Human Pathology (2016) 57, 152–159

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Human PATHOLOGY

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Original contribution

Loss of expression of Plag1 in malignant transformation from pleomorphic adenoma to carcinoma ex pleomorphic adenoma☆,☆☆

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Received 11 March 2016; revised 1 July 2016; accepted 6 July 2016

Keywords:
Pleomorphic adenoma;
Carcinoma ex pleomorphic adenoma;
PLAG1;
Immunohistochemistry;
Malignant transformation;
Carcinogenesis

Summary *PLAG1* (*pleomorphic adenoma gene 1*) is frequently activated in pleomorphic adenoma (PA). Carcinoma ex pleomorphic adenoma (CXPA) arises in PA, and PLAG1 expression is believed to be maintained from PA to CXPA, as it can contribute to the carcinogenesis process. To evaluate if PLAG1 is a good marker of malignant transformation from PA to CXPA as well as to evaluate if PLAG1 expression is associated with progression and histopathologic subtype of CXPA. Forty PAs, 21 residual PAs (without malignant transformation), and 40 CXPAs were analyzed by immunohistochemistry with PLAG1 antibody. The proportion of positive neoplastic cells was assessed according to a 2-tiered scale: >10% to 50%, and >50% positive cells. The CXPA group was classified according to histopathologic subtype and invasiveness degree. Thirty-seven PAs (92.5%), 15 residual PAs (71%), and 14 CXPAs (35%) were positive for PLAG1. In relation to the CXPA group, among the intracapsular cases, myoepithelial carcinoma and epithelial-myoepithelial carcinoma showed the highest level of PLAG1 expression. PLAG1 expression is lost when PA undergoes malignant transformation, possibly due to other pathway activation and different clone cells. In addition, PLAG1 expression seems to be present mainly in low-grade carcinomas and in cases with early

☆ Competing interest: The authors declare that they have no conflict of interest.

☆☆ Funding/Support: This study was supported by the following grants: FAPESP Process: 2014/18312-1, FAPESP Process: 2011/23366-5, and FAPESP Process: 2011/23204-5.

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http://dx.doi.org/10.1016/j.humpath.2016.07.011

phase of invasion, due to its regulation of oncogene-induced cell senescence. In CXPA, PLAG1 expression was most associated with myoepithelial differentiation. This way, loss of PLAG1 expression can be considered a hallmark of CXPA carcinogenesis, mainly when there is only epithelial differentiation.

1. Introduction

Pleomorphic adenoma (PA) or benign mixed tumor is the most common salivary gland neoplasia corresponding to 60% of all salivary gland tumors. The parotid gland is the most commonly affected gland (80%), followed by submandibular (10%) and minor salivary glands (10%) of several sites (oral cavity, nasal cavity, paranasal sinuses, upper respiratory, and alimentary tracts) [1]. Surgical approach is the first choice of treatment with a favorable prognosis. However, 2 adverse clinical courses can affect these patients: recurrence from 0.4% to 45% according to surgical technique and malignant transformation to carcinoma ex pleomorphic adenoma (CXPA), with rates around 25% [2-4]. CXPA accounts for 3% to 5% of all salivary gland tumors and affects mainly major salivary glands (80% in parotid gland). The biological behavior of CXPA varies according to invasiveness degree (intracapsular, minimally invasive, and frankly invasive) and histopathologic subtype, which are acquired during malignant transformation [4,5]. The carcinogenesis mechanism involved in CXPA remains unclear under several aspects, for example: Is there a common target gene involved in all histopathologic subtypes? Which factors are decisive for malignant transformation in a specific histopathologic subtype?

Extensive studies have been performed to characterize cytogenetic alterations involved in PA tumorigenesis and CXPA carcinogenesis. Concerning PA, approximately 39% are tumors with rearrangements involving 8q12, and the target gene in this location is *PLAG1* (*pleomorphic adenoma gene 1*). Translocations involving 8q12 commonly result in promoter swapping/substitution between *PLAG1* and a ubiquitously expressed translocation partner gene (down-regulated), leading to transcriptional up-regulation of *PLAG1*. The protein overexpression, PLAG1, can be detected by immunohistochemistry with potential diagnostic applications [1,6-10]. PLAG1 protein does not only regulate positively several genes that are growth factors and contributes significantly to tumorigenesis, but also regulates genes that inhibit cellular proliferation; therefore, the *PLAG1* gene may play a dual role in tumor formation [6,7,11-13]. In light of these findings, several studies have characterized this antibody as a diagnostic marker for PA and CXPA; indeed PLAG1 seems to be a key feature for PA, but its role in CXPA remains unclear [7,10,11,14].

Because of its low incidence, intense morphologic variety, and different degrees of invasive progression of the CXPA, attempts to identify diagnostic markers and explanations about carcinogenesis are still inconclusive. Investigations encompassing PA and CXPA samples have an interesting carcinogenesis model; once this way becomes possible, the transition between benign and malignant can be evaluated. Therefore, our goal was to perform an immunohistochemical assessment of PLAG1 expression in PA without malignant transformation, residual PA in CXPA, and CXPA in an attempt to answer the following questions: (1) Is this antibody a good diagnostic marker of transformation of PA to CXPA? (2) Is the expression of this protein associated with the progression and histologic type of CXPA?

2. Materials and methods

Forty cases of CXPA, 40 cases of PA (control PA), and 21 areas of PA in CXPA (residual PA) were studied over a 22-year period (January 1990–December 2014) retrieved from the archives of Pathology Department, Medical Sciences Faculty, State University of Campinas (Campinas, SP, Brazil). The original hematoxylin-eosin (H&E)–stained slides were reviewed by 2 pathologists, and the diagnoses of all cases were confirmed following the World Health Organization's 2005 *Histological Typing of Salivary Gland Tumors* guidelines [1]. All cases of CXPA were classified according to invasiveness (intracapsular, minimally, and frankly invasive phase) and to histologic subtype. PLAG1 expression was detected by immunohistochemistry, using the clone 3B7, 1:100 dilution as the primary antibody (Novus Biologicals, Littleton, CO, USA). Positive control was used. EnVision plus (Dako, Glostrup, Denmark, 4001) was used as the detection system, and DAB (Sigma, Darmstadt, Germany) as chromogen. The relative numbers of positive cells for the antibody were considered in relation to all neoplastic cells observed in each stained section. When the proportion of positive cells was more than 10%, the case was judged to be positive. The proportion of positive neoplastic cells was assessed according to a 2-tiered scale: >10% to 50%, and >50% positive cells. The current study was conducted in accordance with the ethical guidelines of our institution (Process number 834.605 from Plataforma Brasil).

3. Results

3.1. Clinicopathological data

In the CXPA group, the most prevalent histopathologic subtype was salivary duct carcinoma (13 patients [32.5%]), followed by adenocarcinoma not otherwise specified in 11