subsequent studies on the

same or related markers yield inconsistent conclusions or stand in direct contradiction to the promising results[25].

The proteins, mainly antibodies, are extensively used as diagnostic tools in a wide array of different analyses. Antibody-based immunoassays are the most commonly used type of diagnostic assay and still one of the fastest growing technologies for investigation of biomolecules[26].

Light-emitting chemical reactions (chemiluminescence - CL) and biological reactions (bioluminescence BL) have a diverse range of analytical applications but relatively few have been adopted by routine clinical laboratories[2].

For example, the principle of CL has been employed in the field of Obstetrics and Gynecology for the early detection of cervical cancer and pre-cancer[6].

Tumor growth and metastases, as well as drug efficacy, have been monitored in living animals by injecting a mouse with luminescence recombinant tumor cells and imaging the produced light[27]. Alternatively, primary tumors and unknown metastases can be revealed in vivo by using engineered light-emitting cells as probes for tumor location[28].

The concentration of carcinoembryonic antigen (CEA) in serum obtained from different carcinosis patients was detected by using the method of double antibody sandwich CL immunoassay and the results obtained by this method are fairly well agreeable to those obtained by a consagrated detection method (RIA)[29].

Zhuang and co-workers (2004)[29] synthesized a new biacridine compound, 10,10-dimethyl-3, 3-disulfo-9, 9-biacridine (DMDSBA) as a CL label and established a sandwich CLIA method for the determination of carcino-embryonic Ag (CEA) in human serum for detection of tumoral diseases.

A chemiluminescence enzyme immunoassay (CLEIA) based on alkaline phosphatase ALP-labeling has been proposed for AFP detection. But ALP-labeling methodology shows two weak points when compared with HRP-labeling methods, which will bring high background and unavoidably, unproportionate or false positive results in the clinical usage[30].

Zhang and co-workers (2009)[31] pretreating the magnetic particles with fluorescein isothiocyanate (FITC) labeled anti-AFP monoclonal antibody (FITC-McAb), a one step CLEIA based on magnetic particles was developed for AFP with high simplicity and sensitivity, as well as wide linear range. The proposed magnetic particles based CLEIA

was used to evaluate AFP in human sera samples and a good correlation was obtainedwhen comparing the results with that from a commercial electrochemiluminescence immunoassay kit.

The α-fetoprotein (AFP) is the most widely used tumor marker through CL for the diagnosis of Hepatocellular carcinoma (HCC)[32,33].

Despite the prostate cancer (PCa) has become a most widespread and stubborn disease and a major cause of death in the old age male population nowadays[34]. Zheng and co-workers (2008)[24] development sensitive chemiluminescence immunosensor was developed for the detection of PSA. A sandwich assay format was established by using a monoclonal antibody pair acting as the capture probe and detecting probe, respectively.

Most of the current PSA detection methods are usually based on immunoassays. The more established approaches include enzyme-linked immunosorbent assays (ELISA)[35], time-resolved immunofluorometric assay[36], surface plasmon fluorescence immunoassay[37], bioluminescent immunoassay[38], electrochemical[39] and surface-enhanced Raman scattering (SERS)[40].

Lately, several new PSA detection methods employing the nanowire electrodes[41], the nanoparticle-based bio bar code[42], and the microcantilever method[43] are proposed. Although they all have their individual strengths, chemiluminescence (CL) is among the most widely used readout modality in virtue of undoubted advantages over other more widely used systems[12,44].

PERSPECTIVES AND FUTURE:

Chemiluminescence is a well-established detection principle in various fields, including liquid chromatography, pharmaceutical and analysis, immuno- and gene probe assays. Nowadays, the CL represents a potent and versatile medical analytical tool suitable for a wide range of applications, because it combines the high delectability of the of the luminescence signal with the possibility to localize and quantify the light emission in a sample[6].

The high detectability and rapidity of CL techniques, along with the availability of microarray-based analytical devices, allows the development of high throughput

screening assays, in which simultaneous, multi-analyte detection is performed on multi samples[17].

Therefore, more and more medical experts and chemists are interested in CLIA. However, the development of CLIA is dependent on the application of the sensitive and selective chemiluminescent probe[29].

Improvements in analytical sensitivity will likely lead to the discovery of new analytes tumours detection. Technical enhancement holds the promise of detecting very low concentrations in serum using nanoparticles as labels and CL detection[45,46].

In conclusion, chemiluminescent immunoassay (CLIA) is a fast and simple method without radioactive pollution, its sensitivity is usually higher than that of fluorescent immunoassay and enzyme immunoassay[29].

Nowadays, the method using nanoparticles, especially metal, as biological labels has attracted considerable interest. As biological labels, NPs present many advantages[45,46,47].

Recently in our laboratory we tested the applications of chemiluminescence immunoassay with galectin-3 acridinum ester conjugated to anti-Galectin3 antibody in prostatic and thyroid tumors and chemiluminescent assays with acridinum ester conjugated with Concanavalin-A in breast lesions.

REFERENCES:

1. Carlson R, Lewis SW, Lim KF, Seeing the light: using chemiluminescence to demonstrate chemical fundamentals. Aust J Chem Educ, 14: 51-53, (2000).

2. Xiao Q, Li H, Lin JM, Development of a highly sensitive magnetic particle-based chemiluminescence enzyme immunoassay for thyroid stimulating hormone and comparison with two other immunoassays. Clinica Chimica Acta, 411 : 1151–1153, (2010).

3. Wilson R, Akhavan-Tafti H, DeSilva R, Schaap AP, Comparison Between Acridan Ester, Luminol, and Ruthenium Chelate Electrochemiluminescence. Electroanalysis, 13 (13); 1083-1092, (2001).

4. King, DW, Flow Injection Analysis of H2O2 in Natural Waters Using Acridinium Ester Chemiluminescence: Method Development and Optimization Using a Kinetic Model. Anal Chem, 79: 4169-4176, (2007).

5. Ram S, Siar CH, Chemiluminescence as a diagnostic aid in the detection of oral cancer and potentially malignant epithelial lesions. Int J Oral Maxillofac Surg, 34: 521-527, (2005).

6. Roda A, Guardiglia M, Pasinia P, Mirasolia M, Michelinia E, Musiani M, Bio- and chemiluminescence imaging in analytical chemistry. Analytica Chimica Acta, 541: 25-26, (2005).

7. Mann W, Lonky N, Nassad S, Scott R, Blanco J, Vasileu S, Papanicolau smear screening augmented by a magnified chemiluminescent exam. Int J Gynecol Obstet, 43: 289-296, (1993).

8. Lin J, Ju H, Eletrochemical and chemiluminescent immunosensors for tumor markers. Biosens Bioelectron, 20: 1461-1470, (2005).

9. Yang M, Liu C, Hu X, He P, Fang Y, Electrochemiluminescence assay for the detection of acridinium esters. Analytica Chimica Acta, 461 (1): 141-146, (2002).

10. Campos LM, Cavalcanti CBL, Lima-Filho JL, Carvalho-Junior LB, Beltrão EI, Acridinium ester conjugated to lectin as chemiluminescent histochemistry marker. Biomarkers, 11 (5): 480-484, (2003).

11. Ferreira EC, Rossi AV, A Quimiluminescência como ferramenta analítica: do mecanismo a aplicações da reação do luminol em métodos de análise. Química Nova, 25 (6): 1003-1011, (2002).

12. Kricka LJ, Clinical applications of chemiluminescence. Analytica Chimica Acta, 500: 279-386, (2003).

13. Roda A, Pasini P, Musiani M, Baraldini M, Guardigli M, Mirasoli M, Bioanalytical applications of chemiluminescent imaging. Baeyens W GarcÍa-Campaña AM eds. Chemiluminescence in analytical chemistry. Marcel Dekker New York, 473-495, (2002).

14. Yang Z, Fu Z, Yan F, Liu H, Huangxian Ju, A chemiluminescent immunosensor based on antibody immobilized carboxylic resin beads coupled with micro-bubble accelerated immunoreaction for fast flow-injection immunoassay. Biosensors and Bioelectronics, 24: 35-40, (2008).

15. Campbell AK, Hallet MB, Weeks I, Chemiluminescence as na analytical tool in cell biology and medicine. Methods in Biochemical Analysis, 31: 317-416, (1985).

16. Wei HB, Lin JM, Wu DN, Zhao LX, Ji Z,Ying XT, Detection of 17β-Estradiol in River Water and Human Urine by Highly Sensitive Chemiluminescence Enzyme Immunoassay. Chinese Journal of Analytical Chemistry. 35(3): 319-324, (2007).

17. Zhao L, Sun L, Chu X, Chemiluminescence immunoassay. Trends in Analytical Chemistry, 28 (4): 404-415, (2009).

18. Terrier N, Bonardet A, Descomps B, Cristol JP, Dupuy AM, Determination of beta2-microglobulin in biological samples using an immunoenzymometric assay (chemiluminescence detection) or an immunoturbidimetric assay: comparison with a radioimmunoassay. Clinical laboratory, 50 (11-12): 675-683, (2004).

19. Roda A, Pasini P, Guardigli M, Baraldini M, Musiani M, Mirasoli M, Bio- and chemiluminescence in bioanalysis. Fresenius J Anal Chem, 366: 752–759, (2002).

20. Lu S, Song J, Campbell-Palmer L, A modified chemiluminescence method for hydrogen peroxide determination in apple fruit tissues. Scientia Horticulturae, 120: 336-341, (2009).

21. Seitz WR, Immunoassay labels based on chemiluminescence and bioluminescence. Clin Biochem, 17: 120-125, (1984).

22. Lee JH, Rhoa JERR, Rhoa THD, Newby JG, Advent of innovative chemiluminescent enzyme immunoassay. Biosensors and Bioelectronics, 26: 377–382, (2010).

23. Scorilas A, Agiamarnioti K, Papadopoulos K, Novel Biotinylated Acridinium Derivatives: New Reagents for Fluorescence Immunoassays and Proteomics. Clin Chim Acta, 357: 159-167, (2005).

24. Zheng Y, Chen H, Liu XP, Jiang JH, Luo Y, Shen GL, Yu RQ, An ultrasensitive chemiluminescence immunosensor for PSA based on the enzyme encapsulated liposome. Talanta, 77: 809-814, (2008).

25. Schilsky RL, Taube SE, Introduction: Tumor markers as clinical cancer tests are we there yet?. Semin Oncol, 29: 211-2 (2002).

26. Borrebaeck CA, Rouge P, Mitogenic properties of structurally related Lathyrus lectins. Archives of Biochemistry and Biophysics, 248: 30-34, (1986).

27. Soling A, Rainov NG, Bioluminescence imaging in vivo application to cancer research. Expert Opin Biol Ther, 3: 1163-1172, (2003).

28. Yu YA, Timiryasova T, Zhang Q, Beltz R, Szalay AA, Optical imaging: bacteria, viruses, and mammalian cells encoding light-emitting proteins reveal the locations of primary tumors and metastases in animals. Anal Bioanal Chem, 377: 964-972, (2003).

29. Zhuang J, Huang JL, Chen GN, Synthesis of a new biacridine and its use as the chemiluminescent probe for immunoassay of carcinoembryonic antigen, Anal Chim Acta, 512: 347-353, (2004).

30. Wang X, Zhang QY, Li ZJ, Ying XT, Lin JM, Development of high-performance magnetic chemiluminescence enzyme immunoassay for α-fetoprotein (AFP) in human serum. Clinica Chimica Acta, 393 (2): 90-94, (2008).

31. Zhang Q, Wang X, Li Z, Lin JM, Evaluation of α-fetoprotein (AFP) in human serum by chemiluminescence enzyme immunoassay with magnetic particles and coated tubes as solid phases. Analytica Chimica Acta, 631 (2): 212-217, (2009).

32. Koteish A, Thuluvath PJ, Screening for hepatocellular carcinoma. J Vasc Interv Radiol, 13: S185-190, (2002).

33. Zhou L, Liu J, Luo F, Serum tumor markers for detection of hepatocellular carcinoma. World J Gastroenterol, 12: 1175–81, (2006).

34. INCA - Instituto Nacional de Câncer Brasil. Ministério da Saúde. Secretaria de Atenção à Saúde. Coordenação de Prevenção e Vigilância. Estimativa 2010-2011: Incidência de câncer no Brasil. Rio de Janeiro: INCA, (2010).

35. Acevedo B, Perera Y, Ruiz M, Rojas G, Benitez J, Ayala M, Gavilondo J, Development and validation of a quantitative ELISA for the measurement of PSA concentration. Clin Chim Acta, 317: 55-63, (2002).

36. Soukka T, Paukkunen J, Härmä H, Lönnberg S, Lindroos H, Lövgren T, Supersensitive Time-resolved Immunofluorometric Assay of Free Prostate-specific Antigen with Nanoparticle Label Technology. Clinical Chemistry, 47: 1269-1278, (2001).

37. Fang Y, Bjorn P, Stefan L, Wolfgang K, Attomolar sensitivity in bioassays based on surface plasmon fluorescence spectroscopy. J Am Chem Soc, 126 (29): 8902–8903, (2004).

38. Seto Y, Iba T, Abe K, Development of ultra-high sensitivity bioluminescent enzyme immunoassay for prostate-specific antigen (PSA) using firefly luciferase Luminescence, 16 (4): 285-290, (2001).

39. Fernandez-Sanchez C, McNeil CJ, Rawson K, Nilsson O, Disposable Noncompetitive Immunosensor for Free and Total Prostate-Specific Antigen Based on Capacitance Measurement. Anal Chem, 76: 5649-5656, (2004).

40. Grubisha DS, Lipert RJ, Park HY, Driskell J, Porter MD, Femtomolar detection of prostate-specific antigen: an immunoassay based on surface-enhanced Raman scattering and immunogold labels. Anal Chem, 75 (21): 5936-5943, (2003).

41. Roberts MA, Kelley SO, Ultrasensitive Detection of Enzymatic Activity with Nanowire electrodes. J Am Chem Soc, 129: 11356-11357, (2007).

42. Nam JM, Thaxton CS, Mirkin CA, Nanoparticles-based bio-bar codes for the ultrasensitive detection of proteins. Science, 301: 1884-1886, (2003).

43. Wu J, Fu Z, Yan F, Huangxian J, Biomedical and clinical applications of immunoassays and immunosensors for tumor markers. Trends in Analytical Chemistry, 26 (7): 679-688, (2007).

44. Chouhan RS, Babu KV, Kumar MA, Neeta NS, Thakur MS, Amitha Rani BE, Pasha A, Karanth GK, Karanth NG, Detection of methyl parathion using immuno-chemiluminescence based image analysis using charge coupled device. Biosens. Bioelectron, 21: 1264-1271, (2006).

45. Hu D, Han H, Zhou R, Dong F, Bei W, Jia F, Chen H, Gold(III) enhanced chemiluminescence immunoassay for detection of antibody against ApxIV of Actinobacillus pleuropneumoniae. Analyst, 133: 768-773, (2008).

46. Jie GH, Huang X, Sun J, Zhu J, Electrochemiluminescence of CdSe quantum dots for immunosensing of humanprealbumin. Biosens Bioelectron, 23 (12): 1896-1899, (2008).

47. Gupta S, Huda S, Kilpatrick PK, Velev OD, Characterization and optimization of gold nanoparticle-based silver-enhanced immunoassays. Anal Chem, 23: 5498-5504, (2007).

CAPÍTULO II

Technical report
CONJUGATION OF ANTI-GALECTIN-3 ANTIBODY TO ACRIDINIUM ESTER FOR CHEMILUMINESCENCE IMMUNOASSAY IN TISSUES

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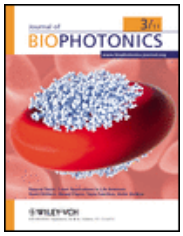

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Keywords:	acridinium ester, galectin-3, chemiluminescent immunoassay, tumors

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CONJUGATION OF ANTI-GALECTIN-3 ANTIBODY TO ACRIDINIUM ESTER FOR CHEMILUMINESCENCE IMMUNOASSAY IN TISSUES

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The chemiluminescence, a process in which excited atoms or molecules through chemical reactions release their excess energy as light, have been the focus of research to broaden its application in various fields of biotechnology. This study aimed to conjugate the acridinium ester to antibody (anti-galectin3), to evaluate the shelf-life testing and promote chemiluminescent immunoassay in tumoral lesions of prostate and thyroid. Our results demonstrate the efficiency of conjugation of anti-galectin-3 and acridinium ester to a shelf-life with a recovery in 96.51% after 12 months. By applying this conjugate in chemiluminescent immunoassay on thyroid and prostate tissues, we found differential patterns between the studied lesions and their normal counterpart.

1. Introduction

Chemiluminescence (CL) is defined as the emission of electromagnetic radiation, usually in the visible or near infrared, produced by a chemical reaction. This phenomenon has many applications in analytical chemistry, especially in the area of flow injection analysis, column chromatography and liquid separation systems by capillary electrophoresis as well as immunoassays [1].

The use of CL reactions has some advantages, among which have low detection limits and wide dynamic range [2]. The application of CL as an instrument of analysis depends on the coupling of the substance of interest to the participants of a chemiluminescent reaction or product in the excited state. The concentration of the unknown sample is proportional to the total production of light emitted or a physical parameter associated with the luminescence, such as color or polarization of light emitted [3].

Laboratory tests based on chemiluminescent principles have been developed for measurements requiring high sensitivity, for exemple, determination of cytokines, epidermal growth factor and vascular endothelial growth factor. Among the wide variety of tests are already commercially available to those used in the evaluation of thyroid function, fertility, tumor markers, therapeutic drug monitoring, hepatitis, specific proteins and steroids [4].

Chemiluminescent substances can be detected in the range of fentomoles or atomoles (10^{-15} to 10^{-18} mol), with sensitivity superior to spectrophotometric assays (10^{-6} to 10^{-9} mol) and fluorometric (10^{-9} to 10^{-12} mol) [5]. Several organic compounds exhibit CL under appropriate conditions, among which stand out luminol, isoluminol, acridinium ester or its derivatives as some of the markers used in chemiluminescent immunoassays [2, 6].

The acridine is a fluorescent compound whose salts can be stimulated to produce light in the presence of hydrogen peroxide in dilute alkalis in the absence of a catalyst [3]. This property is due to the presence of a quaternary nitrogen center and derivatisation at carbon-9 which has a phenyl ester labeled a radical. Among the derivatives of acridine, acridinium ester received a special mention by the easy oxidation with hydrogen peroxide and the formation of an excited product (N-metilacridona), which will emit light [7, 8].

The acridinium ester (AE) was introduced in immunoassays from the need to replace the markers radiosotopic few that were becoming popular because of its short half life, danger to health and potential problems regarding the waste generated [7]. Since then the chemiluminescence of AE has been widely applied in analytical chemistry and biochemistry [9].

The low detection limits and background signal (without catalysts), the high quantum yield and easy conjugation to proteins, allowed the use of EA in many competitive immunoassays, non-competitive for the detection of several analytes at sub-picomolar: growth hormone, tumor markers (α-fetoprotein), interleukins, interferons, antibodies, proinsulin, and apolipoprotein B [10].

After several conjugated molecules, the acridinium ester has been used in the preparation of cytochemical and his-

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tochemical probes. Like acridinium ester, other compounds such as luminol and isoluminol are also used as markers in chemiluminescent immunoassays [4].
This study proposes a combination between the acridinium ester and anti-galectin-3 to build a model of chemiluminescent immunoassay in tissues for differential diagnosis between glandular tumor lesions.

2. Experimental

Characterization of acridinium Ester

Acridine derivatives emit light in the form of flash in a short period of time (3-5 seconds) after starting the chemical reaction. The chemical process involves the absorption of the chemiluminescence reagents, sufficient energy for the generation of an activated complex, which turns into an electronically excited product. The mechanism has been studied in detail by McCapra [11, 12, 13]. The most probable mechanism for the chemiluminescence reaction of AE is illustrated in Fig. 1. AE is stable in acid is rapidly hydrolyzed under alkaline conditions. In the presence of peroxide anion (HOO-) undergoes an AE intermolecular rearrangement forming the unstable intermediate dioxetatone. This compound decomposes rapidly producing N-metilacridona quimiluminesce at 470 nm. All intermediates, except the dioxetatone, have been isolated and characterized. McCapra has also proposed other routes (see Fig. 1 for alternative routes) that do not imply the dioxetatone [13].

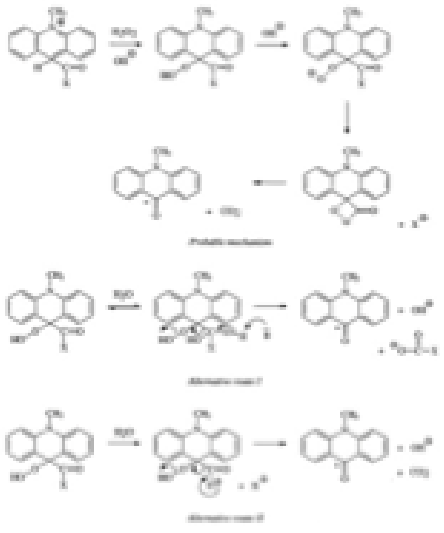

Figure 1. Most probable mechanism of acridinium derivatives and alternative routes [10].

Acridinium ester conjugation to anti-galectin3 (AE-AntiGal3)

The acridinium ester (DMAE-NHS/1966-1-53-2/Organic Lab kindly supplied by Dr. H. H. Weetall) was conjugated to the antibody according to the protocol developed by Weeks [7]. The antibody, anti-galectin-3 (500µl containing 2mg of protein) was incubated with 15µL of the AE solution (0.2 mg in 400µL of N,N-dimethylformamide) for 1 h at 25 °C under constant agitation. The conjugate (AE-AntiGal3) was applied to a column of Sephadex G-25 (10 x 1 cm) previously equilibrated with 10 mM phosphate buffer in 0.15 M NaCl (PBS), pH 7.2 (100ml). Aliquots (1 ml) were collected and the amount of protein determined spectrophotometrically at 280nm.
The chemiluminescence of the samples were measured by the luminometer Modulus Single Tube 9200-001 (Turner BioSystems) and the emission intensity was measured in relative light unit (RLU) using the following system: 50 µL of sample + 50µL H_2O_2 + 50µL NaOH. Protein concentration was determined according to Lowry [14].

3. Results and discussion

Acridinium ester-Antibody conjugate

The conjugate was purified by molecular sieve chromatography (Sephadex G-25) where support resolved the sample into a single protein peak (ABS 280nm) eluted with PBS. We observed the same protein fractions of a peak luminescence (RLU), indicating the conjugation of the AE with antibody (Figure 2). The second peak represents the luminescence of free AE that did not bind to the protein. Ten repetitions were performed, obtaining always the same chromatographic pattern.
The easy coupling to protein using activated esters or imidates explain why the chemiluminogenic acridinium derivatives have been early proposed for ultrasensitive immunoassaysof thyroid stimulating hormone (TSH), immunoglobulins and related compounds [15]. Furthermore, conjugation of AE to antibodies and enzymes has been used in clinical and biomedical research [4, 16]. AE reacts with amino groups of proteins under mild conditions, forming a complex stable and reproducible. The marking procedure is simple for both proteins, as for peptides and low molecular weight molecules [1].

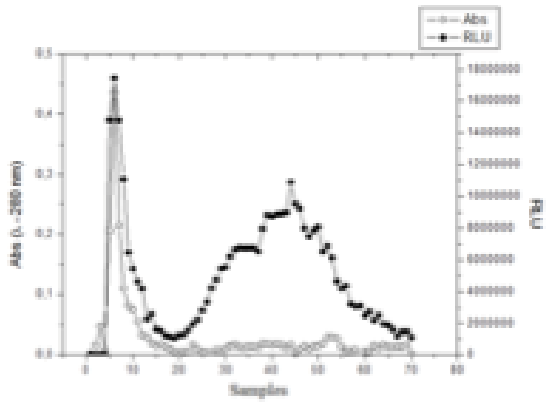

Figure 2: Chromatographic profile of the conjugate (AntiGal3-AE) on a column of Sephadex G-25 (10 x 1cm). Elution performed with 10 mM phosphate buffer in 0.15 M NaCl (PBS), pH 7.2. Fractions (1ml samples) were collected and absorbance and chemiluminescence measured.

The conjugate (AE-AntiGal3) was placed in polypropylene test tubes and stored at -10°C. Its shelf life was investigated by running the emission of luminescence (RLU) and tested for stability according to different time intervals (1, 2, 3, 4, 5, 6 months and 12 months later).
The results demonstrated the stability of the conjugate compared to various time intervals. It was subjected to chemiluminescent assays where its percentage of recovery after a period of one year of storage remained in the range of 96.51% activity.

Application models

Despite all the interrelationships between performed immunohistochemistry with antibodies and tumor cells, there are few studies demonstrating the effectiveness of the use of chemiluminescent immunoassay in the marking of tissues with benign and malignant tumors.
Starting from the combination of acridinium ester with anti-galectin-3, chemiluminescents immunoassays were performed in tissues of prostate and thyroid carcinoma in order to test the immunogenicity of the antibody test to their respective tissue antigens.
Tissue samples of prostate and thyroid were selected, and after being subjected to routine histological complete, slices were obtained with 8um of thickness incubated with AE conjugated AntiGal3 and submitted to chemiluminescent immunoassay as described in Figure 3.

Figure 3. Representation of the steps used in the chemiluminescent immunoassay with AE-AntiGal3 in tissues.

Was standardized an average prostate tissue area (1.8 cm^2) and thyroid (2.5 cm^2), and the concentration of conjugate (100 µg / ml). After the chemiluminescent immunoassay, which occurred at approximately 135 min (incubation + measurement), it was possible to quantify the presence of galectin-3 in the lesions studied by photon emission, expressed as relative light units (RLU) during the breaking the link between the antibody and the ester (Table 1).

Table 1. Comparative profile of chemiluminescent immunoassay for anti-galectin-3 in tumor lesions of the thyroid and prostate.

Tissue	Lesion	Mean diameter (cm^2 tissue area)	Concentration of AE-AntiGal3	Reaction time (min)	Amplitude (RLU)
Prostate	Prostatic carcinoma	1,8	100 µg/ml	135	296.583
Thyroid	Carcinoma Papillary	2,5	100 µg/ml	135	942.190

Inhibition 6.869 RLU (prostate carcinoma); 15.381 RLU (carcinoma papillary thyroid)

These results corroborate those reported in the literature with respect to conventional immunohistochemistry, where is observed an increaded presence of galectin in papillary thyroid carcinoma [17, 18] and a decrease of this expression in prostatic carcinoma [19, 20].
This evidence find that the chemiluminescent immunoassay technique has a high sensitivity and specificity stimulating investigations on the use of antibodies conjugated to chemiluminescent markers for the diagnosis of neoplasms.

4. Conclusion

In conclusion, the combination of acridinium ester to antibody (AE-AntiGal3) proved to be a simple procedure and can be easily repeated.
From these data could be observed that the combination of AE-AntiGal3 can be stored for an average period of 12 months, maintaining good capacity to generate chemiluminescent reactions.
Our tests in lesions of the prostate and thyroid tumors showed that the tissue analysis using chemiluminescent immunoassay with specific markers, may be a valuable

tool, used in association with others procediments, in the differential diagnosis of tumors.
These results demonstrate the stability of the conjugate studied, and adds to it, prospects as regards its use in the manufacture of diagnostic kits, for use in the diagnosis of tumor lesions.

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References

[1] A. M. García-Campaña, W. R. G. Baeyens, X. R. Zhang, E. Smet, G. Van Der Weken and K. Nakashima, A. C. Calokerinos, Biomed. Chomatography, **14**, 166-172 (2003).
[2] L. Zhao, L. Sun and X. Chu, Tren. in Anal. Chem. **28**, 4 (2009).
[3] A. Natrajan, D. Sharpe, J. Costello and Q. Jiang, Analyt. Biochem. **406**, 204–213 (2010).
[4] L. J. Kricka, Analyt. Chim. Act. **500**, 279-386 (2003).
[5] A. K. Campbell, M. B. Hallet and I Weeks, Method. in Biochem. Anal. **31**, 317-416 (1985).
[6] A. Roda, P. Pasini, M. Guardigli, M. Baraldini, M. Musiani, M. Mirasoli and Fresen. J. Analyt. Chem. **366**, 752-759 (2000).
[7] J. Weeks, M. L. Sturgess, R. C. Brown and J. S. Woodhead, Meth. Enzym. **133**, 366-387 (1986).
[8] M. Yang, C. Liu, X. Hu, P. He and Y. Fang, Analit. et Chim. Acta **461**, 141-146 (2002).
[9] D. W. King, W. J. Cooper, S. A. Rusak, B. M. Peake, J. J. Kiddle, D. W. O'Sullivan, M. L. Melamed, C. R. Morgan and S. M. Theberge, Anal. Chem. **79**, 4169-4176 (2007).
[10] C. Dodeigne, L. Thunus and R. Lejeune, Talanta **51**, 415-439 (2000).
[11] F. McCapra and D.G. Richardson, Tetrahedron Lett. **43**, 3167 (1964).
[12] F. McCapra, Acc. Chem. Res. **9**, 201 (1976).
[13] F. McCapra, Pure Appl. Chem. **24**, 611 (1970).
[14] O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, Biol. Chem. **193**, 265-75 (1951).
[15] J. S. Woodhead and I. Weeks, Pure Appl. Chem. **57**, 523-529 (1985).
[16] W. R. Baeyens, S. G. Schulman, A. C. Calokerinos, Y. Zhao, M. Garcia Campana, K. Nakashima and D. De Keukeleire. J Pharm. Biomed. Anal. **17**, 941-953 (1998).
[17] A. Bartolazzi, F. Orlandi, E. Saggiorato, M. Volante, F. Arecco, R. Rossetto, N. Palestini, E. Ghigo, M. Papotti, G. Bussolati, M. P. Martegani, F. Pantellini, A. Carpi, M. R. Giovagnoli, S. Monti, V. Toscano, S. Sciacchitano, G. M. Pennelli, C. Mian, M. R. Pelizzo, G. Troncone, L. Palombini, G. Chiappetta, G. Botti, A. Vecchione and R. Bellocco. Lancet Oncol. **9**, 543–49 (2008).
[18] M. Torregrossa, F. Pinuccia, T. Camacci, G. Materazzi, P. Berti, M. Minuto, R. Elisei, P. Vitti, P. Miccoli and F. Basolo, Hum. Pathol. **38**, 1482– 1488 (2007).
[19] J. L. S. Araújo-Filho, M. R. Melo-Júnior, C. B. L. Antunes, R. A. B. Lins, M. C. F. Machado, L. B. Carvalho-Jr and N. T. Pontes-Filho. J. Bras. Patol. Med. Lab. **42**, 469-475 (2006).
[20] H. Ahmed, P. P. Banerjee and G. R. Vasta. Biochem. and Biophys. Res. Comm. **358**, 241–246 (2007).

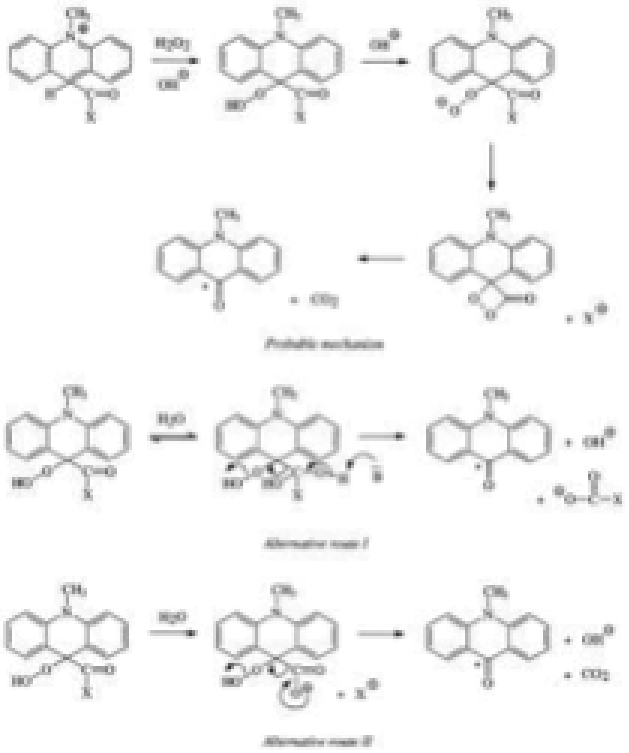

Figure 1. Most probable mechanism of acridinium deriv-atives and alternative routes [10].
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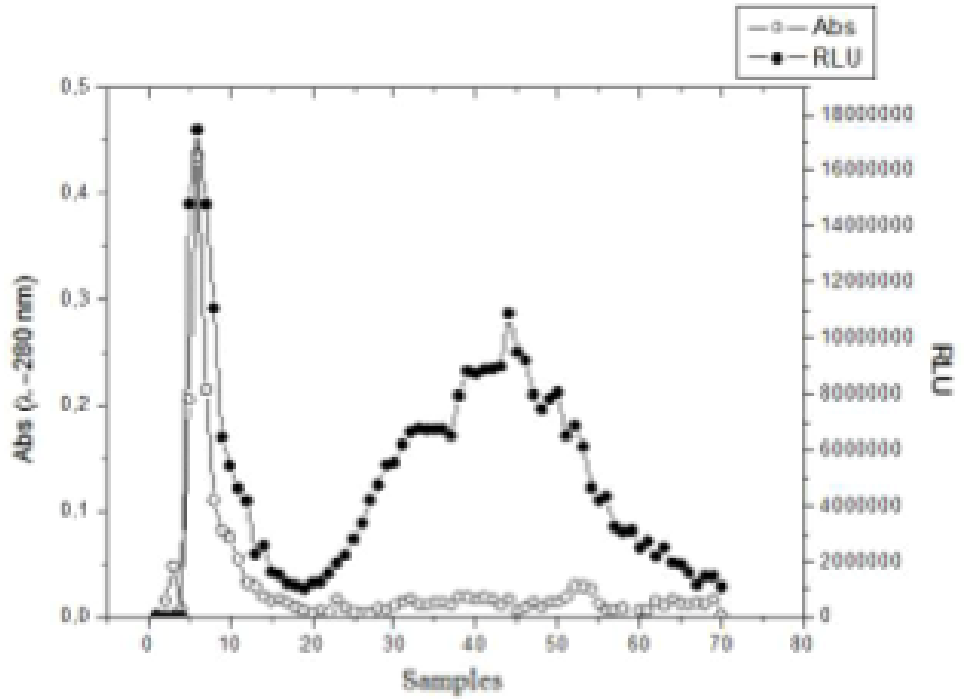

Figure 2: Chromatographic profile of the conjugate (An-tIGal3-AE) on a column of Sephadex G-25 (10 x 1cm). Elution performed with 10 mM phosphate buffer in 0.15 M NaCl (PBS), pH 7.2. Fractions (1ml samples) were collected and absorbance and chemiluminescence meas-ured. 180x131mm (96 x 96 DPI)

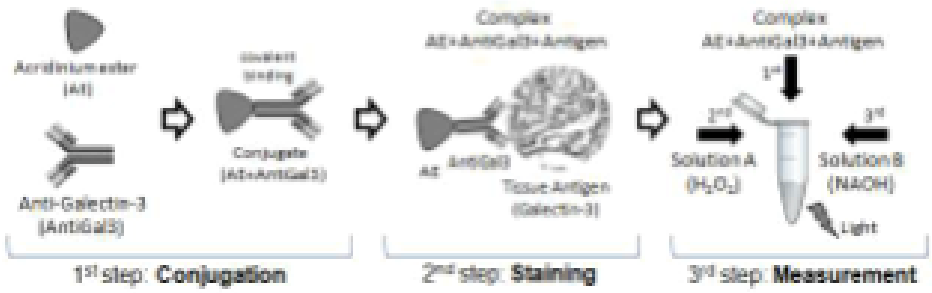

Figure 3. Representation of the steps used in the chemi-luminescent immunoassay with AE-AntiGal3 in tissues.
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CAPÍTULO III

CHEMILUMINESCENT DETECTION OF GALECTIN-3 IN TUMORAL TISSUE FROM PROSTATE

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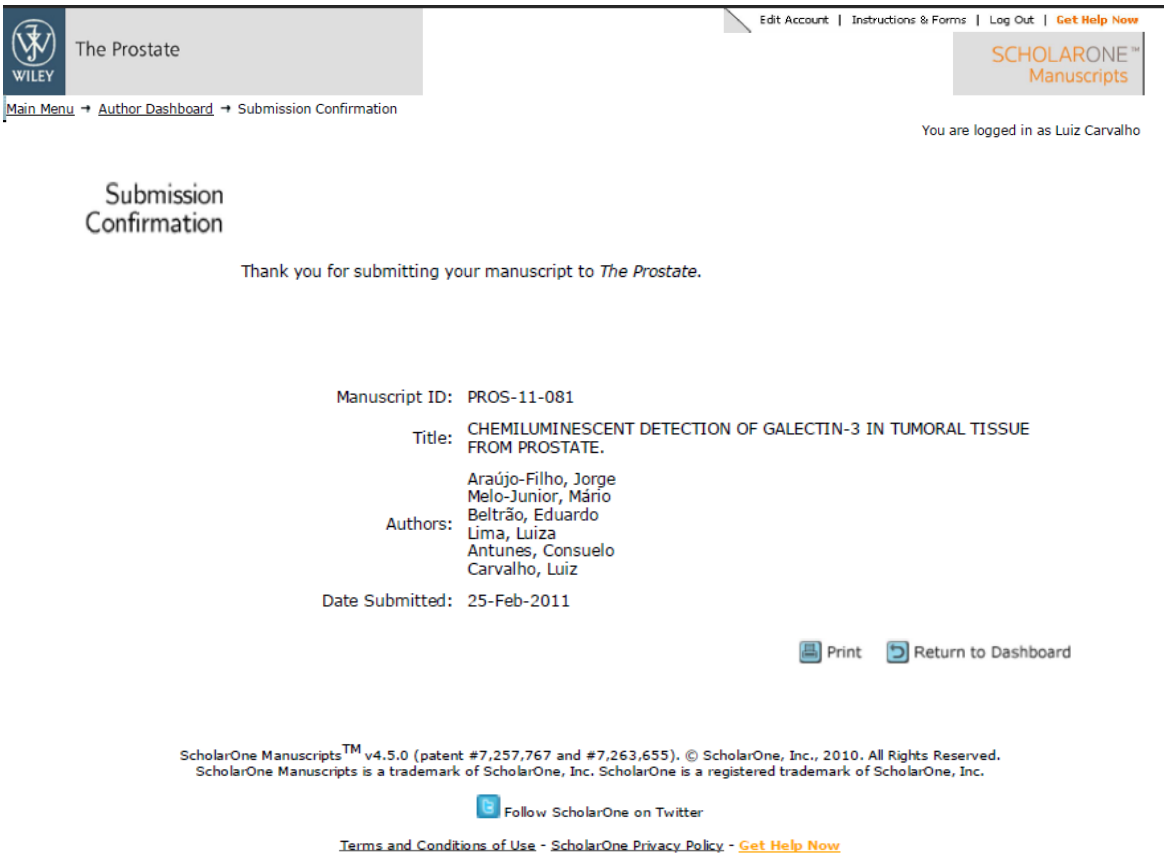

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CHEMILUMINESCENT DETECTION OF GALECTIN-3 IN TUMORAL TISSUE FROM PROSTATE

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Abstract

BACKGROUND. Galectin-3 (Gal3) is involved in immune system and cancer. Immunohistochemical methods to detect it and based on qualitative analysis present disparity and variability depending on the observers. This contribution proposes a chemiluminescent quantitative method.

METHODS. Monoclonal antibody anti-Gal3 was conjugated to acridinium ester (AE) and the complex formed with Gal3 in the prostate tissue was chemiluminescently detected.

RESULTS. The light emission (expressed in Relative Light Unit – RLU) showed mean values higher for benign prostatic hyperplasia ($1.38 \times 10^6 \pm 67{,}799$) than for normal tissues ($1.083 \times 10^6 \pm 81{,}044$) and adenocarcinoma ($214{,}234 \pm 22{,}172$). These values showed to be statistically significant ($p<0.001$). There was a linear relationship between RLU and tissue area. Furthermore, these values were dramatically reduced when the tissue samples were previously incubated with non labeled anti-Gal3. Finally, the anti-Gal3-AE solution in buffer stored at $4^{\circ}C$ and the tested samples with the same solution showed to be stable during twelve months and at least 72 h, respectively.

CONCLUSIONS. The Gal3 expression in prostate tissue was benign prostatic hyperplasia>normal>adenocarcinoma. This quantitative, specific and sensitive method based on labeling antibody to acridinium ester can be applied to detect antigen in tissue.

Key words: chemiluminescence, galectin-3, prostate tissue, acridinium ester.

INTRODUCTION

Galectins, a family of β-galactoside binding lectins, are multifunctional proteins involved in a variety of biological processes such as growth development, immune functions, apoptosis, and cancer metastasis [1]. The galectins have been widely used as tools to describe immunohistochemical changes in the tumor cell surface where these changes are associated with tumor cell growth by inducing apoptosis or metastasis [2]. Galectin-3 (Gal3) is one of the best studied galectins with many proposed functions in the immune system and cancer [3]. It is a small molecular weight (about 30 kDa) protein that is expressed in many types of human cells, in particular epithelial and immune cells [4]. PSA has been reported to regulate Gal3 in human semen and may

regulate Gal3 function during prostate cancer progression [5]. They presented evidences that PSA is a chymotrypsin-like serine protease secreted by the prostatic epithelium and normally functions in liquefaction of semen following ejaculation. Furthermore, PSA is implicated in the promotion of localized prostate tumors and bone metastases by its roles in immunomodulation, invasion, and apoptosis. Preliminary evaluation indicated that Gal3 is highly expressed in normal but not in malignant cells [6].

In prostate cancer, histopathological analysis has a great clinical relevance. However, immunohistochemical methods qualitatively analyzed have often shown great disparity and variability of results among different observers. Thus, in order to provide a numerical scale and reproducible patterns of marking tissue, increasing sensitivity and quality control analysis, it has been increasingly technological refinements using automated morphometric methods [7]. Another approach could be the use of chemiluminescent procedure. The chemiluminescence, a process in which excited atoms or molecules through chemical reactions release their excess energy as light has been the focus of research to broaden its application in various areas including in immunoassays [8].

The aim of this study was to identify the Gal3 in normal, benign hyperplasia and adenocarcinoma prostate tissues by using a specific antibody labeled to acridinium ester (AE). Afterwards the antigen-antibody complex was revealed by chemiluminescence that is capable to detect in the range of fentomoles or atomoles (10^{-15} to 10^{-18} mol) [9]. Additionally, Gal3 was evaluated in these prostate tissues by immunohistological staining using antibody conjugated to peroxidase and digital image analysis as comparison.

MATERIALS AND METHODS

Human prostate specimens. Formalin-fixed and paraffin-embedded benign hyperplasia (15); adenocarcinoma (15) and normal (15) prostate tissues were obtained from the archives of the Clinical Hospital of the Federal University of Pernambuco, Brazil. These diagnostics were established by one of the authors (CABL). The Gleason histological grade ranged from 5 to 9, with the mean of 6.7 and median of 6. Clinical staging was defined according to the American Joint Committee on Cancer classification [10] and histological grade according to Gleason score [11]. Patients' ages varied from 45 to 80 years old.

Anti-Gal3 antibody conjugation with acridinium ester. Acridinium ester was conjugated to anti-Gal3 antibody (Diagnostic Biosystems: clone 9C4) according to Weeks et al. [12]. Briefly, anti-Gal3 (500 µl containing 2 mg of protein) was incubated with 15 µl of acridinium ester solution (0.2 mg diluted in 400 µl of N,N-dimethylformamide) for 1 h at 25°C. The conjugate (anti-Gal3-acridinium ester) was applied to a column of Sephadex G-25 (10 x 1 cm), previously equilibrated with 10 mM phosphate buffer, pH 7.2 (100 ml) and eluted with this buffer. Aliquots (1 ml) were collected and their protein content was spectrophotometrically determined at 280 nm and chemiluminescence assayed. The aliquots containing protein and chemiluminescence were pooled, dialyzed overnight against 10 mM phosphate buffer, pH 7.2, and kept at -10°C until use.

Chemiluminescent immunoassay. Paraffin sections (8 µm) of samples were cut, transferred to glass slides, deparaffinized in xylene (1st for 5 min and 10 dips in 4 successive containers with xylene) and hydrated in graded alcohols (3 x 100% and 1 x 70% - 10 dips each). Afterwards tissue slices were incubated with anti-gal3-AE (100 µL - 100 mg mL^{-1}) for 2 h at 4 °C, followed by washings (2 x 5 min) with 5 mL of 10 mM phosphate buffer, containing 0.15 M NaCl (PBS), pH 7.2. Then the tissues were transferred to polypropylene test tubes with a volume of 50 µL of PBS. Finally, solutions of 0.5% H_2O_2 in 0.1 N HNO_3 (50 µl) and 0.25 M NaOH (50 µl) were added for chemiluminescent measurement using a luminometer Modulus Single Tube 9200-001 (Turner BioSystems, USA). The emission intensity was determined as relative light units (RLU) with a counting time of 5 seconds per sample. Triplicate measurements were carried out throughout in this study. Anti-Gal3-AE binding inhibition assays were accomplished by incubating the tissues with non labeled Anti-Gal3 (100 µL - 100 mg mL^{-1}) for 2 h at 4 °C prior to its incubation with anti-Gal3-AE. Following steps were as described previously for the binding protocol.

Immunohistochemical staining. The immunoreactivity of anti-Gal3 conjugated to peroxidase (Sigma, USA) was established according to the protocol proposed by Hsu et al. [13]. Briefly, the tissues were cut (4 µm), transferred to glass slides and deparaffinized as described above. The sections were incubated with citrate buffer at 100 °C for 30 min under water vapor, washed (5 min) with PBS and incubated with lyophilized bovine serum (to avoid cross-reactions), then incubated with H_2O_2 for 5 min and washed with PBS, incubated with a solution containing monoclonal antibody anti-Gal3 (at 1:50 dilution) for 1 h at 25°C. The prostate tissues were incubated with biotinylated secondary antibody (DAKO LSAB Kit, USA). The revelation of the antibody was obtained by reaction of peroxidase visualized by incubating the tissues in a solution containing the substrate (diaminobenzidine - DAB plus H_2O_2). The sections were counter stained with hematoxylin and examined under a light microscope (Olympus BH-2, Japan).

Digital image analysis. Slices images (magnification 100x) were captured using a digital video-camera (Sony, Japan) connected to a microscope and processed using OPTIMAS™ software version 6.1 (Optimas Corporation, USA). Antibody staining patterns revealed by DAB-peroxidase reaction were obtained in gray value. Image analysis of Gal3 positive cells was carried out adjusting the equipment to consider positive viable cells only those presenting nucleus and visible staining. A correction factor (CF) was used to minimize distortions in values due to the presence of non-stained cells, according to the equation CF=s/S where s means relative surface area and S the total area measured [14]. Analyses of the number of cells per area (12,234 μm^2) were developed in three random areas of stained tumor tissues.

Statistical analysis. The software OriginPro 8 (OriginLab Corporation, One Roundhouse Plaza, Northampton, MA 01060 USA) was used for the chemiluminescence data processing (expressed as mean ± standard deviation) and statistical analysis (Mann-Whitney U-test and Student t-test; $p<0.05$).

RESULTS

Here, AE was conjugated to the anti-Gal3 antibody and used to identify the Gal3 in prostate tissues. Figure 1 shows the RLU mean values obtained for normal ($1.083 \times 10^6 \pm 81044$ RLU), benign prostatic hyperplasia ($1.38 \times 10^6 \pm 67799$ RLU) and adenocarcinoma (214,234 ± 22,172 RLU). These mean values showed to be statistically significant ($p<0.001$). Furthermore, these values were dramatically reduced when the tissue samples were previously incubated with non labeled anti-Gal3 antibody yielding values of 10,617 ± 4,496 RLU; 8,837 ± 969 RLU and 5,687 ± 1,011 RLU, respectively.

The anti-Gal3-AE solution in buffer stored at -10°C showed to be stable during a year presenting (50 µl) chemiluminescence of 16,804,400 ± 431,263 (2.6%). Furthermore, the tissue samples treated with the anti-Gal3-AE were also stable for 72 h at least yielding RLU values of 1,087,250 ± 39,728 (6%); 1,311,200 ± 40,035 (3.0%) and 211,059 ± 12,977 (6%) for the normal, hyperplasia benign prostatic and prostatic adenocarcinoma samples, respectively.

The immunohistochemical staining studies demonstrated significant differences in the patterns of Gal3 expression in the tissues (Table 1). Benign prostatic hyperplasia presented higher average stained area (854.6 ± 49.7 μm^2) than the prostatic adenocarcinoma cells (118.5 ± 21.8 μm^2) and normal prostate (242.9 ± 24.6 μm^2). These results corroborate the chemiluminescence data.

DISCUSSION

In our lab, the use of chemiluminescence has been used to reveal the glycocode of mammary tissues [15]. Concanavalin A was labeled to AE and used to treat biopsies of normal and infiltrating duct carcinoma of mammary tissues. Photon emission, observed during the breakage of the chemical bound between lectin and AE, was quantified, expressed in RLU and correlated to the labeling of the normal and transformed tissues. RLU values were much higher for the infiltrating duct carcinoma than the normal tissue (about 500 times higher). The results also demonstrated that RLU presented a linear relationship with the labeled tissue area. The antibody anti-galectin-3 was also immobilized onto polysiloxane–polyvinyl alcohol semi-interpenetrated network and the antibody-matrix was capable to capture the serum antigen galectin-3 [16]. ELISA procedure was set up to quantify the Gal3 levels in sera from patients with prostatic adenocarcinoma and benign prostatic hyperplasia and healthy individuals. This report describes optical density (Gal3 level) values lower for the sera from patients compared with those found for the healthy individuals. Also, for comparative effect, the Gal3 expression in the prostate tissue through immunohistochemistry was evaluated. Gal3 showed a significant increase and reduction of the cytoplasmatic protein expression in benign prostatic hyperplasia and prostatic adenocarcinoma, respectively, compared with the normal prostate.

Qualitative and quantitative changes in the glycoprotein components of cell membranes are highly significant in the development and progression of many neoplasic processes [17]. In cancer cells the increased expression of surface carbohydrates has been widely documented by histochemistry and immunohistochemistry [18]. The galectins, in particular, are being tested as sensitive tools, stable and easy to use to distinguish transformed cells and non-transformed [19-22].

Galectins are found in many cell types and tissues, and various functions are described to them. The galectins have received increasing scientific attention due to its various functions, not only in biochemistry, but also in medicine with possible pharmacological activity. Among the most extensively studied galectins are Gal3, a protein with diverse biological roles [23].

About functional properties of galectin-3, described so far, it is clear that this galectin has several roles in the pathogenesis of cancer, proliferation and dissemination of metastases. In addition, changes were found not only in the expression of galectin-3 but also in their intracellular distribution in certain types of cancers [24, 25].

Our results are consistent with those described in the literature on prostate cancer, showing a reduced expression of galectin-3 occurs as the disease progresses [23, 26]. And conversely, a higher expression of this protein in tissue fragments of normal when compared to the malignant counterpart [6].

Several evidence have demonstrated the great importance of interactions between cancer cells and residual carbohydrates during cancer progression [19], suggesting the existence of a large number of molecules involved in this biological event.

In the present study the analysis based on immunohistochemical staining identified expression of Gal3 more intense in cases of benign prostatic hyperplasia and normal tissue compared to the prostatic adenocarcinoma. This finding corroborates previous reports [6, 19].

From the results above one can conclude that there was a statistically significant reduction in tissue expression of Gal3 in prostatic adenocarcinoma cells compared to normal tissue. Benign prostatic hyperplasia showed a significant increase in cellular expression of Gal3 when compared with the counterpart malignant and normal tissue. Furthermore, there was a linear relationship between the Gal3 chemiluminescent detection and tissue area in all examined prostate samples. The anti-Gal3-AE conjugate and the treated samples showed to be stables for one year and 72 h, respectively. Thus the antibodies conjugated to acridinium ester can be used as tool to quantify the changes, minimizing the subjectivity of the analysis. This method demonstrated that imunochemiluminescence can be value in tissue analysis, showing high sensitivity and perspective of application in early diagnosis due to its detection at the molecular level.

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References

1. Liu FT, Rabinovich GA. Galectins: regulators of acute and chronic inflammation. Ann N Y Acad Sci 2010;1183:158-182.

2. Dumic J, Dabelic S, Flogel M. Galectin-3: an open-ended story. Biochim Biophys Acta 2006;1760:616-635.

3. Salomonsson E, Carlsson MC, Osla V, Hendus-Altenburger R, Kahl-Knutson B, Oberg CT, Sundin A, Nilsson R, Nordberg-Karlsson E, Nilsson UJ, Karlsson A, Rini JM, Leffler H. Mutational tuning of galectin-3 specificity and biological function. J Biol Chem 2010;285:35079-35091.

4. Yu LG. Circulating galectin-3 in the bloodstream: An emerging promoter of cancer metastasis. World J Gastrointest Oncol 2010;2:177-180.

5. Saraswati S, Block AS, Davidson MK, Rank RG, Mahadevan M, Diekman AB. Galectin-3 is a substrate for prostate specific antigen (PSA) in human seminal plasma. Prostate 2011;71:197-208.

6. Ahmed H, Banerjee PP, Vasta GR. Differential expression of galectins in normal, benign and malignant prostate epithelial cells: silencing of galectin-3 expression in prostate cancer by its promoter methylation. Biochem Biophys Res Commun 2007;358:241-246.

7. Rashbass J. The impact of information technology on histopathology. Histopathology 2000;36:1-7.

8. Zhao L, Sun L, Chu X. Chemiluminescence immunoassay. TrAC Trends in Analytical Chemistry 2009;28:404-415.

9. Campbell AK, Hallett MB, Weeks I. Chemiluminescence as an analytical tool in cell biology and medicine. Methods Biochem Anal 1985;31:317-416.

10. Beahrs OH, Henson DE, Hutter RVP, Kennedy BE. Manual for Staging of Cancer Philadelphia: Lippincott Williams & Wilkins; 1992.

11. Gleason DF. Histologic grading of prostate cancer: a perspective. Hum Pathol 1992;23:273-279.

12. Weeks I, Sturgess M, Brown RC, Woodhead JS. Immunoassays using acridinium esters. Methods Enzymol 1986;133:366-387.

13. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981;29:577-580.

14. Van Bemmel JH, Musen MA. Biostatistical Methods. Handbook of Medical Informatics. Springer-Velag ; 1997. p 387-397.

15. Campos LM, Cavalcanti CL, Lima-Filho JL, Carvalho LB, Beltrao EI. Acridinium ester conjugated to lectin as chemiluminescent histochemistry marker. Biomarkers 2006;11:480-484.

16. Melo-Junior MR, Araujo-Filho JL, Lins CA, de Pontes-Filho NT, de Carvalho LBJ. Immobilization of anti-galectin-3 onto polysiloxane-polyvinyl alcohol disks for tumor prostatic diseases diagnosis. Appl Biochem Biotechnol 2010;160:2198-2207.

17. Bao L, Haque A, Jackson K, Hazari S, Moroz K, Jetly R, Dash S. Increased Expression of P-Glycoprotein Is Associated with Doxorubicin Chemoresistance in the Metastatic 4T1 Breast Cancer Model. Am J Pathol 2011;178:838-852.

18. Kim MS, Kwon S, Kim T, Lee ES, Park JK. Quantitative proteomic profiling of breast cancers using a multiplexed microfluidic platform for immunohistochemistry and immunocytochemistry. Biomaterials 2011;32:1396-1403.

19. van den Brule FA, Waltregny D, Liu FT, Castronovo V. Alteration of the cytoplasmic/nuclear expression pattern of galectin-3 correlates with prostate carcinoma progression. Int J Cancer 2000;89:361-367.

20. Califice S, Castronovo V, van den Brule F. Galectin-3 and cancer (Review). Int J Oncol 2004;25:983-992.

21. Califice S, Castronovo V, Bracke M, van den Brule F. Dual activities of galectin-3 in human prostate cancer: tumor suppression of nuclear galectin-3 vs tumor promotion of cytoplasmic galectin-3. Oncogene 2004;23:7527-7536.

22. van den Brule F, Califice S, Castronovo V. Expression of galectins in cancer: a critical review. Glycoconj J 2004;19:537-542.

23. Wang Y, Nangia-Makker P, Tait L, Balan V, Hogan V, Pienta KJ, Raz A. Regulation of prostate cancer progression by galectin-3. Am J Pathol 2009;174:1515-1523.

24. Stannard KA, Collins PM, Ito K, Sullivan EM, Scott SA, Gabutero E, Darren G, I, Low P, Nilsson UJ, Leffler H, Blanchard H, Ralph SJ. Galectin inhibitory disaccharides promote tumour immunity in a breast cancer model. Cancer Lett 2010;299:95-110.

25. Krzeminski M, Singh T, Andre S, Lensch M, Wu AM, Bonvin AM, Gabius HJ. Human galectin-3 (Mac-2 antigen): defining molecular switches of affinity to natural glycoproteins, structural and dynamic aspects of glycan binding by flexible ligand docking and putative regulatory sequences in the proximal promoter region. Biochim Biophys Acta 2011;1810:150-161.

26. Ellerhorst J, Nguyen T, Cooper DN, Lotan D, Lotan R. Differential expression of endogenous galectin-1 and galectin-3 in human prostate cancer cell lines and effects of overexpressing galectin-1 on cell phenotype. Int J Oncol 1999;14:217-224.

Figure legends.

Figure 1. The light emission (RLU) from normal, benign prostatic hyperplasia (BPH) and Prostatic adenocarcinoma (PA) tissues (n=15 and triplicates). The inhibited columns were obtained by incubating the tissues with non-labeled antibody previous to anti-Gal3-AE incubation. The statistical analyses showed significance between the mean values ($p<0.001$).

Figure 2. Relationship between RLU and the tissue area of benign prostatic hyperplasia (○), normal tissue (●) and adenocarcinoma (■).

Figure 1

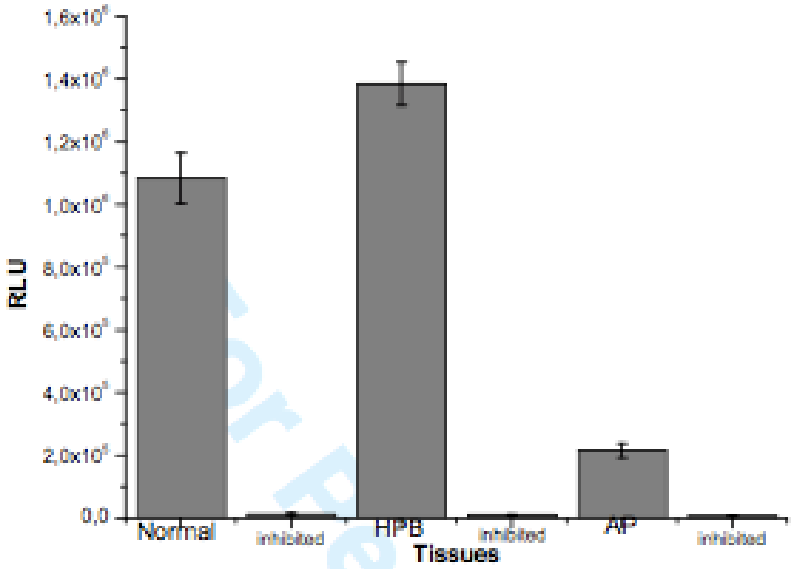

Figure 2

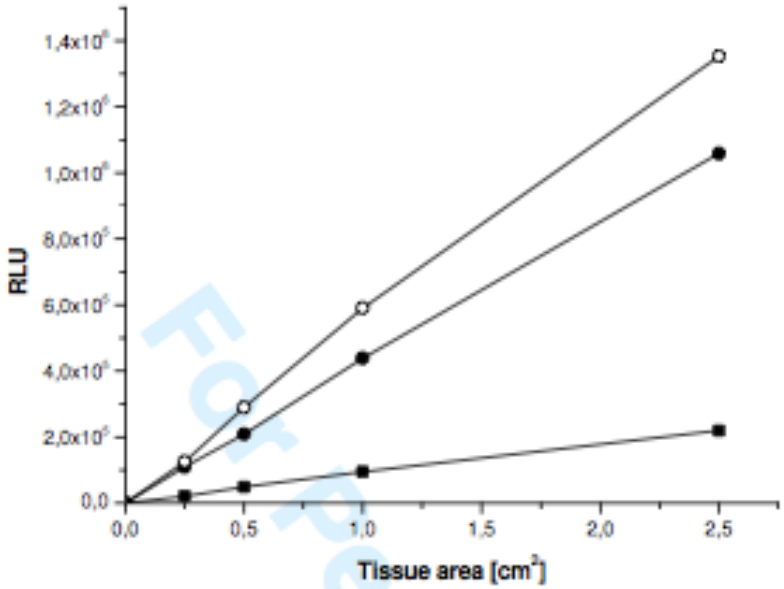

Table 1. Results of the immunohistochemical staining from number of positive Gal3 in prostatic tissues evaluated by digital analysis (total area per field = 12,234 µm2).

Tissue	Average area (µm2)
Benign prostatic hyperplasia	854.6 ± 49.7*
Prostatic adenocarcinoma	118.5 ± 21.8 *
Normal prostate	242.9 ± 24.6

(* p<0.001)

CAPÍTULO IV

COMPARATIVE ANALYSIS BETWEEN IMMUNOHISTOCHEMISTRY AND CHEMILUMINESCENCE IMMUNOASSAY IN THYROID TUMOURS

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COMPARATIVE ANALYSIS BETWEEN IMMUNOHISTOCHEMISTRY AND CHEMILUMINESCENCE IMMUNOASSAY IN THYROID TUMOURS

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Abstract

Galectin-3 has been targeted for researches as specific molecule capable to differentiating benign between malignant lesions that combined with quantitative techniques in order to eliminate the subjectivity of the evaluator, has an important role in more accurate diagnosis. In addition, chemiluminescent assays have also been subject of research. From this, this study was conjugated antibody anti-galectin-3 to acridinium ester for chemiluminescent immunoassay to compare the results obtained with conventional immunohistochemistry. We selected paraffin blocks containing tissues of the thyroid: normal (10), follicular adenoma (9), follicular carcinoma (9) and papillary carcinoma (10), and tested to immunohistochemistry and chemiluminescent immunoassay. The results of chemiluminescent immunoassay showed mean values of light emission (expressed in Relative Light Unit – RLU) higher for papillary carcinoma (911.092 ± 16.350 RLU) than for follicular carcinoma (480.541 ± 181.700 RLU), follicular adenoma (115.249 ± 12.321 RLU) and normal thyroid (77.056 ± 7.091 RLU). These values showed to be statistically significant ($p<0.001$). Finally, the anti-Gal3-AE solution in buffer stored at 4°C showed to be stable during twelve months. Such findings demonstrate that the chemiluminescent immunoassay show promise in quantifying performed stained by specific markers and can become an important technique in evaluating antigenic in small tissue samples. Thus, we conclude that the chemiluminescent immunoassay can help to differentiate malignant papillary lesions of the thyroid, is a viable complementary tool.

Keywords: thyroid, chemiluminescent immunoassay, galectin-3

INTRODUCTION

Palpable thyroid nodules are present in 4% to 7% of the adult population; however, an important fraction of these lesions represents cancer (Hegedus, 2004).

Thyroid cancer is the most common malignancy of the endocrine glands, it is presented as unique nodules in the parenchyma, in most cases (Guarino *et al*., 2010). Thyroid carcinomas are classified by clinical and histological parameters and include the subtypes: papillary, follicular, medullar and undifferentiated (Segev *et al*., 2003).

Studies are being conducted at the molecular level, like genomics, transcriptomics and proteomics in benign and malignant follicular nodules of thyroid that may provide new diagnostic methods in the future (Durand *et al*., 2008).

The most widely used technique for the diagnosis of thyroid lesions is fine needle aspiration biopsy, but like all diagnostic method also has limitations due to the profiles and some biomarkers used in routine are unable to promote the distinction between hyperplasia, follicular adenoma and follicular carcinoma. Thus, specific markers have been investigated as adjuvant tool in this investigation (Bartolazzi *et al*., 2008).

Among the potential markers for thyroid lesions lists galectin-3 (Gal-3), which is a protein of the growing family of animal lectins with affinity for β-galactoside (Argüeso & Panjwani, 2011). The expression of Gal-3 was associated with cancer in several tissues (Araújo-Filho *et al*., 2006; Melo-Junior *et al*., 2010). Previous studies indicated that a high expression of this protein is observed in tumors, suggesting the use of immune detection of Gal-3 as a potential marker of malignancy in thyroid neoplasms (Bartolazzi *et al*., 2008; Inohara *et al*., 2008).

Besides some markers associated to sensitive techniques have been researched, such as chemiluminescence, a process in which excited atoms or molecules through chemical reactions release their excess energy as light (Garcia-Campaña *et al*., 2003), have been the focus of research to broaden its application in different biotechnology areas (Adamezyk *et al*., 2001).

Chemiluminescent methods have several benefits, including: limit of ultrasensitive detection, rapid testing and a wide range of analytical applications, no harm to the environment and specificity (Kricka, 2003).

Chemiluminescent immunoassays require light-emitting elements such as luminol, isoluminol or acridine which is a fluorescent compound whose can be stimulated to produce light in the presence of hydrogen peroxide (King *et al*. 2007). Among the

derivatives of acridine, the acridinium ester (AE) received a special mention by the easy oxidation with hydrogen peroxide and formation of an excited product (N-metilacridona), which emits light (Weeks *et al*., 1983, Yang *et al*., 2002).

After conjugated to various biomarkers, the AE can be employed in the preparation of cyto and histochemical probes for making a variety of tests, as some already available commercially used in the evaluation of thyroid function, fertility, tumor markers, monitoring therapeutic drugs, hepatitis, specific proteins and steroids (Kricka, 2003).

Based on these data, the study has standardized a protocol for detection of Gal-3 from thyroid tissue based on antibody conjugated to AE by performing tests chemiluminescent immunoassay and comparing them to the results with conventional immunohistochemistry.

MATERIALS AND METHODS

Tissue specimens

Paraffin blocks of thyroid tissue biopsies were obtained from patients (both sexes) with adenoma (n=9), carcinoma folicular (n=9) e carcinoma papilar (n=10) and healthy individuals (n=10) at a private laboratory (Recife, Brazil). Patients' ages varied from 45 to 70 year-old, with the mean of 57.5. This work was submitted and approved by the research ethics committee.

Anti-gal3 antibody conjugation with AE

AE was conjugated to anti-Gal-3 antibody (Diagnostic Biosystems: clone 9C4) according to Weeks *et al*. (1986). Briefly, anti-gal3 (500 µl containing 2 mg of protein) was incubated with 15 µl of AE solution (0.2 mg diluted in 400 µl of N,N-dimethylformamide) for 1 h at 25^{o}C. The conjugate (anti-gal3-AE) was applied to a column of Sephadex G-25 (10 x 1 cm), previously equilibrated with 10 mM phosphate buffer, pH 7.2 (100 ml) and eluted with this buffer. Aliquots (1 mL) were collected and their protein content was spectrophotometrically determined at 280 nm. The aliquots corresponding to the protein peak had their chemiluminescence assayed, pooled, dialyzed overnight against 10 mM phosphate buffer, pH 7.2, and kept at 10 ^{o}C until use.

Chemiluminescent immunoassay

Paraffin sections (8 μm) of thyroid samples were cut, transferred to glass slides, deparaffinized in xylene (1st for 5 min and 10 dips in 4 successive containers with xylene) and hydrated in graded alcohols (3 x 100% and 1 x 70% - 10 dips each). Afterwards tissue slices were incubated with anti-galectin3-AE (100 μL - 100 mg mL^{-1}) for 2 h at 4 °C, followed by washings (2 x 5 minutes) with 5 mL of 10 mM phosphate buffer, containing 0.15 M NaCl (PBS), pH 7.2. Then the tissues were transferred to polypropylene test tubes with a volume of 50 μL of PBS. Finally, solutions of 0.5% H_2O_2 in 0.1 N HNO_3 (50 μl) and 0.25 M NaOH (50 μl) were added for chemiluminescent measurement using a luminometer Modulus Single Tube 9200-001 (Turner BioSystems, USA). The emission intensity was determined as relative light units (RLU) with a counting time of five seconds per sample. Triplicate measurements were carried out throughout in all samples tested. The conjugate anti-galectin-3-acridinium ester (Anti-Gal3-AE) binding inhibition assays were accomplished by incubating the tissues with non labeled Anti-gal3 (100 μL - 100 mg mL^{-1}) for 2 h at 4 °C prior to its incubation with anti-gal3-AE.

Immunohistological staining

Tissue sections 4 μm thick were deparaffinized in xylene and rehydrated in a graded ethanol series. Sections were treated with 0.3% methanol containing 0.5 mL H_2O_2 for 30 min. Following extensive rinsing in 0.1 M phosphate buffer, pH 6.0, containing NaCl 1 M (PBS), normal goat serum (diluted 1:20) was added to the section for 30 min. Anti-Gal-3 antibody (1:50 – Novocastra, Norwell MA, USA) was used according to Jakubiak-Wielganowicz and co-workers (2003). Streptavidine-biotine-peroxidase kit (DAKO, USA) was applied for antigen detection and diaminobenzidine solution was used for visualization. In control sections, PBS replaced antibody solution.

Statistical analysis

The software OriginPro 8 (OriginLab Corporation, One Roundhouse Plaza, Northampton, MA 01060 USA) was used for the statistical analysis and data were expressed as mean ± standard deviation (s.d.).

RESULTS AND DISCUSSION

Immunohistochemistry

Even after the histopathological analysis is not always possible to differentiate follicular carcinoma from adenoma (Sherman, 2003). As a consequence, most patients with these lesions are referred for surgery. According to Jogai and co-workers (2004), immunohistochemistry plays an important role in the diagnosis of various thyroid lesions, and some authors list Gal-3 as a good marker for malignant lesions, but also present positivity in normal cells (Coli *et al*., 2002).

There are no perfect diagnostic tests, without false positives or false negatives results. Thus, in interpreting the test results, the analyst is faced with uncertainty about the state of the lesion. When it comes to cancer research, diagnosis wrong decisions can have disastrous consequences for the patient (Giard & Hermans, 1996).

In our study, the results presented in Table 1 show that there was no immunostaining in any of the cases of normal thyroid. Cases of adenoma, 22.25% had moderate, 22.25% had weak immunostaining intensity and 55.5% absent staining (Figure 1A). Among the samples of follicular carcinoma 55.5% had positive staining with moderate intensity staining (22.2%) and weak (33.3%) while 44.5% had absent markings (Figure 2).

Numerous studies have shown that Gal-3 has a weak or absent expression in normal tissue and benign lesions, suggesting an involvement of Gal-3 in tumor progression (Bartolazzi *et al*. 2008; Savin *et al*., 2008) and that data confirmed by this study.

Tabela 1: Qualitative analysis of the intensity of staining for Gal-3 in thyroid tissues by immunohistochemistry.

Histopathology	**Number patients**	**Staining level***			
		-	**+**	**++**	**+++**
Normal	10	10	0	0	0
Follicular adenoma	9	5	2	2	0
Papillary carcinoma	10	0	0	2	8
Follicular carcinoma	9	4	3	2	0

* - absent, + weak, ++ moderate, +++ intense

All cases of papillary carcinoma were positive for Gal-3 also had a higher cytoplasmatic expression, with little nuclear staining (Figure 1A). Early studies, also observed a high expression of Gal-3 in papillary carcinoma, characterizing it as one that presents less difficulty in their cytological diagnosis (Coli *et al*., 2002; Inohara *et al*., 2008).

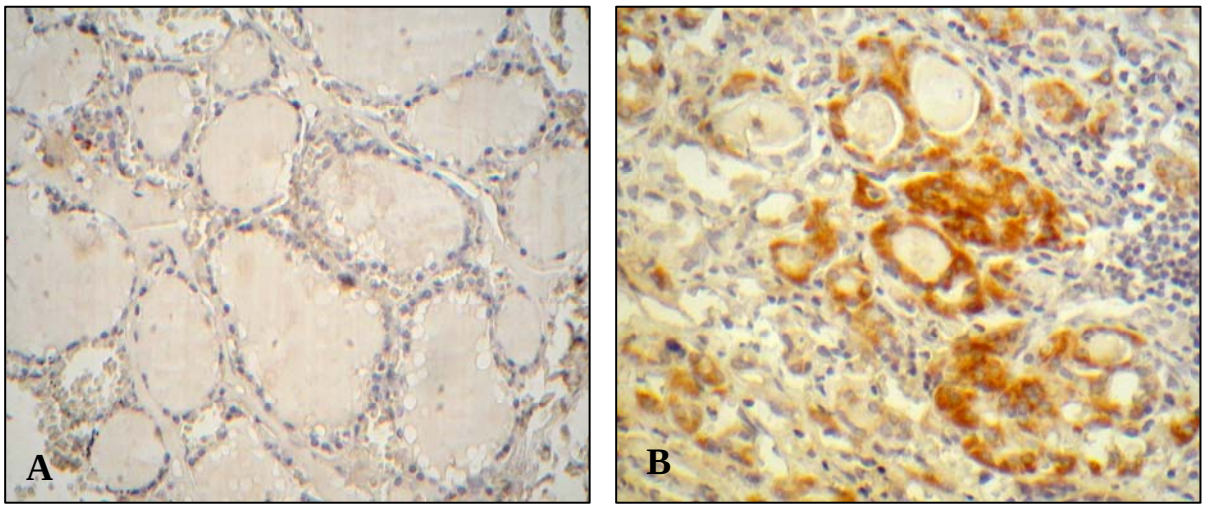

Figure 1. Immunohistochemical profile of galectin-3 protein in the thyroid lesions. A) weak tissue expression in the adenoma. (Magnification, 200x); B) Intense reactivity into papilar carcinoma.

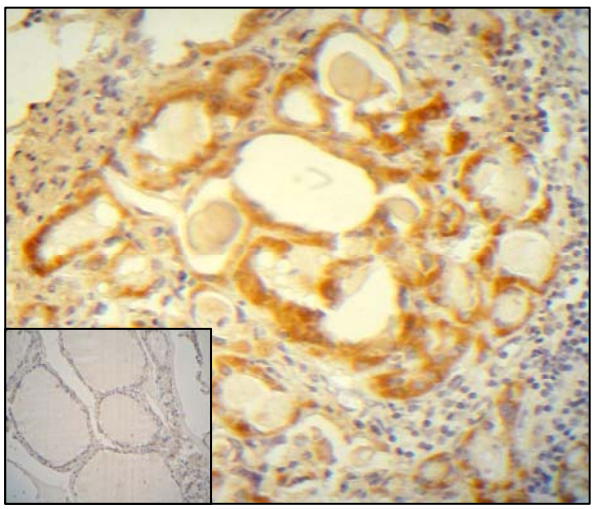

Figure 2. Positive imunostain for galectin-3 protein in the thyroid follicular carcinoma. On the other hand, there is not tissue staining in normal thyroid (small figure) (Magnification, 200x).

The results of the follicular carcinoma showed that 44.45% of the sample showed no immunostaining, 33.35% showed weak and 22.20% moderate reactivity. On cases that presented immunostaining were probably false positives.

Regarding the staining of galectin-3 in cases of adenoma, Bartolazzi and co-workers (2001) presented the hypothesis that follicular adenomas with expression of galectin-3 are actually represented by malignantly transformed cells, in which the capsular invasion and / or vascular damage has not occurred. These lesions are likely to be a malignancy in its earliest stage, or a potentially malignant lesion, but data from this study do not help elucidate this hypothesis.

A fine needle aspiration biopsy of thyroid is the most used test in the evaluation of thyroid nodules, but there are borderline cases that can not be defined cytologically as malignant or benign. Before that, thyroidectomy is performed, not by a therapeutic action, but for the diagnosis. With this most suspicious nodules are operated, and after histopathological examination is performed, more than two-thirds are classified as benign (Dean & Gharib, 2008).

Despite having high sensitivity and specificity, the expression of Gal-3 in some cases may not occur as expected, or as in cases of follicular adenoma it is observed without the lesion being malignant. So the research for other markers to be used in conjunction with the Gal-3 may increase the diagnostic accuracy.

However, considering that not exist immunohistochemical staining specific markers for malignancy of thyroid, it is advisable to use Gal-3 on a panel, always associated to morphological characteristics, combined with the expression of each marker (Matos *et al*., 2005). Still, there are cases of morphological and immunohistochemical evaluation difficult, necessitating the other helper methods that provide sensitive and quantitative data in order to minimize the subjectivity of the analyst.

Chemiluminescent immunoassay

Among the current diagnostic techniques, chemiluminescent assays have been optimized for laboratory tests that require a high sensitivity as well as for protein determination (Xiao *et al*., 2010).

In our laboratory using chemiluminescent compounds exhibit chemiluminescence in appropriate conditions, such as AE was used in conjunction with anti-Gal-3 for chemiluminescent immunoassay. Earlier the combination of tumor markers specific to the AE was held, and chemiluminescent assays with lectins have been tested previously in tissues (Campos *et al*., 2006).

The acridine ester was conjugated to anti-galectin-3, and its shelf life test has shown its stability at different time intervals, totaling 12 months, and maintained activity in the range of 96.51%.

Once purified, the conjugate was incubated with tissue containing tumor lesions of the thyroid and chemiluminescent tests was performed to evaluate the suitability of this technique as a diagnostic tool, and its ability to distinguish between benign and malignant thyroid tumors.

The expression of Gal-3 was quantified by chemiluminescent immunoassay in tissues of 28 patients with lesions of the thyroid: follicular adenoma (115.249 ± 12.321 RLU), papillary carcinoma (911.092 ± 16.350 RLU) and follicular carcinoma (480.541 ± 181.700 RLU), and 10 patients with normal thyroid (77.056 ± 7.091 RLU) were compared with results of conventional immunohistochemistry and the results are shown in Figure 3. Furthermore, these values were dramatically reduced when the tissue samples were previously incubated with non labeled anti-Gal3 antibody.

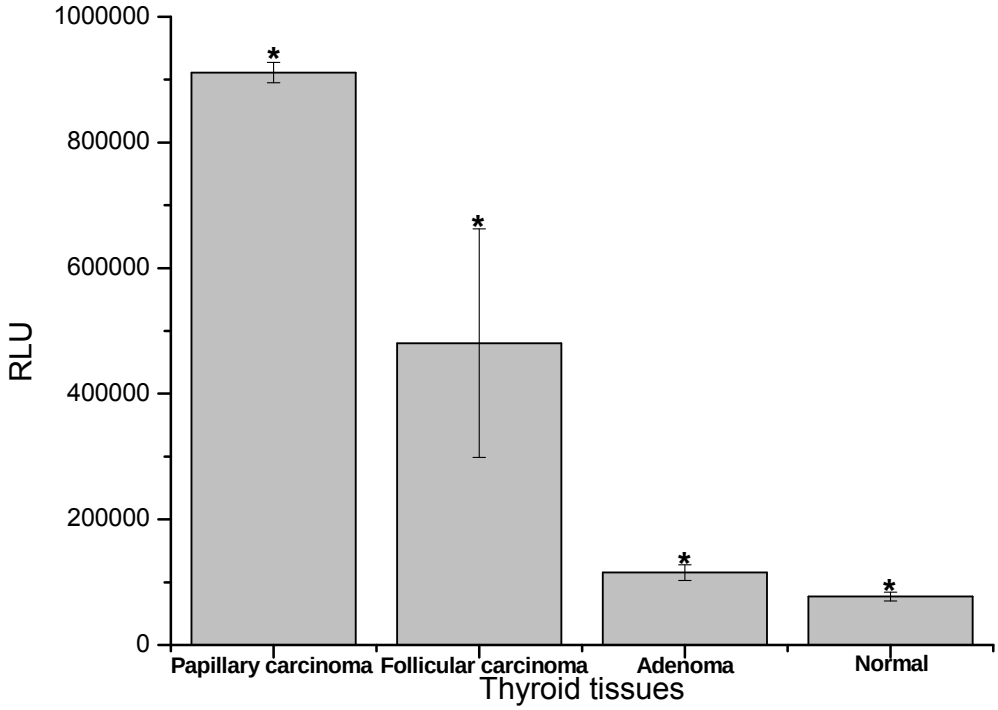

Figure 3. Chemiluminescent immunoassay in normal tissue of the thyroid (n=10), adenoma (n=9), follicular carcinoma (n=9) and papillary carcinoma (n=10) incubated with the conjugate AE-Gal-3. Data of relative light unit (RLU) obtained in triplicate tissue.

Chemiluminescent immunoassay, tissues in the normal from thyroid showed a low expression of Gal-3, since its presence in papillary carcinoma was significantly higher when compared with other thyroid lesions analyzed.

There is a wide standard deviation in RLU in the samples of follicular carcinoma (37,89%), complicating the establishment of Gal-3 immunoexpression pattern in this lesion. This finding are consistent with the reported findings, which show a variation in the pattern of expression of Gal-3 in cases of follicular carcinoma, makes the diagnosis difficult in this type of lesion (Torregrossa *et al*., 2007).

In conclusion, the technique developed to perform chemiluminescent immunoassay in tissues using specific markers conjugated to AE allowing a quantitative analysis of Gal-3 was successful. Immunostaining by AE conjugated to anti-Gal-3 behaved as a marker of malignancy in thyroid tumors with a clear expression of preference for malignant cases of papillary carcinoma may be useful for definitive

diagnosis of this lesion independent of cell morphology, and complements conventional immunohistochemistry.

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References

Araújo-Filho JLS, Melo-Júnior MR, Antunes CBL, Barreto-Lins, RAB, Machado MCFP, Carvalho Jr LB, Pontes-Filho NT. Galectin-3 in prostatic tumours: immunohistochemistry and digital image analysis. J Bras Patol Med Lab. 42(6): 469-475, 2006.

Argüeso P, Panjwani N. Focus on Molecules: Galectin-3. Experimental Eye Research. 92: 2-3, 2011.

Bartolazzi A, Gasbarri A, Papotti M, Bussolati G, Lucante T, Khan A. Application of an immunodiagnostic method for improving preoperative diagnosis of nodular thyroid lesions. Lancet. 357(9269): 1644-50, 2001.

Bartolazzi A, *et al*. Galectin-3-expression analysis in the surgical selection of follicular thyroid nodules with indeterminate fi ne-needle aspiration cytology: a prospective multicentre study. for the Italian Thyroid Cancer Study Group (ITCSG). Lancet Oncol. 9: 543–49, 2008.

Campos LM, Cavalcanti CBL, Lima-Filho JL, Carvalho-Junior LB, Beltrão EI. Acridinium ester conjugated to lectin as chemiluminescent histochemistry marker. Biomarkers. 11(5): 480-484, 2006.

Coli A, Bigotti G, Zucchetti F, Negro F, Massi G. Galectin-3, a marker of welldifferentiated thyroid carcinoma, is expressed in thyroid nodules with cytological atypia. Histopathology. 40(1): 80-7, 2002.

Dean D.S, Gharib H. Epidemiology of thyroid nodules. Best Practice & Research Clinical Endocrinology & Metabolism. 22(6): 901–911, 2008.

Durand S, Ferraro-Peyret C, Selmi-Ruby A. Evaluation of gene expression profi les in thyroid nodule biopsy material to diagnose thyroid cancer. J Clin Endocrinol Metab. 93: 1195–202, 2008.

Garcia-Campaña AM, Baeyens WRG, Zhang XR, Smet E, Van Der Wken G, Nakashima K, Calokerinos AC. Detection in the liquid phase applying chemiluminescence. Biomedical Chomatography. 14: 166-172, 2003.

Giard RW, Hermans J. The diagnostic information of tests for the detection of cancer: the usefulness of the likelihood ratio concept. Eur J Cancer. 32A(12): 2042-8, 1996.

Guarino V, Castellonea MD, Avilla E, Melillo RM. Thyroid cancer and inflammation. Molecular and Cellular Endocrinology. 321: 94–102, 2010.

Hegedus L. Clinical practice. The thyroid nodule. N Engl. J Med. 351: 1764-71, 2004.

Inohara H, Segawa T, Miyauchi A, Yoshii T, Nakahara S, Raz A, Maeda M, Miyoshi E, Kinoshita N, Yoshida H, Furukawa M, Takenaka Y, Takamura Y, Ito Y, Taniguchi N. Cytoplasmic and serum galectin-3 in diagnosis of thyroid malignancies. Biochemical and Biophysical Research Communications. 376; 605–610, 2008.

Jakubiak-Wielganowicz M, Kubiak R, Sygut J, Pomorski L, Kordek R. Usefulness of galectin-3 immunohistochemistry in differential diagnosis between thyroid follicular carcinoma and follicular adenoma. Pol. J. Pathol. 54(2); 111-115, 2003.

Jogai S, Adesina AO, Temmim L, Al-Jassar A, Amir T, Amanguno HG. Follicular variant of papillary thyroid carcinoma – a cytological study Cytopathology. 15: 212-6, 2004.

King DW, Cooper WJ, Rusak SA, Peake BM, Kiddle JJ, O'Sullivan DW, Melamed ML, Morgan CR, Theberge SM. Flow Injection Analysis of H2O2 in Natural Waters Using Acridinium Ester Chemiluminescence: Method Development and Optimization Using a Kinetic Model. Anal. Chem. 79: 4169-4176, 2007.

Kricka LJ. Clinical applications of chemiluminescence. Analytica Chimica Acta. 500: 279-386, 2003.

Matos PS, Ferreira AP, de Oliveira Facuri F, Assumpção LV, Metze K, Ward LS. Usefulness of HBME-1, cytokeratin 19 and galectin-3 immunostaining in the diagnosis of thyroid malignancy. Histopathology. 47: 391-401, 2005.

Melo-Júnior MR, Araújo-Filho JLS, Antunes CBL, Pontes-Filho NT, Carvalho Jr LB. Immobilization of Anti-Galectin-3 onto Polysiloxane Polyvinyl Alcohol Disks for Tumor Prostatic Diseases Diagnosis. Appl Biochem Biotechnol. 160: 2198–2207, 2010.

Nakahara S, Oka N, Wang Y, Hogan V, Inohara H, Raz A. Characterization of the nuclear import pathways of galectin-3. Cancer Res. 66(20): 9995-10006, 2006.

Savin S, Cvejic D, Isic T, Paunovic I, Tatic S, Havelka M. Thyroid peroxidase and galectin-3 immunostaining in differentiated thyroid carcinoma with clinicopathologic correlation. Human Pathology. 39: 1656–1663, 2008.

Segev DL, Umbricht C, Zeiger MA. Molecular pathogenesis of thyroid cancer. Surg Oncol. 12(2): 69-90, 2003.

Sherman SI. Thyroid carcinoma. Lancet. 361(9356): 501-11, 2003.

Stannard KA, Collins PM, Ito K, Sullivan EM, Scott SA, Gabutero E, Grice ID, Low P, Nilsson UF, Leffler H, Blanchard H, Ralph SJ. Galectin inhibitory disaccharides promote tumour immunity in a breast cancer model. Cancer Letters. 299: 95-110, 2010.

Torregrossa M, Pinuccia F, Camacci T, Materazzi G, Berti P, Minuto M, Elisei R, Vitti P, Miccoli P, Basolo F. Galectin-3 is highly expressed in nonencapsulated papillary thyroid carcinoma but weakly expressed in encapsulated type; comparison with Hector Battifora mesothelial cell 1 immunoreactivity. Human Pathology. 38: 1482– 1488, 2007.

Weeks I, Beheshti I, McCapra F, Campbell AK, Woodhead JS. Acridinium esters as high-specific-activity labels in immunoassay. Clinical Chemistry. 29: 1474-1479, 1983.

Xiao Q, Li H, Lin JM, Development of a highly sensitive magnetic particle-based chemiluminescence enzyme immunoassay for thyroid stimulating hormone and comparison with two other immunoassays. Clinica Chimica Acta. 411: 1151–1153, 2010.

Yang M, Liu C, Hu X, He P, Fang Y. Electrochemiluminescence assay for the detection of acridinium esters. Analytica Chimica Acta. 461: 141-146, 2002.

8. CONCLUSÕES

A partir dos resultados obtidos, podemos concluir que:

- A conjugação do éster de acridina ao anticorpo (EA-AntiGal3) demonstrou ser um procedimento simples fácil de ser repetido, o conjugado pode ser armazenado por um período médio de 12 meses, mantendo boa capacidade para gerar reações quimiluminescentes;
- No tecido prostático, os resultados da imunoistoquímica foram semelhantes aos da imunoquimiluminescência que demonstraram uma redução estatisticamente significante na expressão tecidual da galectina-3 em células de adenocarcinoma em comparação com tecido normal, e um aumento dessa expressão na hiperplasia prostática benigna quando comparado com os tecidos malignos e normais;
- Além disso, houve uma relação linear entre a quimiluminescência e a área de tecido em todas as amostras examinadas da próstata e o conjugado anti-Gal3-EA previamente incubados nos tecidos prostáticos apresentaram estabilidade quando submetidas a imunoquimiluminescência até após o período de 72 h, assim os anticorpos conjugados com éster de acridina pode ser usado como ferramenta para quantificar a mudanças, minimizando a subjetividade da análise;
- Nos tecidos tireoidianos, a imunocoloração pelo conjugado EA-Antigal3 se comportou como um marcador de malignidade em tumores da tiróide com uma expressão clara de preferência para os casos malignos de carcinoma papilar, podendo ser útil no o diagnóstico definitivo da lesão independentes da morfologia das células, e complementa imunoistoquímica convencional;
- A imunoquimiluminescência apresentou resultados reprodutíveis na análise de tecidos, mostrando alta sensibilidade e perspectiva de aplicação no diagnóstico precoce, devido à sua detecção em nível molecular.

9. PERSPECTIVAS

- Proceder à imobilização de outros marcadores tumorais ao éster de acridina, como o anticorpo Ki-67 para a realização de ensaios imunoquimiluminescentes em lesões da tireóide e a Alpha-methyl-coenzyme-A-racemase (AMACR) ou a proteína B7-H3 que vem sendo associadas à progressaõ do câncer de próstata;

- Desenvolver estratégias metodológicas para a imobilização de marcadores em outras moléculas quimiluminescentes para possíveis testes de diagnóstico diferencial das lesões tumorais;

- Testar a eficiência de imunoquimiluminescência com os conjugados desenvolvidos no soro dos pacientes;

- Promover a interface entre a imunoquimiluminescência em tecidos com patologistas, afim de inserir a técnica na rotina do diagnóstico.

9. REFERÊNCIAS

Adamczyk M, Gebler JC, Shreder K, Wu J. Quantitative determination of noncovalently bound acridinium in protein conjugates by liquid chromatography/electrospray ion trap mass spectrometry. Rapid Commun Mass Spectrom. 15(9): 670-4, 2001.

Ahmed H, Banerjee PP, Vasta GR. Differential expression of galectins in normal, benign and malignant prostate epithelial cells: Silencing of galectin-3 expression in prostate cancer by its promoter methylation Biochemical and Biophysical Research Communications. 358: 241–246, 2007.

Albertin R, Arribas MAG, Bastos EL, Röpke S, Sakai PN, Sanches AMM, Stevani, CV, Umezu IS, YU J, Baader WJ. Quimioluminescência orgânica: alguns experimentos de demonstração para a sala de aula. Química Nova. 21(6): 772-779, 1998.

Alves CM, Silva DA, Azzolini AE, Marzocchi-Machado CM, Carvalho JV, Pajuaba AC, Lucisano-Valim YM, Chammas R, Liu FT, Roque-Barreira MC, Mineo JR. Galectin-3 plays a modulatory role in the life span and activation of murine neutrophils during early Toxoplasma gondii infection. Immunobiology, Amsterdam. 215(6): 475-85, 2010.

Amrikachi M, Ramzy I, Rubenfeld S, Wheeler TM. Accuracy of fine-needle aspiration of thyroid. Arch Pathol Lab Med. 125(4): 484-8, 2001.

Bacchi CE, Almeida CC, Franco M. Manual de padronização de laudos histopatológicos (Sociedade Brasileira de Patologia), 2a Edição, Reichmann & Affonso (Ed.), Rio de Janeiro. 266p. 1999.

Barondes SH, Cooper DNW, Gitt MA, Leffler H. Galectins. Structure and function of a large family of animal lectins. J Biol Chem. 269: 20807-10, 1994.

Bartolazzi A, Gasbarri A, Papotti M, Bussolat G, Lucante T, Khan A, Inohara H, Marandino F, Orlandi F, Nardi F, Vecchione A, Tecce R, Larsson O. Thyroid Cancer Study Group. Application of an immunodiagnostic method for improving preoperative diagnosis of nodular thyroid lesions. Lancet. 26;357(9269): 1644-50, 2001.

Bartolazzi A, Orlandi F, Saggiorato E. Galectin-3-expression analysis in the surgical selection of follicular thyroid nodules with indeterminate fine-needle aspiration cytology: a prospective multicentre study. Lancet Oncol. 9: 543–49, 2008.

Basílio-de-Oliveira CA., Barretto Netto M. Tireóide. In: Brasileiro Filho G. Patologia. Rio de Janeiro: Guanabara Koogan; 2006. p.1080-1100.

Brasileiro Filho, G. Alterações do crescimento e da diferenciação celular. In: Bogliolo, L. Patologia, 6ª edição, Rio de Janeiro, Guanabara Koogan, 2000.

Califice S, Castronovo V, Van Den Brule F. Galectin-3 and cancer (Review). Int J Oncol. 25(4):983-92, 2004.

Campbell AK, Hallet MB, Weeks I. Chemiluminescence as na analytical tool in cell biology and medicine. Methods in Biochemical Analysis. 31: 317-416, 1985.

Campos LM, Cavalcanti CBL, Lima-Filho JL, Carvalho-Junior LB, Beltrão EI. Acridinium ester conjugated to lectin as chemiluminescent histochemistry marker. Biomarkers. 11(5): 480-484, 2006.

Cao Z, Said N, Amin S, Wu HK, Bruce A, Garate M, Hsu DK, Kuwabara I, Liu FT, Panjwani N. Galectins-3 and -7, but not galectin-1, play a role in re-epithelialization of wounds, J. Biol. Chem. 277: 42299–42305, 2002.

Carpi A, Nicolini A, Gross MD, Fig LM, Shapiro B, Fanti S. Controversies in diagnostic approaches to the indeterminate follicular thyroid nodule. Biomed Pharmacother. 59(9):517-20, 2005.

Castronovo V, Van Den Brule FA, Jackers P, Clausse N, Liu FT, Gillet C, Sobel ME. Decreased expression of galectin-3 is associated with progression of human breast cancer, J. Pathol. 179: 43–48, 1996.

Catalani LH. Quimiluminescência: Sistemas geradores, mecanismos e aplicações. 1996. 151f. Tese de livre-docência - Universidade de São Paulo, São Paulo.

Chan FL, Choi HL, Ho SM. Analysis of glycoconjugate patterns of normal and hormone-induced dysplastic noble rat prostates and an androgen-independent noble rat prostate tumor by lectin and protein blotting. Prostate. 46(1): 21-32, 2001.

Chan JK. Tumors of the Thyroid and Parathyroid Glands. In: Fletcher CDM, editor. Diagnostic Histopathology of Tumors. 2nd ed. Churchill Livingstone. 2003. p. 959-1056.

Christoph F, Grunbaum M., Wolkers F., Muller M., Miller K. Prostate cancer metastatic to the stomach. urology 63: 778.e14–778.e15, 2004.

Cooper DN. Galectinomics: finding themes in complexity. Biochim Biophys Acta. 1572: 209-231, 2002.

Cooper DN, Boulianne RP, Charlton S, Farrell EM, Sucher A Lu BC. Fungal galectins, sequence and specificity of two isolectins from Coprinus cinereus. J Biol Chem 272: 1514-1521, 1997.

Dabelsteen E, Clausen H, Mandel U. Carbohydrate changes in squamous cell carcinomas. APMIS. 100(27): 130-138, 1992.

Danguy A, Camby I, Kiss R. Galectins and cancer. Biochim Biophys Acta. 1572(2-3): 285-93, 2002.

Davidson PJ, Davis MJ, Patterson RJ, Ripoche MA, Poirier F, Wang JL. Shuttling of galectin-3 between the nucleus and cytoplasm. Glycobiology. 12(5): 329-37, 2002.

Dey A, Lane DP, Verma CS. Modulating the p53 pathway. Seminars in Cancer Biology. 20(1): 3-9, 2010.

Djavan B. The correlation between inflammation, BPH and prostate cancer. European Urology Supplements. 8:(13) 863-864, 2009.

Dreveny D, Klammer , Michalowsky J, Gübitz G. Flow-injection and sequential-injection immunoassay for triidothyronine using acridinium ester chemiluminescence detection. Analytica Chemica Acta. 398: 183-190, 1999.

Duffy N, O'Donovan Crown J. Use of molecular markers for predicting therapy response in cancer patients. Cancer Treatment Reviews, in press, 2010.

Dumic J, Dabelic S, Flögel M. Galectin-3: An open-ended story. Biochimica et Biophysica Acta. 1760(4): 616-35, 2006.

Durand S, Ferraro-Peyret C, Selmi-Ruby A. Evaluation of gene expression profi les in thyroid nodule biopsy material to diagnose thyroid cancer. J Clin Endocrinol Metab. 93: 1195–202, 2008.

Elola MT, Chiesa ME, Alberti AF, Mordoh J, Fink NE. Galectin-1 receptors in different cell types. J Biomed Sci. 12(1):13-29, 2005.

Faggiano A, Talbot M, Baudin , Bidart JM, Schlumberger M, Caillou B. Differential expression of galectin 3 in solid cell nests and C cells of human thyroid. Journal of Clinical Pathology. 56:142-143, 2003.

Falvo L, Giacomelli L, D'Andrea V, Marzullo A, Guerriero G, de AE. Prognostic importance of sclerosing variant in papillary thyroid carcinoma. Am Surg. 72(5):438-44, 2006.

Fanggui Ye, Ming Shi, Yong Huang, Shulin Zhao. Noncompetitive immunoassay for carcinoembryonic antigen in human serum by microchip electrophoresis for cancer diagnosis Clinica Chimica Acta. 411(15-16): 1058-1062, 2010.

Faria JL. Carcinomas. In: Patologia Especial com aplicações clínicas. 2a ed. Guanabara-Koogan. Rio de Janeiro. 1999. 687p.

Fautsch MP, Silva AO, Johnson DH. Carbohydrate binding proteins galectin-1 and galectin-3 in human trabecular meshwork, Exp. Eye Res. 77: 11–16, 2003.

Fernandez PL, Merino MJ, Gomez M, Campo E, Medina T, Castronovo V, *et al*. Galectin-3 and laminin expression in neoplastic and non neoplastic thyroid tissue. J Pathol. 181: 80-6, 1997.

Ferrazzo KL, Alves SM Jr, Santos E, Martins MT, de Sousa SM. Galectin-3 immunoprofile in adenoid cystic carcinoma and polymorphous low-grade adenocarcinoma of salivary glands. Oral Oncol. 43(6): 580-5, 2007.

Ferreira, EC; Rossi, AV. A Quimioluminescência como ferramenta analítica: do mecanismo a aplicações da reação do luminol em métodos de análise. Química Nova, 25(6): 1003-1011, 2002.

Garcia-Campaña AM, Baeyens WRG, Zhang XR., Smet E, Van Der Wken G, Nakashima K, Calokerinos AC. Detection in the liquid phase applying chemiluminescence. Biomedical Chomatography, 14:166-172, 2003.

Garnero, P. Markers of bone turnover in prostate cancer. Cancer Treatement Reviews. 27: 187-192, 2001.

Gasbarri A, Martegani MP, Del PF, Lucante T, Natali PG, Bartolazzi A. Galectin-3 and CD44v6 isoforms in the preoperative evaluation of thyroid nodules. J Clin Oncol. 17(11):3494-502, 1999.

Gillenwater A, Xu XC, El-Naggar AK, Clayman GL, Lotan R. Expression of galectins in head and neck squamous cell carcinoma. Head Neck.18:422-32, 1996.

Gimm O. Thyroid cancer. Cancer Letters. 26;163(2): 143-56, 2010.

Gleason DF. The Veteran's Administration Cooperative Urologic Research Group: histologic grading and clinical staging of prostatic carcinoma. Urologic Pathology: The Prostate. Lea and Febiger, Philadelphia, In Tannenbaum M, 171-198, 1977.

Goo YA, Goodlett DR. Advances in proteomic prostate cancer biomarker discovery. Journal of Proteomics. 73: 1839-850, 2010.

Gua Y, Li H, Miki J, Kima KH, Furusato B, Sesterhenn IA, Chub WS, McLeoda DG, Srivastava S, Ewingd CM, Isaacs WB, Rhima JS. Phenotypic characterization of telomerase-immortalized primary non-malignant and malignant tumor-derived human prostate epithelial cell lines. Experimental cell research. 312: 831-843, 2006.

Guarino V, Castellonea MD, Avilla E, Melillo RM. Thyroid cancer and inflammation. Molecular and Cellular Endocrinology. 321: 94–102, 2010.

Gupta S, Huda S, Kilpatrick PK, Velev OD. Characterization and optimization of gold nanoparticle-based silver-enhanced immunoassays. Anal Chem, 23: 5498-5504, 2010.

Gupta SK, Masinick S, Garrett M, Hazlett LD. Pseudomonas aeruginosa lipopolysaccharide binds galectin-3 and other human corneal epithelial proteins, Infect. Immun. 65: 2747–2753, 1997.

Hanson H, Hodgson S. Cancer genetics and reproduction. Best Practice & Research Clinical Obstetrics and Gynaecology. 24: 3–18, 2010.

Hegedus L. Clinical practice. The thyroid nodule. N Engl. J Med. 351:1764-71, 2004.

Heilmann S, Hummel T, Margolis FL, Kasper M, Witt M. Immunohistochemical distribution of galectin-1, galectin-3, and olfactory marker protein in human olfactory epithelium, Histochem. Cell Biol. 113: 241–245, 2000.

Herrmann ME, LiVolsi VA, Pasha TL, Roberts SA, Wojcik EM, Baloch ZW. Immunohistochemical expression of galectin-3 in benign and malignant thyroid lesions. Arch Pathol Lab Med. 126(6): 710-3, 2002.

Herzig M, Christofori G. Recent advances in cancer research: mouse models of tumorigenesis. Biochimica et Biophysica Acta. 1602: 97-113, 2002.

Ho MK and Springer TA. Mac-2, a novel 32,000 Mr mouse macrophage subpopulation-specific antigen defined by monoclonal antibodies. J Immunol. 128: 1221-1228, 1982.

Honjo Y, Inohara H, Akahani S, Yoshii T, Takenaka Y, Yoshida J, Hattori K, Tomiyama Y, Raz A and Kubo T. Expression of cytoplasmic galectin-3 as a prognostic marker in tongue carcinoma. Clin Cancer Res. 6: 4635-4640, 2000.

Houzelstein D, Goncalves IR, Fadden AJ, Sidhu SS, Cooper DN, Drickamer K, Leffler H, Poirier F. Phylogenetic analysis of the vertebrate galectin family, Mol. Biol. Evol. 21: 1177–1187, 2004.

Hrdlickova-Cela E, Plzak J, Smetana Jr K, Melkova Z, Kaltner H, Filipec M, Liu FT, Gabius HJ. Detection of galectin-3 in tear fluid at disease states and immunohistochemical and lectin histochemical analysis in human corneal and conjunctival epithelium, Br. J. Ophthalmol. 85: 1336–1340, 2001.

Hughes R.C. The galectin family of mammalian carbohydratebinding molecules. Biochem Soc Trans. 25: 1194-1198, 1997.

Hughes RC. Secretion of the galectin family of mammalian carbohydrate-binding proteins. Biochim Biophys Acta 1473: 172-185, 1999.

Hughes RC. Galectins as modulators of cell adhesion. Biochimie. 83:667-676, 2001.

INCA - Instituto Nacional de Câncer Brasil. Ministério da Saúde. Secretaria de Atenção à Saúde. Coordenação de Prevenção e Vigilância. Estimativa 2010-2011: Incidência de câncer no Brasil. Rio de Janeiro: INCA, 2010.

Inohara H, Honjo Y, Yoshii T, Akahani S, Yoshida J, Hattori K, *et al*. Expression of galectin-3 in fine-needle aspirates as a diagnostic marker differentiating benign from malignant thyroid neoplasms. Cancer. 85(11): 2475-84, 1999.

Inufusa H, Nakamura M, Adachi D, Aga M, Kurimoto M, Nakatani Y, Wakano T, Miyake M, Okuno K, Shiozaki H, Yasutomi H. Role of galectin-3 in adenocarcinoma liver metastasis. Inter. J. Oncology. 19: 913-919, 2001.

Isaacs, W.; De Marzo, A.; Nelson, W. G. Focus on prostate cancer. Cancer Cell. 2: 113-116, 2002.

Jiang WG, Martin TA, Parr C, Davies G, Matsumoto K, Nakamura T. Hepatocyte growth factor, its receptor, and their potential value in cancer therapies. Critical Reviews in Oncology/Hematology. 53 (1), 35-69, 2005.

Kasai K, Hirabayashi J. Galectins: a family of animal lectins that decipher glycocodes. J Biochem. 119: 1-8, 1996.

Kasper M, Hughes RC. Immunocytochemical evidence for a modulation of galectin 3 (Mac-2), a carbohydrate binding protein, in pulmonary fibrosis, J. Pathol. 179: 309–316, 1996.

Kawachi K, Matsushita Y, Yonezawa S, Nakano S, Shirao K, Natsugoe S, *et al*. Galectin-3 expression in various thyroid neoplasms and its possible role in metastasis formation. Hum Pathol. 31(4):428-33, 2000.

Kebebew E, Ituarte PH, Siperstein AE, Duh QY, Clark OH. Medullary thyroid carcinoma: clinical characteristics, treatment, prognostic factors, and a comparison of staging systems. Cancer. 1;88(5): 1139-48, 2000.

Kondo T, Ezzat S, Asa SL. Pathogenetic mechanisms in thyroid follicular-cell neoplasia. Nat Rev Cancer. 6(4): 292-306, 2006.

Kricka LJ. Clinical applications of chemiluminescence. Analytica Chimica Acta. 500: 279-386, 2003.

Krzeslak A, Lipinska A. Galectin-3 as a multifunctional protein. Cell Mol Biol Lett. 9: 305-28, 2004.

Krzeslaka A, Pomorskib L, Gaja Z, Lipinska A. Differences in glycosylation of intracellular proteins between benign and malignant thyroid neoplasms. Cancer Letters. 196: 101–107, 2003

Kumar V, YU S, Toback FG, Lieske JC. Renal epithelial cells constitutively produce a protein that blocks adhesion of crystals to their surface. American Journal of Physiology and Renal Physiology. 1: 34-38, 2004.

Lahm H, Andre S, Hoeflich A, Kaltner H, Siebert HC, Sordat B, Von der Lieth CW, Wolf E, Gabius HJ. Tumor galectinology: Insights into the complex network of a family of endogenous lectins. Glycoconjugate Journal. 20: 227–238, 2004

Leffler H. Introduction to galectins. Trends Glycosci Glycotech. 45: 9-19, 1997.

Li SY, Davidson PJ, Lin NY, Patterson RJ, Wang JL, Arnoys EJ. Transport of galectin-3 between the nucleus and cytoplasm. II. Identification of the signal for nuclear export. Glycobiology. 16(7): 612-22, 2006.

Lin Chi-Iou, Whang EE, Abramson MA, Donner DB, Bertagnolli MM, Moore Jr FD, Ruan DT, Galectin-3 regulates apoptosis and doxorubicin chemoresistance in papillary thyroid cancer cells. Biochemical and Biophysical Research Communications. 379: 626–631, 2009.

Lipinska A. Comparison of the lectin-binding pattern in different human melanoma cell lines. Melan. Res. 11(3): 205-212, 2001.

Liu FT, Patterson RJ, Wang JL. Intracellular functions of galectins. Biochim Biophys Acta. 1572: 263-73, 2002.

Livolsi VA, Albores-Saavedra J, Asa SL. Papillary Carcinoma. In: DeLellis RA, Lloyd RV, Heitz PU, Eng C, editors. Pathology and Genetics of Tumours of Endocrine Organs.Lyon, France: IARC Press; 2004. p. 57-66.

Lotz MM, Andrews Jr CW, Korzelius CA, Lee EC, Steele Jr GD, Clarke A, Mercurio AM. Decreased expression of Mac-2 (carbohydrate binding protein 35) and loss of its nuclear localization are associated with the neoplastic progression of colon carcinoma, Proc. Natl. Acad. Sci. U.S.A. 90: 3466–3470, 1993.

Maia LC, Figuerêdo-Silva J, Silva ECC, Souza BR, Cavalcanti CLB. Estudo morfométrico da rede arteriolar miocárdica em indivíduos alcoolistas crônicos. An. Fac. Med. Univ. Fed. Pe. 44: 31-33, 1999.

Maitra A, Abbas AK. O sistema endócrino. In: Kumar VK, Abbas AK, Fausto N. Robbins & Cotran Patologia – Bases Patológicas das Doenças. 7ª ed. Rio de Janeiro: Elsevier Editora Ltda; 2005. p.1207-1282.

Martinez VG, Pellizzari EH, Díaz ES, Cigorraga SB, Lustig L, Denduchis B, Wolfenstein-Todel C, Iglesias MM. Galectin-1, a cell adhesion modulator, induces apoptosis of rat Leydig cells in vitro. Glycobiology. 14(2): 127-137, 2004.

Matera L. The choice of the antigen in the dendritic cell-based vaccine therapy for prostate cancer. Cancer Treatment Reviews. 36: 131–141, 2010.

Melo-Junior MR, Cavalcanti CLB, Pontes-Filho NT, CARVALHO JR LB, Beltrão EIC. Lectin staining patterns in human gastric mucosae with and without exposure to Helicobacter pylori. Brazilian Journal of Microbiology, 39, 238-240, 2008.

Melo-Júnior MR, Araújo-Filho JLS, Patu, VJRM, Machado MCFP, Beltrão EIC, Carvalho-Júnior LB. Digital image analysis of skin neoplasms evaluated by lectin histochemistry: potential marker to biochemical alterations and tumour differential diagnosis. J Bras Patol Med Lab. 42(6): 455-460, 2006.

Miyazaki J, Hokari R, Kato S, Tsuzuki Y, Kawaguchi A, Nagao S, Itoh K, Miura S. Increased expression of Galectin-3 in primary gastric cancer and metastatic lymph nodes. Oncology reports. 9: 1307-1312, 2002.

Montenegro MR & Franco M (1999) Carcinogênese. In: Patologia processos gerais. Capítulo 13, 4a edição, Editora Atheneu, São Paulo, 320p.

Moon BK, Lee YJ, Battle P, Jessup JM, Raz AK. Galectin-3 protects human breast carcinoma cells against nitric oxide induced apoptosis. Implication of galectin-3 function during metastasis. Am J Pathol. 159: 1055-60, 2001.

Moutsatsos IK, Wade M, Schindler M and Wang J.L. Endogenous lectins from cultured cells: nuclear localization of carbohydratebinding protein 35 in proliferating 3T3 fibroblasts. Proc Natl Acad Sci USA. 84: 6452-6466, 1987.

Muller WE. Origin of metazoan adhesion molecules and adhesion receptors as deduced from cDNA analyses in the marine sponge Geodia cydonium: a review. Cell Tissue Res 289: 383-395, 1997.

Nakahara S, Oka N, Raz A. On the role of galectin-3 in cancer apoptosis. Apoptosis. 10(2): 267-75, 2005.

Narayan P, Gajendran V, Taylor SP, Tewari A, Presti JC, Leidich R. The role of transrectal ultrasond-guided biopsy-based staging, preoperative serum prostate-specific antigen, and biopsy Gleason score in prediction of final pathologic diagnosis in prostate cancer. Urology. 46:205-12, 1995.

Novellino ATN, Amorim RFB, Queiroz LMG. Análise da imunoexpressão do PCNA e p53 em carcinoma de células escamosas oral: correlação com a gradação histológica de malignidade e características clínicas. Acta Cirurgica. Brasileira. 18 (5): 458-464, 2003.

Openo KP, Kadrofske MM, Patterson RJ, Wang JL. Galectin-3 expression and subcellular localization in senescent human fibroblasts. Exp Cell Res. 255:278-90, 2000.

Orlandi F, Saggiorato E, Pivano G, Puligheddu B, Termine A, Cappia S, De Giuli P, Angeli A. Galectin-3 is a presurgical marker of human thyroid carcinoma. Cancer Res. 58: 3015-20, 1998.

Pacis RA, Pilat MJ, Pienta KJ, Wojno K, Raz A, Hogan V, Cooper CR. Decreased galectin-3 expression in prostate cancer. The Prostate. 44(2): 118-123, 2000.

Pavelic K, Dedivitis RA, Kapitanovic S, Ca¡cev T, Guirado CR, Danic D, Rado¡sevic S, Brkic K, Pegan B, Kri¡zanac S, Kusic Z, Spaventi S, Bura M. Molecular genetic alterations of FHIT and p53 genes in benign and malignant thyroid gland lesions. Mutation Research. 599: 45-57, 2006.

Phillips B, Knisley K, Weitlauf KD, Dorsett J, Lee VH, Weitlauf H. Differential expression of two beta-galactoside-binding lectins in the reproductive tracts of pregnant mice, Biol. Reprod. 55: 548–558, 1996.

Phipps S, Yang THJ, Habib FK, Reuben RL, McNeill SA. Measurement of tissue mechanical characteristics to distinguish between benign and malignant prostatic disease. Urology. 66: 447–450, 2005.

Plzak J, Betka J, Smetana K Jr, Chovanec M, Kaltner H, Andre S, *et al*. Galectin-3 - an emerging prognostic indicator in advanced head and neck carcinoma. Eur J Cancer. 40: 2324-30, 2004.

Rabinovich GA, Rubinstein N, Toscano M. Role of Galectins in inflamatory and immunomodulatory processes. Biochimica et Biophysica Acta. 1572: 274-284, 2002.

Robins SL, Cotran RS, Kumar V. Bases Patológicas das Doenças. 7a Edição; Ed. Guanabara-Koogan, Rio de Janeiro. 338p, 2005.

Roda A, Pasini P, Guardigli M, Baraldini M, Musiani M, Mirasoli M. Bio-and chemiluminescence in bioanalysis. Fresenius J Analyt Chem. 366:752-759, 2000.

Rosa MP, Kanamura CT, Carvalho MB. Expression of galectin-3 and cytokeratin 19 in the epithelial neoplasm of the thyroid gland and histopathological correlation. J. Bras. Patol. Med. Lab. 41(1): 61-70, 2005.

ROSS, M.H., Pawlina, W. Histologia, Texto e Atlas - Em correlação com Biologia Celular e Molecular. 5ª ed. Ed. Panamericana- GEN Guanabara-Koogan, 2008. 908p.

Saggiorato E, Aversa S, Deandreis D, Arecco F, Mussa A, Puligheddu B, *et al*. Galectin-3: presurgical marker of thyroid follicular epithelial cell-derived carcinomas. J Endocrinol Invest. 27(4):311-7, 2004.

Sasaki S, Bao Q, Hughes RC. Galectin-3 modulates rat mesangial cell proliferation and matrix synthesis during experimental glomerulonephritis induced by anti-Thy1.1 antibodies, J. Pathol. 187: 481–489, 1999.

Schaffert C, Pour PM, Chaney WG. Localization of galectin-3 in normal and diseased pancreatic tissue. Int J Pancreatol. 23: 1-9, 1998.

Sclabas GM, Staerkel GA, Shapiro SE, Fornage BD, Sherman SI, Vassillopoulou-Sellin R, *et al*. Fine-needle aspiration of the thyroid and correlation with histopathology in a contemporary series of 240 patients. Am J Surg. 186(6): 702-9, 2003.

Scott LJ, Clarke NW, George NJR, Shanks JH, Testa NG, Lang SH. Interactions of human prostatic epithelial cells with bone marrow endothelium: binding and invasion. British Journal of Cancer. 84: 1417-1423, 2001.

Segev DL, Umbricht C, Zeiger MA. Molecular pathogenesis of thyroid cancer. Surg Oncol. 12(2):69-90, 2003.

Shekhar MP, Nangia-Makker N, Tait L, F. Raz M. Alterations in galectin-3 expression and distribution correlate with breast cancer progression: functional analysis of galectin-3 in breast epithelial– endothelial interactions. Am. J. Pathol. 165 1931–1941, 2004.

Shimonishi T, Miyazaki K, Kono N, Sabit H, Tuneyama K, Harada K, Hirabayashi J, Kasai K, Nakanuma Y. Expression of endogenous galectin-1 and galectin-3 in intrahepatic cholangiocarcinoma, Hum. Pathol. 32: 302–310, 2001.

Sobrinho Simões M, Asa SL, Kroll TG. Follicular Carcinoma. In: DeLellis RA, Lloyd RV, Heitz PU, Eng C, editors. Pathology and Genetics of Tumours of Endocrine Organs.Lyon, France: IARC Press. p. 67-72, 2004.

Stavridi F., Karapanagiotou E.M., Syrigos K.N. Targeted therapeutic approaches for hormone-refractory prostate câncer. Cancer Treatment Reviews. 36: 122–130, 2010.

Takenaka Y, Fukumori T, Raz A. Galectin-3 and metastasis. Glycoconj J. 19: 543–9, 2004.

Takenaka Y, Inohara H, Yoshii T, Oshima K, Nakahara S, Akahani S, *et al*. Malignant transformation of thyroid follicular cells by galectin-3. Cancer Lett. 195(1): 111–9, 2003.

Taniguchi, C. M.; Emanuelli, B.; Kahn, R. Critical nodes in signaliling pathways: insight sinto insulin action. Nature Reviews / Molecular Cell Biology. 7: 85-96, 2006.

Teymoortash A, Pientka A, Schrader C, Tiemann M, Werner JA. Expression of galectin-3 in adenoid cystic carcinoma of the head and neck and its relationship with distant metastasis. J Cancer Res Clin Oncol.132:51-6, 2006.

Thompson IM, Ankerst DP. Prostate-specific antigen in the early detection of prostate cancer. CMAJ JAMC. 176(13): 1853-1858, 2007.

Torregrossa M, Pinuccia F, Camacci T, Materazzi G, Berti P, Minuto M, Elisei R, Vitti P, Miccoli P, Basolo F. Galectin-3 is highly expressed in nonencapsulated papillary thyroid carcinoma but weakly expressed in encapsulated type; comparison with Hector Battifora mesothelial cell 1 immunoreactivity. Human Pathology, 38: 1482– 1488, 2007.

Van den Brule FA, Engel J, Stetler-Stevenson WG, Liu FT, Sobel ME and Castronovo V. Genes involved in tumor invasion and metastasis are differentially modulated by estradiol and progestin in human breast-cancer cells. Int J Cancer. 52: 653-657, 2002.

Van den Brule FA, Waltregny D, Liu FT and Castronovo V. Alteration of the cytoplasmic/nuclear expression pattern of galectin-3 correlates with prostate carcinoma progression. Int J Cancer 89: 361-367, 2000.

Van Dyke, K. Bioluminescent and Chemioluminescence: Instruments and Aplications. CRC Press Inc. v. 1, 1985, pp 241.

Veiga, RKA; Melo-Júnior, MR; Araújo Filho, JLS; Lins CAB; Pontes-Filho NT. Avaliação digital comparativa da expressão tecidual da proteína cerbB-2 em mulheres portadoras de doenças tumorais da mama. Jornal Brasileiro de Patologia e Medicina Laboratorial, v. 45, p. 131-137, 2009.

Wang Y, Nangia-Makker P, Tait L, Balan V, Hogan V, Pienta KJ and Raz A. Regulation of Prostate Cancer Progression by Galectin-3. The American Journal of Pathology, 174(4), 2009.

Wang X, Zhang QY, Li ZJ, Ying XT, Lin JM, Development of high-performance magnetic chemiluminescence enzyme immunoassay for α-fetoprotein (AFP) in human serum. Clinica Chimica Acta. 393(2): 90-94, 2008.

Wawroschek F, Wagner T, Hamm M, Weckermann D, Vogt H, Märkl B, Gordijn R, Harzmann R. The influence of serial sections,immunohistochemistry, and extension of pelvic lymph node dissection on the lymph node status in clinically localized prostate cancer. Eur Urol. 43: 132-7, 2003.

Weeks I, Beheshti I, McCapra F, Campbell AK, Woodhead JS. Acridinium esters as high-specific-activity labels in immunoassay. Clinical Chemistry. 29: 1474-1479, 1983;

Weeks J, Sturgess ML, Brown RC, Woodhead JS. Immunoassay using acridinium esters. Meth Enzymology,133:366-387, 1986.

Weinberger PM, Adam B-L, Gourin CG, Moretz III WH, Bollag RJ, Wang BY, *et al*. Association of Nuclear, Cytoplasmic Expression of Galectin-3 With β-Catenin/Wnt-Pathway Activation in Thyroid Carcinoma. Arch Otolaryngol Head Neck Surg. 133: 503-10, 2007.

World Cancer Report. World Health Organization Report, 2008.

Xu XC, el-Naggar AK, Lotan R. Differential expression of galectin-1 and galectin-3 in thyroid tumors. Potential diagnostic implications. Am J Pathol. 1996; 147:815-22.

Xu XC, Sola Gallego JJ, Lotan R, El-Naggar AK. Differential expression of galectin-1 and galectin-3 in benign and malignant salivary gland neoplasms. Int J Oncol. 17: 271-6, 2000.

Yang M, Liu C, Hu X, He P, Fang Y. Electrochemiluminescence assay for the detection of acridinium esters. Analytica Chimica Acta. 461: 141-146, 2002.

Yang RY, Hsu DK, Liu FT. Expression of galectin-3 modulates T-cell growth and apoptosis. Proc Natl Acad Sci USA. 93: 6737-42, 1996.

Yang RY, Liu FT. Galectins in cell growth and apoptosis. Cell Mol Life Sci. 60(2): 267-76, 2003.

Yoshii T, Inohara H, Takenaka Y, Honjo Y, Akahani S, Nomura T, *et al*. Galectin-3 maintains the transformed phenotype of thyroid papillary carcinoma cells. Int J Oncol. 18(4): 787-92, 2001.

Yang Z, Fu Z, Yan F, Liu H, Huangxian Ju,. A chemiluminescent immunosensor based on antibody immobilized carboxylic resin beads coupled with micro-bubble accelerated immunoreaction for fast flow-injection immunoassay. Biosensors and Bioelectronics. 24: 35–40, 2008.

Zhao L, Sun L, Chu X. Chemiluminescence immunoassay. Trends in Analytical Chemistry. 28(4): 404-415, 2009.

ANEXOS

ATIVIDADES CIENTÍFICAS DESENVOLVIDAS DURANTE O DOUTORADO (2007-2011)

Artigos publicados

1. Melo-Júnior, Mario Ribeiro ; Araújo-Filho, Jorge Luiz Silva ; Lins, Consuelo Antunes Barreto ; Pontes-Filho, Nicodemos Teles ;Carvalho, Luiz Bezerra . **Immobilization of Anti-Galectin-3 onto Polysiloxane Polyvinyl Alcohol Disks for Tumor Prostatic Diseases Diagnosis**. Applied Biochemistry and Biotechnology JCR, v. 160, p. 2198-2207, 2010.

2. MELO-JÚNIOR, M. R. ; ARAÚJO-FILHO, J. L. S. ; TELLES, Adriana Maria da Silva ; PONTES FILHO, Nicodemos Teles de . **Avaliação histomorfométrica de micronúcleos e colágeno como métodos adicionais no diagnóstico diferencial de neoplasias cutâneas.** Arquivos de Ciências da Saúde (FAMERP), v. 16, p. 48-50, 2009

3. VEIGA, Renata Kelly de Araújo ; MELO-JÚNIOR, M. R. ; Araújo Filho, Jorge Luiz Silva ; Lins, Consuelo Antunes Barreto ; Teles, Nicodemos . **Avaliação digital comparativa da expressão tecidual da proteína cerbB-2 em mulheres portadoras de doenças tumorais da mama.** Jornal Brasileiro de Patologia e Medicina Laboratorial, v. 45, p. 131-137, 2009.

4. Lins, R. A. B. ; Cavalcanti, C.L.B. ; ARAÚJO-FILHO, J. L. S. ; MELO-JÚNIOR, M. R. ; CHAVES, M. E. C. . **A distribuição dos eosinófilos nas diferentes fases de evolução do granuloma hepático em camundongos infectados pelo Schistosoma mansoni.** Revista da Sociedade Brasileira de Medicina Tropical JCR, v. 41, p. 1-6, 2008

5. GUENDLAR, J. A. ; ALVES, R. M. R. ; MELO-JÚNIOR, M. R. ; ARAÚJO-FILHO, J. L. S. ; PONTES FILHO, Nicodemos Teles de . **Early swimming effects in the motor cortex of malnourished rats.** Neurobiologia (Recife), v. 71, p. 75-85, 2008

6. MELO-JÚNIOR, M. R. ; MACHADO, M. C. F. P. ; ARAÚJO-FILHO, J. L. S. ; PATÚ, Vasco José Ramos Malta ; TELLES, Adriana Maria da Silva . **Análise morfométrica de micronúcleos e colágeno intersticial em tumores de mama.** Revista de Ciências Médicas e Biológicas, v. 7, p. 49-56, 2008.

7. LIMA NETO, Reginaldo Gonçalves de ; ARAÚJO FILHO, Jorge Luiz da Silva ; MELO-JÚNIOR, M. R. . **Avaliação dos micronúcleos de células inflamatórias em pacientes com esporotricose e cromomicose**. Revista de Ciências Médicas e Biológicas, v. 7, p. 175-181, 2008

8. MELO-JÚNIOR, M. R. ; ARAÚJO FILHO, Jorge Luiz da Silva ; PATÚ, Vasco José Ramos Malta ; MACHADO, M. C. F. P. ; PONTES FILHO, Nicodemos Teles de . **Integrando o Ensino da Patologia às Novas Competências Educacionais.** Ciências & Cognição (UFRJ), São Paulo/Brasil, v. 12, p. m347177, 2007

9. MELO-JÚNIOR, M. R. ; FIGUEIREDO, José Luiz de ; ARAÚJO FILHO, Jorge Luiz da Silva ; BRANDT, Carlos Teixeira ; PONTES FILHO, Nicodemos Teles de . **Hipertensão porta na esquistossomose mansônica: repercussões do tratamento cirúrgico no perfil histomorfométrico da mucosa gástrica.** Revista da Sociedade Brasileira de Medicina Tropical[JCR], v. 40, p. 71-75, 2007

10. MELO-JÚNIOR, M. R. ; ARAÚJO FILHO, Jorge Luiz da Silva ; PATÚ, Vasco José Ramos Malta ; BELTRÃO, Eduardo Isidoro Carneiro ; Cavalcanti, C.L.B. ; CARVALHO JR, Luiz Bezerra de . **Detection of S100 protein from prostatic cancer patients using anti-S100 protein antibody immobilized on POS-PVA discs.** Biotechnology and Bioengineering[JCR], v. 97, p. 182-187, 2007.

11. VEIGA, Renata Kelly de Araújo ; MELO-JÚNIOR, M. R. ; ARAÚJO FILHO, Jorge Luiz da Silva ; MELLO, Luciano Albuquerque ; PONTES FILHO, Nicodemos Teles de . **Alterações morfométricas no timo, baço e placas de Peyer durante a exposição pré e pós-natal ao álcool.** Revista Eletrônica da Faculdade de Farmácia, v. 4, p. 32-42, 2007

12. MELO-JÚNIOR, M. R. ; PATÚ, Vasco José Ramos Malta ; ARAÚJO FILHO, Jorge Luiz da Silva ; MACHADO, M. C. F. P. ; PONTES FILHO, Nicodemos Teles de . **Efeitos do etanol e desnutrição crônicos sobre o perfil histológico do pâncreas de ratos.** Revista Paraense de Medicina, v. 21, p. 23-29, 2007

13. SILVA, Cynarha Daysy Cardoso ; MELO-JÚNIOR, M. R. ; PATÚ, Vasco José Ramos Malta ; ARAÚJO-FILHO, J. L. S. ; MACHADO, M. C. F. P. ; PONTES FILHO, Nicodemos Teles de . **A exposição crônica ao álcool e a desnutrição interfere no desenvolvimento do fígado durante o período perinatal?.** Revista Brasileira de Nutrição Clínica, v. 22, p. 220-225, 2007

14. ARAÚJO FILHO, Jorge Luiz da Silva ; MELO-JÚNIOR, M. R. ; VEIGA, Renata Kelly de Araújo ; MACHADO, M. C. F. P. ; PATÚ, Vasco José Ramos Malta ; PONTES FILHO, Nicodemos Teles de . **Análise histomorfométrica do coração de ratos expostos indiretamente ao etanol e à desnutrição crônica durante o período perinatal.** Revista de Ciências Médicas e Biológicas, v. 6, p. 17-25, 2007.

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