# The expression and immunohistochemical localization of periostin in odontogenic tumors of mixed epithelial/mesenchymal origin

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**Objective.** The object of this study was to determine the expression and localization of periostin in the major mixed odontogenic tumors and to correlate any differential staining of the mesenchymal components to the interrelationship of these tumors.
**Study design.** Five ameloblastic fibromas, 8 ameloblastic fibro-odontomas and 10 odontomas were assessed immunohistochemically for periostin staining. Because mesenchymal tissues were consistently present in all studied cases, these tissues were selected for statistical analysis of differential periostin staining.
**Results.** Periostin was variably localized to the mesenchymal component of the tumors as well as to preameloblasts and ameloblasts. Analysis of the mesenchymal staining intensity was statistically significantly different between ameloblastic fibro-odontomas and odontomas ($P < .001$; Dunn multiple comparisons test).
**Conclusions.** Our results document periostin staining in human mixed odontogenic tumors. Statistical analysis of differential stromal staining supports the concept that the ameloblastic fibroma is a histogenetically distinct neoplasm as compared to ameloblastic fibro-odontoma and odontoma. (Oral Surg Oral Med Oral Pathol Oral Radiol 2013;116:214-220)

Periostin was discovered by Takeshita et al.[1] in 1993 as an 811 amino acid protein in mouse osteoblasts. This novel protein was initially named "osteoblast specific factor-2" and was shown to be required for preosteoblast adhesion. In 1999, the protein was renamed "periostin" following the discovery of its heavy localization to the periodontal ligament and periosteum in mice.[2] In 2007, it was classified as a matricellular protein based on its role in modulation of cell−matrix interactions and cell function.[3]

There has been limited research on periostin in oral and odontogenic tissues, especially in humans. Until recently[4,5] identification of periostin in the periodontal ligament had been confined to adult mice[2] and in developing dental follicles in fetal mice.[6] Much of what is known has been discovered in periostin-null mice. Rios et al.[7] found being periostin-null was non-lethal in utero but was associated with abnormalities after birth and higher mortality rates. Surviving mice were dwarfs with phenotypical defects, including the formation of gnarled incisor enamel, which was thought to result from the failure of a periostin dependent developmental interaction between the periodontal ligament and the dental follicle. Furthermore, periostin-null mice were afflicted with a periodontal disease-like condition that prevented them from eating standard mouse diet and forced them to feed on powdered food. This finding suggested that periostin was crucial for the normal development of the periodontal ligament to withstand the stresses of mastication.[7] Further investigations on mice found perisotin was crucial in maintaining enamel integrity and was inhibitory to the ability of odontoblasts to produce dentine postnatally when subjected to mechanical forces.[8] These results suggested periostin was important not only in regulating enamel development and periodontal ligament function in mice, but also the postnatal development of teeth.

Human periostin is produced from a gene located on chromosome 13 (13q13.3).[9] As a result of alternative splicing of the C-terminal domain, at least 5 different isoforms have been found.[10] Periostin is crucial during development but believed to be limited to remodeling and wound repair in adults. It is found to be upregulated

## Statement of Clinical Relevance

This manuscript documents the presence and localization of periostin in mixed odontogenic tumors. The results have significance in the understanding of odontogenesis, and by extrapolation, suggest that ameloblastic fibromas are likely neoplasms rather than early stages of odontoma formation.

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in areas recovering from injury, including the myocardium,[11,12] blood vessels,[13] pulmonary vasculature,[14,15] bone marrow[16] and epidermis.[17,18] Furthermore, alterations in periostin expression and regulation have been linked to human tumorigenesis in breast,[19,20] lung,[21,22] colorectal,[23] ovarian,[24] pancreatic,[25] and oral squamous cell[26] carcinomas.

In 2009, Kashima et al.[4] reported periostin in the human periodontal ligament around teeth in 5 human jaws. They localized periostin to the periodontal ligament with heavy expression in Sharpey's fibers, and expression in blood vessel walls and alveolar periosteum. These findings were confirmed and extended by Wen et al.[5] in 2010, in human periodontal ligament. In human periodontal ligament cell cultures, periostin levels were observed to increase with cyclical stress.[5] This observation added more evidence to periostin's role in the maintenance of the periodontal ligament in humans.

Since periostin is heavily localized to the periodontal ligament in humans,[4,5] and known to be involved in enamel formation in mice,[7,8] we speculated that periostin would be expressed in both epithelium and stroma of human mixed odontogenic tumors and that differential staining of periostin in the stroma should increase with increasing maturity of the tumor type. In the process of odontogenesis, odontogenic epithelium interacts with ectomesenchymal connective tissues to form teeth. Initially, there are only soft tissues involved, but with further induction and differentiation, dental hard tissues − enamel (derived from epithelial tissues) and dentine (derived from mesenchymal tissues) − begin to develop. Eventually the tooth is formed and the entire process is halted. Tumors that mimic this odontogenic process are known to occur. The ameloblastic fibroma is a neoplasm of children and adolescents, with a relatively high recurrence rate (reported by Zallen et al.[27] to be 18.3% in a cumulative literature review), composed only of soft tissues that microscopically mimic the enamel epithelium in an embryonic mesenchyme which resembles dental papilla.[28] The ameloblastic fibro-odontoma is a neoplasm, which occurs in children, with a very low recurrence rate, that appears similar to the ameloblastic fibroma, but contains dental hard tissues.[28] Odontomas are hamartomas with limited growth and no potential for recurrence, seen either as a group of small misshapen teeth (compound odontoma) or a gnarled mass of dental hard and soft tissues (complex odontoma).[28]

This group of tumors exhibits behavioral characteristics that are proportional to the stage of odontogenesis mimicked: the earlier the stage − the more aggressive. Compounding these observations is the knowledge that odontomas must pass through stages that look microscopically identical to the ameloblastic fibroma, and then the ameloblastic fibro-odontoma. Currently, a pathologist looking at tissues that resemble a small ameloblastic fibroma, cannot tell if the tumor is an actual neoplasm, or just the initial stage of a developing hamartoma − an odontoma.

We compare the mixed odontogenic tumors: ameloblastic fibroma, ameloblastic fibro-odontoma and odontoma, to determine the expression, if any, and localization of periostin and to consider the role of periostin in human odontogenesis as mimicked in these tumors. We also examine the interrelationship of the major mixed odontogenic tumors relative to differential periostin staining.

## MATERIALS AND METHODS

Hematoxylin-eosin (H&E) stained sections of 5 cases of ameloblastic fibroma from 3 females and 2 males with mean age of 8.0 ± 1 years, median of 8 and range from 7 to 9 years; 8 cases of ameloblastic fibro-odontoma from 4 females and 4 males with mean age of 8.1 ± 3 years, median of 8 and range from 3 to 13 years; and 10 cases of odontoma, 2 compound and 8 complex, from 4 males and 6 females with mean age 14.1 ± 6 years, median of 16 and range from 5 to 26 years, were reviewed. The anonymized tissue blocks of the formalin fixed, paraffin embedded and decalcified (when necessary, in disodium ethylenediaminetetraacetate dihydrate and hydrogen chloride) tissues of these cases were obtained from the archives of the Oral Pathology Diagnostic Service, at Western University, London, Ontario. The Ethics Board stated that this type of research did not require formal approval since the study used only anonymized secondary archived paraffin embedded tissues.

Serial 5 micron sections to the H&E stained sections were rehydrated, quenched in 3% hydrogen peroxide in methanol for 5 min, then rinsed in water and phosphate buffered saline (PBS) for 5 min. The sections were then blocked in 10% horse serum in PBS for 30 min at room temperature in a humidified chamber. Excess horse serum was drained and sections were incubated with goat anti-human periostin antibody (Periostin S-15: sc-49480, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) at 1/200 dilution overnight in a humidified chamber in a 4 °C refrigerator for a standardized time of 14 h. Following overnight incubation, these sections were rinsed in PBS on a microagitator for 5 min. Rinsed samples were then incubated with ImmPress (Vector Laboratories, Burlington, Ontario, Canada) anti-goat antibody for 30 min in a humidified chamber at room temperature. Sections were then rinsed in PBS on a microagitator for 5 min. Diaminobenzidene (DAB) substrate kit for peroxidase (Vector Laboratories, Burlington, Ontario, Canada) was used to achieve a brown stain in positive areas of the tissues.