The inactive form of glycogen synthase kinase-3 beta is associated with the development of carcinomas in galectin-3 wild-type mice, but not in galectin-3-deficient mice

INTERNATIONAL JOURNAL OF CLINICAL AND EXPERIMENTAL PATHOLOGY, MADISON, v. 5, n. 6, supl., Part 3, pp. 547-554, DEC, 2012
http://www.producao.usp.br/handle/BDPI/35593

Int J Clin Exp Pathol 2012;5(6):547-554
www.ijcep.com /ISSN:1936-2625/IJCEP1203005

Original Article

The inactive form of glycogen synthase kinase-3β is associated with the development of carcinomas in galectin-3 wild-type mice, but not in galectin-3-deficient mice

Daniella Fernandes Mendonça[1], Roger Chammas[2], Fu-Tong Liu[3], Suely Nonogaki[4], Sergio Vitorino Cardoso[5], Adriano Mota Loyola[5], Paulo Rogério de Faria[6]

[1]Uberlândia Federal University, School of Medicine, Uberlândia, Brazil; [2]São Paulo University, School of Medicine, São Paulo, Brazil; [3]Department of Dermatology, University of California, Davis, School of Medicine, Sacramento, CA, USA; [4]Adolfo Lutz Institute, Molecular and Quantitative Pathology, São Paulo, Brazil; [5]Uberlândia Federal University, School of Dentistry, Uberlândia, Brazil; [6]Uberlândia Federal University, Biomedical Science Institute, Department of Morphology, Uberlândia, Brazil

Received March 8, 2012; Accepted June 23, 2012; Epub July 17, 2012; Published August 15, 2012

Abstract: Galectin-3 has been implicated in the tumor development via its mediation of the Wnt signaling pathway. Likewise, glycogen synthase kinase-3beta (GSK3β) also plays a role in the Wnt signaling pathway by controlling the levels of cytoplasmic beta-catenin. Altered GSK3β expression has been described in various tumors, but to date, there are no studies evaluating its expression in models of oral carcinogenesis. Additionally, it is unknown whether the absence of galectin-3 regulates the expression of GSK3β. To this end, Gal3-deficient ($Gal3^{-/-}$) and wild-type ($Gal3^{+/+}$) male mice were treated with 4NQO for 16 weeks and sacrificed at week 16 and 32. The tongues were removed, processed, and stained with H&E to detect dysplasias and carcinomas. An immunohistochemical assay was performed to determine the level of P-GSK3β-Ser9 expression in both groups. Carcinomas were more prevalent in $Gal3^{+/+}$ than $Gal3^{-/-}$ mice (55.5% *vs*. 28.5%), but no statistical difference was reached. In the dysplasias, the proportion of cells positive for P-GSK3β-Ser9 was slightly higher in $Gal3^{+/+}$ than $Gal3^{-/-}$ mice (63% *vs.* 61%). In the carcinomas, a significant difference between $Gal3^{+/+}$ and $Gal3^{-/-}$ mice was found (74% *vs.* 59%; p=0.02). P-GSK3β-Ser9-positive cells slightly decreased from the progression of dysplasias to carcinomas in $Gal3^{-/-}$ mice (61% *vs.* 59%; p>0.05). However, a significant increase in P-GSK3β-Ser9 expression was observed from dysplasias to carcinomas in $Gal3^{+/+}$ mice (63% *vs*. 74%; p=0.01). In conclusion, these findings suggest that fully malignant transformation of the tongue epithelium is associated with increased P-GSK3β-Ser9 expression in $Gal3^{+/+}$ mice, but not in $Gal3^{-/-}$ mice.

Keywords: Oral carcinogenesis, immunohistochemistry, galectin-3, P-GSK3β-Ser9, tongue, mice

Introduction

Galectins are a mammalian family of lectins involved in various physiological and pathological processes [1, 2]. Among the 15 recognized types of galectins, galectin-3 is one of the most widely studied [3]. As galectin-3 has been implicated in a number of functions inside and outside the cells, including apoptosis, RNA splicing, cell proliferation, angiogenesis, cell adhesion, and survival, it is not surprising that it is involved with malignant transformation and tumor progression [4, 5]. Although the mechanism underlying its role in the tumor formation has not been firmly established, previous study shed light on this showing galectin-3 acting as a mediator of the Wnt signaling pathway [6]. Additionally, it was also recently reported that galectin-3 modulates the Wnt signaling pathway by regulating AKT phosphorylation levels and thus, GSK3β activity in human colon cancer cells [7].

It is well known that genes belonging to the Wnt signaling pathway play important roles in the development of a variety of malignancies [8]. Likewise, GSK3, a serine/threonine kinase and member of the Wnt signaling pathway, has

become one of the most attractive molecules because it exerts a wind spectrum of functions inside cells [9]. Despite presenting two isoforms, GSK3α and GSK3β, the latter is crucial in the regulation of diverse cellular functions, thereby its activity is much better characterized than the former [10]. About GSK3β, it phosphorylates a spectrum of proteins, including beta-catenin, a key member of the Wnt signaling pathway, driving it for degradation via ubiquitin-proteosome [9-11]. On the other hand, in the presence of a Wnt signal, GSK3β is inactivated and the level of beta-catenin is stabilized in the cytoplasm until its translocation into the nucleus, which is aided by galectin-3, to regulate transcriptional activity of some genes including c-myc and cyclin D1 [6].

It is widely accepted that GSK3β may cause malignant transformation by regulating a wide range of signaling pathways which in part are involved in different processes, including proliferation [11]. One possible explanation came from previous reports showing that phosphorylation of GSK3β on the Ser9 residue (P-GSK3β-Ser9) is implicated with tumor development by preventing phosphorylation and degradation of various proteins, and as a result leading to the activation of the signaling pathways [9, 12]. For example, in gastric carcinoma, high P-GSK3β-Ser9 expression was observed and correlated with vascular invasion, loco-regional metastasis, and tumoral staging [13]. Similarly, it was shown that P-GSK3β-Ser9 expression is increased in colorectal carcinomas when compared to their benign counterparts [14]. However, whether P-GSK3β-Ser9 is implicated with malignant transformation of oral tissues is unknown.

We recently reported that absence of galectin-3 is not critical for tongue-induced tumor in galectin-3-deficient mice ($Gal3^{-/-}$), as was also shown by us in another study that Wnt signaling pathway was activated in dysplasias and carcinomas from both $Gal3^{-/-}$ and wild-type ($Gal3^{+/+}$) mice [15, 16]. Here, trying to understand these previous findings, we ask whether P-GSK3β-Ser9, a negative regulator of the Wnt pathway and tumor inducer, whose activity is indirectly regulated by galectin-3 via AKT signaling, may be involved with malignant transformation of tongue epithelium. For this, we use the same approach previously reported, i.e., treating $Gal3^{-/-}$ and $Gal3^{+/+}$ mice with the carcinogen 4-nitroquinoline-1-oxide (4NQO) to evaluate P-GSK3β Ser9 expression in dysplasias and carcinomas as a way to answer whether the number $Gal3^{+/+}$ and $Gal3^{-/-}$ mice with tongue carcinomas were related with the level of this protein.

Material and methods

Animals

The Committee on Animal Experimentation of the Universidade Federal de Uberlândia approved the use of the animals for the present study (CEUA/UFU # 038/09).

The $Gal3^{-/-}$ mice were designed and kindly provided the Hsu group [17]. Twenty male $Gal3^{-/-}$ mice at 6 weeks of age and weighing 23 g were used in this study. Another group of 20 $Gal3^{+/+}$ mice with the same background were used as controls. Both groups of mice were split into two groups, which were sacrificed immediately after 4NQO treatment (at week 16; $Gal3^{-/-}$ n = 10; $Gal3^{+/+}$ n = 10) or 16 weeks after the end of 4NQO treatment (at week 32; $Gal3^{-/-}$ n = 10; $Gal3^{+/+}$ n = 10). All mice were maintained in controlled temperature (22°C) and light-dark periods of 12 h. All animals were permitted to have free access to a commercial diet.

Experimental protocol

Treatment with the carcinogen 4NQO was based on the protocol described by Tang et al. [16]. 4NQO was initially diluted in propylene glycol (5 mg/ml) and then dissolved in filtered water to achieve a concentration of 100 μg/ml. The final solution was given to the mice for 16 weeks in the drinking water *ad libitum*. Throughout the treatment period, the final solution was prepared weekly and administered for each group. At the end of the treatment, half the mice from $Gal3^{+/+}$ and $Gal3^{-/-}$ groups were sacrificed immediately and the remaining were permitted to drink only filtered water until the euthanasia at week 32.

Microscopic examination

After ether anesthesia, all mice were killed by cervical dislocation after their lost

consciousness. The tongues from each mouse were removed and immediately fixed in 4% formalin for about 24 h. After this period, the tongues were cut transversely into five slices, routinely processed and embedded in paraffin. During this procedure, great care was taken by us when a lesion was observed on the tongue surface to avoid its omission in the microscopic view. In this case, the lesion was carefully cut to permit its representation on histological slide. Paraffin-embedded, 5-μm thick tissue sections were stained using a standard hematoxylin and eosin method to detect dysplasias and carcinomas according to the criteria established by Lumerman et al. [18] and Cardesa et al. [19], respectively. All histological slides were independently examined by three well-trained pathologists (PRF, AML, and SVC) to diagnose of the lesions. Discrepancies were solved after achieve a consensus scoring by the three pathologists. When two or more epithelial alterations were seen in the same histological slide, the highest aggressive lesion was taken to define the pathologic condition of the mouse.

Immunohistochemistry

A standard streptavidin-biotin peroxidase method using a polyclonal antibody to P-GSK3β-Ser9 (Santa Cruz Biotechnology, USA; catalog number # sc-11757) was performed to evaluate its expression in the dysplasias and carcinomas from both groups of mice. Briefly, 3-μm thick tongue paraffin sections were mounted on silanized slides (Sigma Chemical Co, St Louis, MO, USA), deparaffinized in xylene and dehydrated in a series of decreasing concentrations of ethanol solution. Next, the tissue sections were submitted to antigen retrieval in 10 nM, pH 8.0 citric acid buffered saline solution using a pressure cooker for 4 minutes. Subsequently, the tissue sections were treated with 3% hydrogen peroxide three times for 10 minutes each in order to block peroxidase activity. Following this, all tissues were washed once in tap and distilled water and then in phosphate buffered saline (10 mM, pH 7.4) for 10 minutes. The tissue sections were then incubated with a goat polyclonal anti-body against P-GSK3β-Ser9 (1:200) diluted in PBS, 1% BSA and 0.1% sodium azide, for 30 min at 37°C and 18 h at 4°C in a humid chamber. Next, the slides were rinsed three times for 3 minutes each in PBS and incubated for 30 minutes at 37°C with a rabbit anti-goat IgG secondary antibody (Vector, CA, USA; catalog number # BA 5000) diluted at 1:500 and then with streptavidin-HRP complex (Dako, USA; catalog number # 377) at 1:200 for 30 minutes at 37°C. After a PBS rinse, the reaction was then revealed with 3,3'-diaminobenzidine hydrochloride (Sigma, USA; catalog number # D-5637) diluted in 1 ml dimethyl sulfoxide and 6% hydrogen peroxide for 5 minutes at room temperature followed by counterstaining with Harris hematoxylin. Lymph node sections were used as a positive control. The diluted primary antibody solution alone was used as a negative control.

Immunohistochemical staining was assessed through a standard method of cells-quantification using the Image Processing and Analysis Software (Image J). To quantify the immunohistochemical staining, the ration of the number of labeled cells to the total number of cells was determined in the dysplasias and carcinomas from both groups of mice. The resulting index was expressed as the percentage of cytoplasmic and/or nuclear P-GSK3β-Ser9-positive cells in a median of 800 cells counted. When two or more dysplasias were found in the same histological slide, the lesion of highest degree was chosen for immunohistochemical analysis. Likewise, when two or more carcinomas were observed in the same histological slide, the lesion with the higher horizontal dimension was chosen for immunohistochemical analysis. Furthermore, due to the small size of lesions, all the possible fields obtained for each lesion in the 400x magnification by light microscopic were taken for analysis.

Statistical analysis

Statistical analysis of the incidence of dysplasias and carcinomas between $Gal3^{+/+}$ and $Gal3^{-/-}$ mice was performed using Fisher's exact test. The mean index of positive cells between dysplastic lesions and carcinomas from $Gal3^{+/+}$ and $Gal3^{-/-}$ mice was determined using the Mann-Whitney non-parametric test. All data were analyzed using GraphPad Prism software (San Diego, USA, version 5.0). All values were expressed as means ± standard deviation, and a value of $p<0.05$ was considered statistically significant.

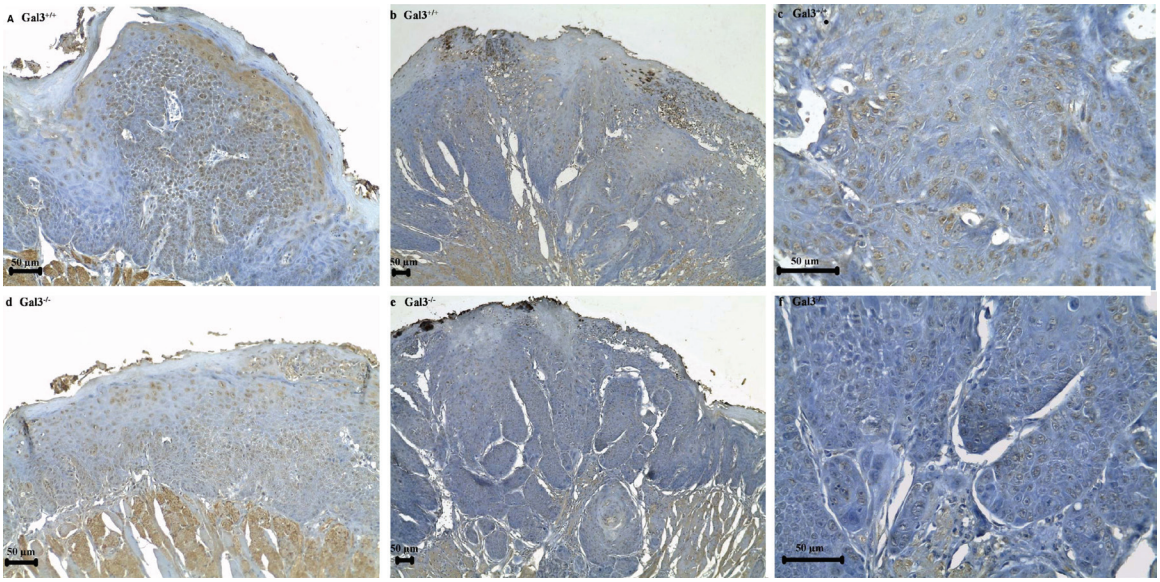

Figure 1. Immunostaining for P-GSK3β-Ser9. A. Dysplasia from Gal3+/+ mice presenting a specific nuclear expression. (B and C) Carcinoma from Gal3+/+ mice. As in the dysplasia, a nuclear expression inside tumor cells was observed. D. Dysplastic lesion from Gal3-/- mice showing a nuclear expression. (E and F) Carcinoma from Gal3-/- mice exhibiting a nuclear expression in the tumor cells. Magnification lens is x200 for a and d, x100 for b and e, and x400 for c and f.

Results

Unfortunately, 2 of the $Gal3^{+/+}$ mice and 6 of the $Gal3^{-/-}$ mice died prior to the end of the experiment and were thus excluded from the results of this study. However, the *causa mortis* could not be determined because of autolysis of tissues on microscopic view.

The data from the carcinogenesis could be seen elsewhere [16]. In summary, the oral carcinogenesis occurred in both groups. Overall, 55.5% of the $Gal3^{+/+}$ mice developed tongue carcinomas, while only 28.5% of the $Gal3^{-/-}$ group developed carcinomas. However, no statistical difference could be achieved, suggesting that absence of galectin-3 does not affect the malignant transformation of the tongue epithelial cells in mice.

Immunohistochemistry

The immunostaining for P-GSK3β-Ser9 in the samples of dysplasias and carcinomas from $Gal3^{+/+}$ and $Gal3^{-/-}$ mice is shown in **Figure 1**.

To evaluate whether the absence of galectin-3 influences the expression of P-GSK3β-Ser9 in this model of carcinogenesis, a standard immunohistochemistry protocol was used. P-GSK3β-Ser9 immunostaining was quantified in the dysplastic lesions of highest degree and/or the tongue carcinomas with the highest horizontal dimension in the histological sections from the mice, as aforementioned. In the dysplasias, the mean index of cells positive for P-GSK3β-Ser9 was slightly higher in $Gal3^{+/+}$ than $Gal3^{-/-}$ mice (63±12% *vs.* 61±16%; respectively) with no statistical difference reached (**Figure 2A**). In the carcinomas, the percentage of P-GSK3β-Ser9-positive cells was significantly higher in $Gal3^{+/+}$ than $Gal3^{-/-}$ mice (74±6% *vs.* 59±19%, respectively) (**Figure 2B**). In the intragroup analysis, a statistical difference between the dysplasias and carcinomas from $Gal3^{+/+}$ mice was determined (p=0.01), but not when the same analysis was applied to the lesions in $Gal3^{-/-}$ mice (**Figure 2C** and **2D**). Taken together, these data suggest a possible role for P-GSK3β-Ser9 in the malignant transformation of lingual epithelium of $Gal3^{+/+}$ mice as well as the high incidence of carcinomas in this group when compared to $Gal3^{-/-}$ mice.

Discussion

Carcinogenesis is a multistep process that occurs due to a progressive accumulation of

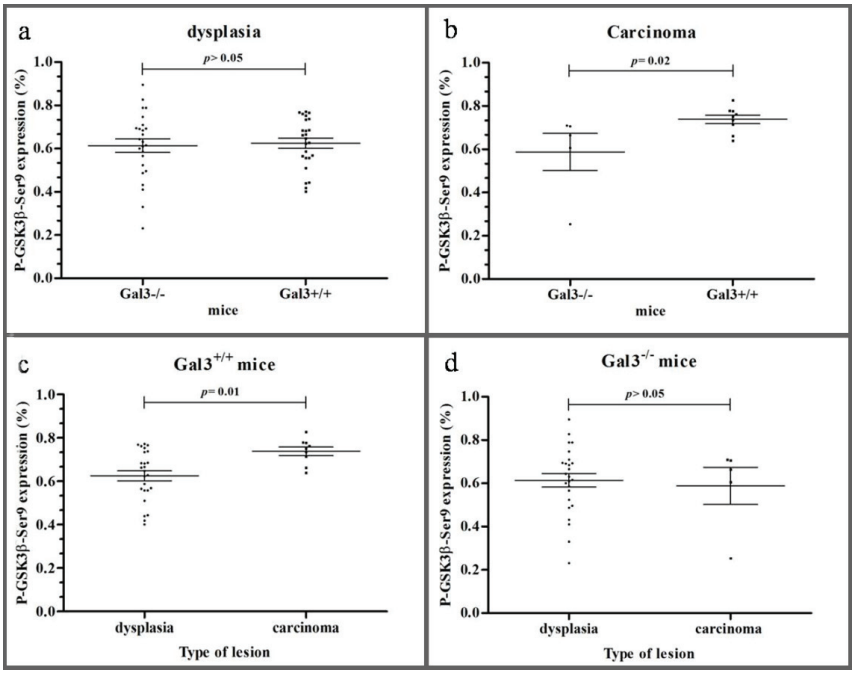

Figure 2. Mean index of cells positive for P-GSK3β-Ser9. (A and B) Comparative analysis between dysplasias and carcinomas developed in Gal3+/+ and Gal3-/- mice. C. Comparative analysis between dysplasias and carcinomas from Gal3+/+ mice. D. Comparative analysis between dysplasias and carcinomas from Gal3-/- mice.

genetic alterations that drives cells to an oncogenic phenotype [20]. There are countless animal models of carcinogenesis, and all are intended to develop lesions similar to those that occur in humans [21, 22]. One of these models is based on the ingestion of carcinogenic substances by rodents for a period of time. All of the substances employed in these experimental models, 7, 12-dimethylbenz(a) anthracene (DMBA) and 4NQO are the most widely used [23]. Although chemically different, both compounds have mutagenic and carcinogenic effects and induce epithelial changes in a similar way as observed in the oral carcinogenesis in humans [24]. Here, we use a mouse model of carcinogenic induction described by Tang et al., who administered 4NQO diluted in propylene glycol for 16 weeks [21]. As observed by them, in our study premalignant lesions and carcinomas developed in the tongues from $Gal3^{+/+}$ and $Gal3^{-/-}$ mice, indicating that this model is a reliable and effective approach to study oral carcinogenesis.

We did not observe any difference in the incidence of dysplasias and carcinomas between $Gal3^{+/+}$ and $Gal3^{-/-}$ mice. Overall, the percentage of $Gal3^{+/+}$ mice with carcinoma was higher than $Gal3^{-/-}$ mice (55.5% vs. 28.5%). Besides, while approximately 89% of $Gal3^{+/+}$ mice developed tumors at week 32, only 50% of $Gal3^{-/-}$ mice did. Different results were reported by Abdel-Aziz *et al.* [25] who found a difference statistically significant in the incidence of lung tumors between $Gal3^{+/+}$ and $Gal3^{-/-}$ mice at the end of week 32 ($p < 0.05$) [25]. In contrast, we recently showed that the absence of galectin-3 is not considered an important mediator of tongue carcinogenesis in mice [15]. Similar conclusions have been reported by Eude-Le Parco *et al.* [26] who using galectin-3 null mice, revealed that galectin-3 is not pivotal in the promotion of lung tumorigenesis [26]. Previous studies have shown that galectin-3 is strikingly important in diverse biological processes inside cells and therefore, its disruption has the potential to drive cells to malignant transformation ([1]. Of particular interest, studies showing the involvement of galectin-3 in the Wnt signaling pathway have enlightened some aspects of its tumor-inducing role [1, 6, 27, 28]. Although our results highlight the idea stated by us in another study, namely, that galectin-3 does not exert any role in the development of tongue carcinomas in mice, we cannot completely exclude a possible association between increased incidence of tumors and more activated Wnt signaling pathway in $Gal3^{+/+}$ mice than in $Gal3^{-/-}$ mice, as recently described by us and another group [15, 16, 25].

In line with this observation, we sought to evaluate the expression of the inactive form of GSK3β, P-GSK3β-Ser9, in dysplasias and carcinomas developed from $Gal3^{+/+}$ and $Gal3^{-/-}$ mice

by immunohistochemistry. First, because it was recently shown that increased galectin-3 expression in RKO-gal3 cells is associated with increased levels of AKT protein, P-GSK3β-Ser9 and beta-catenin, an important mediator of the Wnt signaling pathway [7]. Second, because it is well recognized that the site-specific phosphorylation on GSK3β regulates its enzymatic activities, and phosphorylation of GSK3β at Ser9 residue is considered the most important modification for its full activity [11, 29]. Moreover, it has been reported that increased expression of P-GSK3β-Ser9 is associated with development of several diseases, including malignancies originating from epithelial tissues [9, 30]. Indeed, we found a significant difference in the mean index of P-GSK3β-Ser9-positive cells in carcinomas from $Gal3^{+/+}$ as compared to carcinomas from $Gal3^{-/-}$ mice ($p=0.02$). In addition, we observed a significant difference between the dysplasias and carcinomas from $Gal3^{+/+}$ mice, but not between the same lesions arising in $Gal3^{-/-}$ mice. These data indicate that P-GSK3β-Ser9 is associated with the conversion of preneoplastic condition into fully malignant transformation in $Gal3^{+/+}$, but not in $Gal3^{-/-}$ mice.

Likewise, other studies have also reported an oncogenic role for P-GSK3β-Ser9. For example, a recent study found increased P-GSK3β-Ser9 expression in 70% of cases of gastric carcinoma [13]. Additionally, during skin carcinogenesis in mice, a drastic increase of P-GSK3β-Ser9 expression was observed during the progression of papillomas to squamous cell carcinomas [12]. Furthermore, it was showed in transgenic mice that the inactive form of GSK3β was also implicated in the development of mammary tumors [31]. However, data directly implicating GSK3β in the malignant transformation of the oral tissues are rarely seem and sometimes contradictories [9, 32]. Here, we show for the first time that GSK3β- inactive form may drive oncogenic transformation of the tongue epithelium from $Gal3^{+/+}$ mice, but not $Gal3^{-/-}$ mice. One possible mechanism for this may be related to the role of galectin-3 in the Wnt signaling pathway, especially by regulating GSK3β activity via AKT phosphorylation, a key regulator of GSK3β [7]. With this in mind, it is plausible to hypothesize that the results found in the present study may be closely associated not only the presence of the activated Wnt signaling pathway but also because of the presence of galectin-3 in the oral tissues from $Gal3^{+/+}$ mice, instead of $Gal3^{-/-}$ mice.

In conclusion, up-regulation of the inactive form of GSK3β is associated with malignant transformation of the tongue epithelium of $Gal3^{+/+}$ mice. However, our results do not permit to state a complete lack of GSK3β activity in the tongue from $Gal3^{-/-}$ mice because P-GSK3β-Ser9-positive cells were observed in the dysplasias and carcinomas from this group, which seems to indicate the involvement of another signaling pathway in the regulation of this protein in this group. On the other hand, it is unknown whether the highest level of P-GSK3β-Ser9 expression in $Gal3^{+/+}$-developed carcinomas is linked with the elevated expression of others Wnt target proteins, such as cyclin D1 and c-myc, which need to be elucidate. Lastly, further studies are warranted to enlighten the mechanism governing the difference in P-GSK3β-Ser9 expression between $Gal3^{+/+}$ and $Gal3^{-/-}$ mice and how that expression is promoting the neoplastic transformation of tongue epithelium differently between the groups of mice.

Acknowledgements

The Authors would like to acknowledge the Research Supporting Foundation of Minas Gerais (FAPEMIG: CDS-APQ-00397-09) for financial support.

Financial support

The Research Supporting Foundation of Minas Gerais (FAPEMIG: CDS-APQ-00397-09)

Address correspondence to: Prof. Paulo Rogério de Faria, DDS, PhD, Universidade Federal de Uberlândia, Instituto de Ciências Biomédicas, Avenida Pará 1720, Bloco 2B, Laboratório de Histologia, sala 2B-256, CEP: 38405-320, Uberlândia-MG, Brazil, Phone number: +55 34 3218-2240, Fax number: +55 34 3218-2430, E-mail: paulorfaria@icbim.ufu.br

References

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

[2] Cvejic D, Savin S, Petrovic I, Selemetjev S, Paunovic I, Tatic S and Havelka M. Galectin-3 and proliferating cell nuclear antigen (PCNA) ex-

pression in papillary thyroid carcinoma. Exp Oncol 2005; 27: 210-214.
[3] Kobayashi T, Shimura T, Yajima T, Kubo N, Araki K, Tsutsumi S, Suzuki H, Kuwano H and Raz A. Transient gene silencing of galectin-3 suppresses pancreatic cancer cell migration and invasion through degradation of beta-catenin. Int J Cancer 2011.
[4] Liu FT and Rabinovich GA. Galectins as modulators of tumour progression. Nat Rev Cancer 2005; 5: 29-41.
[5] Nakahara S, Oka N and Raz A. On the role of galectin-3 in cancer apoptosis. Apoptosis 2005; 10: 267-275.
[6] Shimura T, Takenaka Y, Fukumori T, Tsutsumi S, Okada K, Hogan V, Kikuchi A, Kuwano H and Raz A. Implication of galectin-3 in Wnt signaling. Cancer Res 2005; 65: 3535-3537.
[7] Song S, Mazurek N, Liu C, Sun Y, Ding QQ, Liu K, Hung MC and Bresalier RS. Galectin-3 mediates nuclear beta-catenin accumulation and Wnt signaling in human colon cancer cells by regulation of glycogen synthase kinase-3beta activity. Cancer Res 2009; 69: 1343-1349.
[8] Ma C, Wang J, Gao Y, Gao TW, Chen G, Bower KA, Odetallah M, Ding M, Ke Z and Luo J. The role of glycogen synthase kinase 3beta in the transformation of epidermal cells. Cancer Res 2007; 67: 7756-7764.
[9] Mishra R. Glycogen synthase kinase 3 beta: can it be a target for oral cancer. Mol Cancer 2010; 9: 144.
[10] Li J, Xing M, Zhu M, Wang X, Wang M, Zhou S, Li N, Wu R and Zhou M. Glycogen synthase kinase 3beta induces apoptosis in cancer cells through increase of survivin nuclear localization. Cancer Lett 2008; 272: 91-101.
[11] Luo J. Glycogen synthase kinase 3beta (GSK3beta) in tumorigenesis and cancer chemotherapy. Cancer Lett 2009; 273: 194-200.
[12] Leis H, Segrelles C, Ruiz S, Santos M and Paramio JM. Expression, localization, and activity of glycogen synthase kinase 3beta during mouse skin tumorigenesis. Mol Carcinog 2002; 35: 180-185.
[13] Zheng HC, Xu XY, Xia P, Yu M, Takahashi H and Takano Y. Involvement of inactive GSK3beta overexpression in tumorigenesis and progression of gastric carcinomas. Hum Pathol 2010; 41: 1255-1264.
[14] Wang J, Wang X, Gong W, Mi B, Liu S and Jiang B. Increased expression of beta-catenin, phosphorylated glycogen synthase kinase 3beta, cyclin D1, and c-myc in laterally spreading colorectal tumors. J Histochem Cytochem 2009; 57: 363-371.
[15] de Faria PR, Chammas R, de Melo TL, Hsu DK, Liu FT, Nonogaki S, Cardoso SV and Loyola AM. Absence of galectin-3 does not affect the development of experimental tongue carcinomas in mice. Exp Mol Pathol 2011; 90: 189-193.
[16] Sant' Ana JMA, Chammas R, Liu F, Nonogaki S, Cardoso SV, Loyola AM and de Faria PR. Activation of the Wnt/beta-catenin signaling pathway during oral carcinogenesis process is not influenced by the absence of galectin-3 in mice. Anticancer Research 2011; 31: 2805-2811.
[17] Hsu DK, Yang RY, Pan Z, Yu L, Salomon DR, Fung-Leung WP and Liu FT. Targeted disruption of the galectin-3 gene results in attenuated peritoneal inflammatory responses. Am J Pathol 2000; 156: 1073-1083.
[18] Lumerman H, Freedman P and Kerpel S. Oral epithelial dysplasia and the development of invasive squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995; 79: 321-329.
[19] Cardesa A, Gale N, Nadal A and Zidar N. Tumors of the Hypopharynx, Larynx and Trachea. In: Barnes L, Eveson JW, Reichart P, Sidransky D, editors. World Health Organization Classification of Tumors. Pathology and Genetics of Head and Neck Tumors. Lyon: IARC; 2005. p. 118-121.
[20] Peters J, Loud J, Dimond E and Jenkins J. Cancer genetics fundamentals. Cancer Nurs 2001; 24: 446-461; quiz 462.
[21] Tang XH, Knudsen B, Bemis D, Tickoo S and Gudas LJ. Oral cavity and esophageal carcinogenesis modeled in carcinogen-treated mice. Clin Cancer Res 2004; 10: 301-313.
[22] Steidler NE and Reade PC. Initiation and promotion of experimental oral mucosal carcinogenesis in mice. J Oral Pathol 1986; 15: 43-47.
[23] Fronza G, Campomenosi P, Iannone R and Abbondandolo A. The 4-nitroquinoline 1-oxide mutational spectrum in single stranded DNA is characterized by guanine to pyrimidine transversions. Nucleic Acids Res 1992; 20: 1283-1287.
[24] Kandarkar SV, Sawant SS and Reade PC. Ultrastructural changes to the palatal mucosa of rats following the application of 4-nitroquinoline-1-oxide (4NQO) and vitamin C. Oral Oncol 1998; 34: 247-252.
[25] Abdel-Aziz HO, Murai Y, Takasaki I, Tabuchi Y, Zheng HC, Nomoto K, Takahashi H, Tsuneyama K, Kato I, Hsu DK, Liu FT, Hiraga K and Takano Y. Targeted disruption of the galectin-3 gene results in decreased susceptibility to NNK-induced lung tumorigenesis: an oligonucleotide microarray study. J Cancer Res Clin Oncol 2008; 134: 777-788.

[26] Eude-Le Parco I, Gendronneau G, Dang T, Delacour D, Thijssen VL, Edelmann W, Peuchmaur M and Poirier F. Genetic assessment of the importance of galectin-3 in cancer initiation, progression, and dissemination in mice. Glycobiology 2009; 19: 68-75.
[27] Shimura T, Takenaka Y, Tsutsumi S, Hogan V, Kikuchi A and Raz A. Galectin-3, a novel binding partner of beta-catenin. Cancer Res 2004; 64: 6363-6367.
[28] Weinberger PM, Adam BL, Gourin CG, Moretz WH, 3rd, Bollag RJ, Wang BY, Liu Z, Lee JR and Terris DJ. Association of nuclear, cytoplasmic expression of galectin-3 with beta-catenin/Wnt-pathway activation in thyroid carcinoma. Arch Otolaryngol Head Neck Surg 2007; 133: 503-510.
[29] Rask K, Nilsson A, Brannstrom M, Carlsson P, Hellberg P, Janson PO, Hedin L and Sundfeldt K. Wnt-signalling pathway in ovarian epithelial tumours: increased expression of beta-catenin and GSK3beta. Br J Cancer 2003; 89: 1298-1304.
[30] Takahashi-Yanaga F and Sasaguri T. GSK-3beta regulates cyclin D1 expression: a new target for chemotherapy. Cell Signal 2008; 20: 581-589.
[31] Farago M, Dominguez I, Landesman-Bollag E, Xu X, Rosner A, Cardiff RD and Seldin DC. Kinase-inactive glycogen synthase kinase 3beta promotes Wnt signaling and mammary tumorigenesis. Cancer Res 2005; 65: 5792-5801.
[32] Goto H, Kawano K, Kobayashi I, Sakai H and Yanagisawa S. Expression of cyclin D1 and GSK-3beta and their predictive value of prognosis in squamous cell carcinomas of the tongue. Oral Oncol 2002; 38: 549-556.