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Short communication

Expression of the transcription factor *PITX2* in ameloblastic carcinoma

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ABSTRACT

Ameloblastic carcinoma is a rare odontogenic tumour that combines the histological features of ameloblastoma with cytological atypia. Until 2005, the incidence of ameloblastic carcinoma was unknown, and since then, fewer than 60 cases have been reported. These tumours may originate from pre-existing tumours or cysts, or they arise de novo from the activation or transformation of embryological cells. *PITX2* is a transcription factor that is a product and regulator of the WNT cell signalling pathway, which has been involved in development of several tumours. To analyse whether *PITX2* could be involved in the biological behaviour of ameloblastic carcinoma, we analysed the expression of this transcription factor in a sample of this tumour and nine benign ameloblastomas to compare. The results of Western blotting and RT-PCR analyses were positive, and considering the hundreds of genes that *PITX2* regulates, we believe that its expression could be intimately linked to the behaviour of ameloblastic carcinoma and possibly other odontogenic lesions.

1. Introduction

Ameloblastic carcinoma (AC) is a rare lesion of odontogenic origin. It is defined as a malignant epithelial odontogenic tumour that histologically retains the features of ameloblastic differentiation and exhibits cytological features of malignancy, such as cellular atypia and mitotic activity.[1] AC may appear de novo (ameloblastic carcinoma primary type) or originates from pre-existing ameloblastoma or odontogenic lesions (ameloblastic carcinoma secondary type).[2,3]

PITX2 is a transcription factor that belongs to the bicoid-related homeobox family of transcription factors, and its expression is a product of the Wnt cell signalling pathway.[4,5] In the odontogenesis process, *PITX2* is selectively expressed at early stages of morphogenesis in the oral ectoderm and in epithelial cells.[6–8] When *PITX2* expression is altered during embryogenesis, it may lead to developmental abnormalities,

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such as enamel hypoplasia and anodontia, and may have a direct association with Axenfeld–Rieger syndrome.[10] In vitro studies in human and mouse cells have shown that a mesenchymal signal is required to maintain *PITX2* expression in the epithelium throughout tooth development.[8,11,12] On the other hand, Huang et al.[9] identified, for the first time, that *PITX2* promotes thyroid carcinogenesis. Later, this factor was also identified in other types of cancer.

In the case of odontogenic tumours, including AC, the origin cells are unknown, although it is believed that these lesions are derived from the dental lamina or the epithelial rests of odontogenesis.[13]

In this work, we analyse the *PITX2* expression in AC, because it is an odontogenic tumour that possibly originates after the activation or transformation of embryological remnants of odontogenesis. We hypothesized that ameloblastic carcinoma, and perhaps other odontogenic tumours, is product of the activation of the remnants cells of odontogenesis. During this process, *PITX2* may have an oncogenic role in inducing the proliferation of these cells, which may continue beyond odontogenesis. Additionally, some molecules of the Wnt cell signalling pathway have been analysed with regards to their activation or activity in odontogenic tumours,[14,15] but particularly *PITX2* has not been previously analysed in any odontogenic tumour.

2. Materials and methods

2.1. Tissue samples and histopathologic findings

Ten fresh tumours specimens were analysed, one AC and nine benign ameloblastomas (seven unicystic ameloblastomas (UAs), and two solid/multicyst ameloblastomas (SMAs)). Samples were provided from the Department of Maxillofacial Surgery, Hospital Juarez de Mexico (Mexico City, Mexico). The present study followed the Declaration of Helsinki for the medical protocol and ethics, and the institutional committee of research and ethics approved the study (registration no. HJM 1996/11.03.08). To analyse the morphologic aspects of each neoplasm, the samples were sectioned, mounted on microscope slides, stained with haematoxylin and eosin,[16] and examined using optical microscopy (Nikon H550 L, Yokohama, Japan). The samples were reviewed in at least 5 high-powered fields by two qualified oral and maxillofacial pathologists and classified according to the Histological Classification of Tumours criteria of the World Health Organization.[17]

For Western blot and RT-PCR assays, we analysed the samples. The specimens were removed from the tumour parenchyma during surgery. Next, the samples were cleaned using a solution of 0.9% sodium chloride (Baxter, Mexico City, Mexico), frozen in liquid nitrogen, and stored at −70 °C until use. For haematoxylin and eosin staining assays, the pieces of the specimens were fixed in 10% buffered formalin and embedded in paraffin. Tissue sections 5 μm thick were obtained and stained with Mayer's haematoxylin solution and visualized using optical microscopy (Nikon H550 L, Yokohama, Japan). Next, paraffin embedded tissue sections were examined by the pathologist.

2.2. Protein extraction

Protein extraction of ameloblastic carcinoma and ameloblastoma was based on the selective extraction method previously described.[18] Samples were rinsed in 0.9% sodium chloride (Baxter, Mexico City, Mexico), frozen in liquid nitrogen, mechanically pulverized and suspended (400 mg tissue/ml) in sample buffer (7 M urea, 2 M tiourea, 4% CHAPS, 2% IPG buffer, 40 mM DTT) containing complete™ protease inhibitor cocktail (Roche, Germany). Then, samples were disrupted by sonication. Insoluble material was removed by centrifugation (20,000 × *g* for 5 min at 4 °C), and the supernatant was preserved. Additionally, proteins were precipitated with acetone-TCA and the 2D Clean-Up Kit (Amersham Biosciences, USA). The precipitate was diluted in rehydration solution (7 M urea, 2 M thiourea, 2% CHAPS, 0.5% IPG buffer and 0.1% bromophenol blue) supplemented with 2 mM DTT. Protein concentration was measured using 2D Quant Kit (Amersham Biosciences, USA) according to the manufacturer's recommendations.

2.3. Western blot

To analyse the *PITX2* expression in AC and ameloblastoma we performed Western blot assays on fresh samples of AC, UA, and SMA. These assays were done as previously described.[19] Briefly, 35 μg of proteins in rehydration solution were separated by 10% SDS-PAGE and transferred to nitrocellulose membranes. After blocking for 2 h in 5% nonfat milk in Tris-buffered saline (TBS) containing 0.05% Tween-20, membranes were incubated with polyclonal antibodies against the *PITX2* protein (dilution 1:5000) (Abcam, Cambridge, MA, USA), followed by an anti-rabbit secondary antibody conjugated to horseradish peroxidase (Invitrogen, Carlsbad, CA, USA) (dilution1:8000). Antibody detection was developed by chemiluminescence (ECL, GE Healthcare Life Sciences, Switzerland). As internal control, samples were probed with antibodies against actin (kindly provided by Dr. Manuel Hernández-Hernández, CINVESTAV-IPN, Mexico).

2.4. RT-PCR analysis

To corroborate the *PITX2* expression in AC and benign ameloblastoma we analysed the samples by RT-PCR assays as previously described.[20] Briefly, total RNA was extracted from the samples using TRI Reagent (Ambion, TX) following the manufacturer's recommendations. Then, cDNA was synthesized using an oligo dT and Superscript Reverse Transcriptase (Invitrogen, Mexico). The primers used for PCR assays were as follows: forward: 5′-CAGCGGACTCACTTTACC-3′ and reverse: 5′-GGAGTCGGCGGCGCG-3′. The PCR parameters used were: 94 °C for 5 min; 28 cycles of 94 °C for 30 s, 56 °C for 30 s and 70 °C for 1 min; and a 10 min extension at 72 °C. Finally, the amplified products were analysed using agarose gels (1%).

3. Results

3.1. Histopathological findings in the AC sample

The microscopic analysis of the AC sample showed islands and nests of tumour odontogenic cells, similar to ameloblasts,