TGF-β1, Smad-2/-3, Smad-1/-5/-8, and Smad-4 signaling factors are expressed in ameloblastomas, adenomatoid odontogenic tumors, and calcifying cystic odontogenic tumors: an immunohistochemical study

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OBJECTIVES: The TGF-β/Smad signaling pathway regulates diverse cellular functions, including tooth development, and is involved in numerous pathological processes such as tumorigenesis. The aim of this study was to investigate the immunoexpression of the TGF-β/Smad signaling pathway members in ameloblastoma (AM), calcifying cystic odontogenic tumor (CCOT), and adenomatoid odontogenic tumor (AOT).

MATERIALS AND METHODS: This retrospective cross-sectional study included 65 tissue specimens: 34 AMs, 13 CCOTs, and 18 AOTs. Serial sections were immunohistochemically stained with TGF-β1, Smad-4, Smad-1/-5/-8, and Smad-2/-3 antibodies, and a semiquantitative measurement of the positive cells was carried out by two oral pathologists using a 0–3 scale (0: no immunoreactivity, 1: <20% positive cells, 2: 20–50% positive cells, 3: >50% positive cells).

RESULTS: All biomarkers studied were found significantly decreased in AM compared to CCOT and AOT. AOT and CCOT expressed Smad-1/-5/-8 more strongly compared to AM (OR = 11.66, P < 0.001 and OR = 5.34, P = 0.013, respectively), and Smad-2/-3 immunostaining was found significantly increased in CCOT (OR = 10.42, P = 0.001) and AOT (OR = 5.16, P < 0.004) compared to AM. Similarly, Smad-4 was expressed more strongly in AOT and CCOT compared to AM (P = 0.001), while AOT demonstrated a fivefold higher chance to express TGF-β1 compared to AM (P = 0.011).

CONCLUSION: TGF-β/Smad signaling pathway is activated in AM, AOT, and CCOT. The statistically significant reduced TGF-β1/Smad immunoexpression in AM compared to AOT/CCOT could be associated with the more aggressive biological behavior of AM including increased cell proliferation and reduced apoptosis and differentiation. Thus, the biomarkers TGF-β, Smad-4, Smad-1/-5/-8, and Smad-2/-3 could serve as supplementary diagnostic indices between odontogenic tumors of high and low neoplastic dynamics.


Keywords: adenomatoid odontogenic tumor; ameloblastoma; calcifying cystic odontogenic tumor; Smad; TGF-β

Introduction

The transforming growth factor beta (TGF-β) superfamily comprises a large group of structurally related molecules that are involved in critical cellular functions, such as proliferation, differentiation, motility, extracellular matrix production, angiogenesis, and apoptosis (1–5). The main members of this superfamily are TGF-β, bone morphogenetic proteins (BMP), activin, nodals, and anti-Mullerian hormone (6–8).

Human TGF-β has three isoforms (TGF-β1, -β2, and -β3) encoded by distinct genes (9–12), with TGF-β1 being the most common (6). After binding to type I (TβRI) and type II (TβRII) serine/threonine kinase receptors, TGF-β signals are transferred to cell nucleus through Smad transcriptional factors, causing stimulation or inhibition of target genes (13). Smads, named after the founder members Mad (the Drosophila gene Mothers Against Decapentaplegic) (14) and Sma (the Mad homologs observed in the nematode Caenorhabditis elegans) (15), may, also, be activated by numerous upstream molecules of pleiotropic function and are thought to constitute the core-point for transcriptional regulation of multiple genes with diverse activities (6,16).

Smads are categorized into R-Smads, the receptor-activated Smads that include Smad-1, Smad-2, Smad-3, Smad-5 and Smad-8; Co-Smad or Smad-4 that forms...
transfer complexes with activated phosphorylated R-Smads to bring them into the nucleus and initiate the transcriptional activity; and I-Smads, the inhibitory Smad-6 and Smad-7 that inhibit the signaling activity of the R-Smads (16). In general, Smad-2 and Smad-3 act as substrates for TGF-β, activin and nodal receptors, and Smad-1, Smad-5, and Smad-8 are activated by BMP-2, BMP-4, and BMP-7 (16). R-Smads are activated via receptor-mediated phosphorylation and are subject to a constant nucleocytoplasmic shuttling, as phosphorylation decreases their affinity for cytoplasmic anchors and increases their affinity for nuclear factors (16,17). When R-Smads are dephosphorylated, they translocate into the cytoplasm for another round of receptor-mediated phosphorylation, according to the receptor activation state (16,18).

TGF-β is involved in all stages of tooth development, mostly through paracrine and autocrine regulation of epithelial–mesenchymal interactions that result in terminal differentiation of ameloblasts and odontoblasts, as well as the regulation of proliferation and homeostasis of odontogenic epithelium (19–23). In vitro studies show that TGF-β1, TGF-β2, and TGF-β3 isoforms are co-expressed in developing teeth (22–24). TGF-β1 is localized in the stellate reticulum and dental lamina; TGF-β2 in pre-ameloblasts, ameloblasts, and the epithelial cells of ameloblastomas; and TGF-β3 in ameloblasts and dental papilla (22). TGF-β1 is also expressed in the developing alveolar bone, cementum, and periodontal ligament (25). Smads are essential for the control of tooth formation and growth (26,27). Smad-4 is expressed in all stages of odontogenesis in the dental epithelium and mesenchyme (27), as well as in Hertwig’s epithelial root sheath (HERS) and the epithelial rests of Malassez (ERM) (27,28). Smad-2, Smad-3, and Smad-4 normally show low expression in odontoblasts and pulp cells (29), but Smad-2 and -3 expression increases in reparative dentin, probably due to the stimulation of TGF-β1 signaling (30). Smad-1, Smad-5, and Smad-8 are strongly expressed by the dental epithelium and mesenchyme (26,27).

The TGF-β/Smad signaling pathway is involved in oncogenesis with a dual action: It terminates cell cycle and induces apoptosis during initiation and early tumor formation (31,32), but it later promotes tumorigenesis and enhances invasiveness and tumor progression (31–33). The TGF-β-induced oncogenesis is usually caused by mutations of TGF-β, its receptors, or Smads, which disable components of the TGF-β signaling pathway (31,32,34). Alterations in various points of the TGF-β/Smad signaling pathway have been described in many cancer types (33,35–41). As Smad-4 is a key point of the pathway that acts as a tumor suppressor gene (42), its alteration or dysfunction negatively affects the TGF-β-induced tumor suppression and may lead to carcinogenesis (43). In fact, Smad-4 mutations have been found in various cancers, such as pancreatic, breast, lung, or colorectal (44–47), and decreased Smad-4 expression is associated with a poor prognosis in patients with squamous cell carcinoma of the esophagus and the oral mucosa (48,49). A role for Smad-2 and Smad-3 in carcinogenesis is, also, strongly suggested. For example, a missense mutation in Smad-2 and Smad-3 genes was found in colorectal cancer. This genetic alteration leads to inhibition of the Smad-2/-3 protein nuclear transfer and subsequent decrease of its function during TGF-β-induced transcriptional activation (50,51).

As the TGF-β/Smad signaling pathway is involved in both tooth development and oncogenesis, we investigated the immunohistochemical expression of TGF-β1, phosphorylated Smad-2/-3 (pSmad-2/-3), phosphorylated Smad-1/-5/-8 (pSmad-1/-5/-8), and Smad-4 in ameloblastomas (AM), adenomatoid odontogenic tumors (AOT), and calcifying cystic odontogenic tumors (CCOT).

### Materials and methods

Sixty-five cases of epithelial odontogenic tumors were retrospectively studied: 34 AM (7 follicular, 6 plexiform, 5 follicular/plexiform, 8 follicular/acanthomatous, 5 unicystic luminal, and 3 unicystic intraluminal), 18 AOT, and 13 CCOT. Diagnosis was based on standard microscopic criteria (52,53). The main clinical features of all cases are summarized in Table 1.

### Immunohistochemistry

Five-micron-thick serial sections of formalin-fixed and paraffin-embedded tissues were immunostained in the Leica BOND-MAX™ fully automated immunohistochemistry system (Leica Biosystems Newcastle Ltd, Newcastle Upon Tyne, UK), by applying the NovoLink™ Polymer Detection System (Leica Biosystems Newcastle Ltd). For epitope retrieval, a high temperature technique with citrate buffer was utilized. The sections were incubated in 3% hydrogen peroxide (Novocasta™ Peroxidase Block; Novocasta™ Leica Biosystems) to neutralize endogenous peroxidase activity; treated with Novocasta™ Protein Block to reduce non-specific binding of primary and polymer; incubated with primary antibodies; and treated with Novocasta™ Post Primary Block, containing 10% (v/v) animal serum in tris-buffered saline, to enhance penetration of the subsequent polymer reagent. Consequently, poly-HP anti-mouse/rabbit IgG reagent (NovoLink™ Polymer)

| Table 1 Main clinical features of 34 cases of AM, 18 cases of AOT, and 13 cases of CCOT |
|---------------------------------|-----|-----|-----|-----|
|                                | AM  | AOT | CCOT | Total |
| Gender                         |     |     |      |       |
| Male                           | 13  | 7   | 8    | 28    |
| Female                        | 17  | 10  | 4    | 31    |
| N/A                            | 4   | 1   | 1    | 6     |
| Age                            |     |     |      |       |
| 7–14                           | 2   | 7   | 2    | 11    |
| 15–29                          | 3   | 7   | 4    | 14    |
| 30–44                          | 9   | 3   | 2    | 14    |
| 45–82                          | 16  | 0   | 3    | 19    |
| N/A                            | 4   | 1   | 2    | 7     |
| Location                       |     |     |      |       |
| Mandible                      | 20  | 7   | 5    | 32    |
| Maxilla                       | 8   | 10  | 7    | 25    |
| N/A                            | 6   | 1   | 1    | 8     |

AM, ameloblastoma; AOT, adenomatoid odontogenic tumor; CCOT, calcifying cystic odontogenic tumor; N/A, not available.
containing 10% (v/v) animal serum in tris-buffered saline was applied to localize the primary antibody, and the reaction product was visualized by incubation with the substrate/chromogen 3,3′-diaminobenzidine (DAB) prepared from Novocastra™ DAB Chromogen and NovoLink™ DAB Substrate Buffer (Polymer), as a brown precipitate. Finally, the sections were counterstained with Novocastra™ Hematoxylin (0.02%).

Primary antibodies used were mouse monoclonal antibody against human TGF-β1 (1:20, TGFβ17; Novocastra™ Leica Microsystems); rabbit polyclonal antibody against phosphorylated Smad-1/5/8 (1:100, anti-phospho Smad-1/Smad5/Smad8, phospho-specific Ser463/465; Millipore™, Billerica, MA, USA); goat polyclonal antibody against phosphorylated Smad-2/3 (1:50, sc-11769, p-Smad-2/-3 Ser463/465; Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA); and rabbit monoclonal antibody against Smad-4 (1:100, EP618Y; Millipore™). Positive internal controls for TGF-β1, Smad-4, and Smad-2/3 primary antibodies were normal oral mucosa sections (54). Smad-1/5/8 expression in squamous epithelium, including oral epithelium, has not been mapped. However, in our samples, pSmad-1/5/8 expression was nuclear as expected. For negative control, the primary antibodies were substituted with non-immune serum of the same specificity.

Immunohistochemical scoring
Each section was scored blindly by two investigators, and the extent of immunoreactivity in the epithelium was recorded using a 0–3 scale, according to the method of Schepere et al. (55): 0, negative; 1, low (<20% positive cells); 2, moderate (20–50% positive cells); and 3, strong (>50% positive cells). Discordant results were reviewed by both investigators, and a consensus was reached.

Statistical analysis
For statistical analysis, Stata10.1 software package (Stata Corp., TX, USA) was used, with statistical significance at \( P < 0.05 \). The immunoreactivity scores for TGF-β1, Smad-1/5/8, Smad-2/3, and Smad-4 among the three tumor types were compared with Chi-square test. The potential correlation of each score with the type of odontogenic tumor, age, and gender of the patients, and location of the lesion was studied by univariable and multivariable ordinal logistic regression (proportional odds model), and the correlation between the scores was further evaluated with the Kendall’s tau-b coefficient and its corresponding \( P \)-value.

Results
Results regarding the odontogenic epithelium immunoreactivity are summarized in Table 2. Reaction of stromal cells was not evaluated.

**TGF-β1**
Normal oral epithelium (Fig. 1a) exhibited a predominantly granular cytoplasmic TGF-β1 immunoeexpression. A similar TGF-β1 expression pattern, low (35.3%), moderate (20.6%), or strong (32.4%), was seen in 30/34 AM in the ameloblast-like and stellate-recticular-like cells (Fig. 1b).

**Table 2** Immunoeexpression score for TGF-β1, pSmad-2/-3, pSmad-1/-5/-8, and Smad-4

<table>
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<th>AOT</th>
<th>CCOT</th>
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<tr>
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<td></td>
<td></td>
<td></td>
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<tr>
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AM, ameloblastoma; AOT, adenomatoid odontogenic tumor; CCOT, calcifying cystic odontogenic tumor. N/A: not available, due to insufficient material.

\( 0 \): no positive cells; 1: <20% positive cells; 2: 20–50% positive cells; 3: >50% positive cells.

Almost all AOT (17/18) demonstrated varying TGF-β1 expression in the cells of the duct-like and rosette-like structures that were chiefly strong (53.8%) (Fig. 1c). The majority of CCOT (11/13) expressed TGF-β1 in the ameloblast-like basal cells and overlying epithelial cells that was chiefly strong (66.7%) (Fig. 1d). The possibility for strong TGF-β1 expression was increased almost fivefold in AOT (\( OR = 4.85, P\)-value = 0.011) and twofold in CCOT (\( OR = 2.44, P\)-value = 0.178), compared to AM.

**pSmad-2/3**
pSmad-2/3 immunostaining in normal oral epithelium (Fig. 2a) was predominantly nuclear. Both the ameloblast-like and stellate-reticulum-like cells were stained in 15/34 AM (Fig. 2b), but reaction was mostly low (32.4%). Most AOT (61.1%) demonstrated low pSmad-2/3 expression in the duct-like and rosette-like structures (Fig. 2c), while 11/13 CCOT showed expression throughout their lining that was chiefly moderate (46.2%) (Fig. 2d). The possibility for moderate/strong pSmad-2/3 expression was increased almost fivefold in AOT (\( OR = 5.16, P = 0.004 \)) and 10-fold in CCOT (\( OR = 10.42, P = 0.001 \)), compared to AM.

**pSmad-1/-5/-8**
pSmad-1/-5/-8 immunostaining was predominantly nuclear and localized in all epithelial components of AM (Fig. 3a), AOT (Fig. 3b), and CCOT (Fig. 3c), when positive. Considering AM, 29/32 were positive and chiefly low or moderately reactive (35.3% and 38.2%, respectively), while
all AOT and CCOT were positive, mostly strongly or moderately (50% and 53.8%, respectively). The possibility for strong pSmad-1/-5/-8 expression was increased almost 12-fold in AOT (OR = 11.66, \( P \)-value = 0.001) and five-fold in CCOT (OR = 5.34, \( P \)-value = 0.013), compared to AM.

**Smad-4**

Smad-4 immunostaining was nuclear/cytoplasmic in normal oral epithelium (Fig. 4a). In 33/34 AM, Smad-4 overall expression was mostly moderate (44.1%) and localized at the ameloblast-like and stellate-reticulum-like cells (Fig. 4b). In AOT (Fig. 4c) and CCOT (Fig. 4d), Smad-4 immunostaining was mostly strong (82.3% and 92.3%, respectively), while ghost cells were non-reactive. Non-parametric test for trend showed that CCOT and AOT were more likely to demonstrate strong reactivity for Smad-4 compared to AM (\( P < 0.001 \)).

**Correlations**

Overall, the percentage of the TGF-\( \beta \)-1-positive cells was significantly higher compared to the pSmad-2/-3-positive cells (\( P < 0.001 \)), and their correlation was not statistical significant (Kendall’s tau-b = 0.199, \( P = 0.073 \)). In particular, TGF-\( \beta \)-1 immunostaining was significantly increased in AM and AOT (\( P < 0.001 \)), compared to pSmad-2/-3. The overall correlation between TGF-\( \beta \)-1 and Smad-4 expression was statistically significant (Kendall’s tau-b = 0.430, \( P < 0.001 \)), with Smad-4 being significantly stronger than TGF-\( \beta \)-1 (\( P < 0.001 \)).

The percentages of the pSmad-2/-3- and Smad-4-positive cells showed a statistically significant positive correlation of moderate power (\( P < 0.001 \), Kendall’s tau-b = 0.425), while in most cases Smad-4 immunoreactivity was significantly higher than pSmad-2/-3 (\( P < 0.001 \)).

Similarly, the percentages of the pSmad-1/-5/-8- and Smad-4-positive cells showed a statistically significant positive correlation of moderate power (\( P < 0.001 \), Kendall’s tau-b = 0.582), while in most cases Smad-4 immunoreactivity was significantly higher or equal to pSmad-1/-5/-8 (\( P < 0.001 \)).

No correlation was found between the histological type of ameloblastoma and TGF-\( \beta \)-1, pSmad-2/-3, pSmad-1/-5/-8, or Smad-4 expression.

**Discussion**

In the present study, TGF-\( \beta \)-1, functional pSmads-2/-3 and pSmads-1/-5/-8, and Smad-4 proteins were immunohistochemically detected in the epithelial cells of AMs, AOTs,
Immunoreactivity in the normal oral epithelium for the antibodies tested was similar to that previously reported (55,56) except for pSmad-1/-5/-8 that has not been studied immunohistochemically in normal oral mucosa before, and it demonstrated a nuclear immunostaining pattern along the epithelial layers of the oral mucosa. pSmads immunolocalization was primarily nuclear, although some cytoplasmic reaction was also observed, possibly due to the nucleocytoplasmic shuttling of pSmads (16,17).

TGF-β and TGF-β1 have been previously studied in odontogenic tumors (20,21,34,57). Heikinheimo et al. (57) found decreased expression of TGF-β1 in AM, compared to human fetal tooth germs. In the study of Takata et al. (21), marked nuclear TGF-β expression was seen in the epithelial cells of six of seven desmoplastic AMs, but not in 10 follicular and plexiform variants, suggesting involvement of TGF-β in the desmoplastic matrix formation. Kumamoto et al. (20) reported marked cytoplasmic TGF-β1 immunoreactivity in the ameloblast-like cells of AMs, the pseudoglular cells of duct-like structures in AOTs, and in the epithelial cells of CCOTs. Iezzi et al. (34) reported cytoplasmic TGF-β1 immunoreactivity in both ameloblast-like and stellate-reticulum-like cells in 4 of 10 unicystic and peripheral AMs, and 11 of 19 solid AMs. TGF-β1 expression was significantly elevated in stromal cells of solid AMs, compared to unicystic/peripheral ones, and this was associated with the ‘acquisition of an aggressive behavior’. The results of our study are comparable to those of previous reports (22,36). In addition, the percentage of cells expressing TGF-β1 in AM was significantly lower than that in AOT and CCOT, five- and twofold, respectively. In oral epithelium, loss of TGF-β1 expression is an early event during malignant transformation, as TGF-β1 normally acts as a tumor suppressor gene, and has stronger inhibitory effect on epithelial growth compared to the other TGF-β.

Figure 2 (a) pSmad-2/-3 predominantly nuclear and sporadically cytoplasmic immunostaining in normal oral mucosa. (b) pSmad-2/-3 predominantly nuclear and sporadically cytoplasmic immunostaining in follicular ameloblastoma. (c) pSmad-2/-3 predominantly nuclear and sporadically cytoplasmic immunostaining in adenomatoid odontogenic tumour. (d) pSmad-2/-3 predominantly nuclear and sporadically cytoplasmic immunostaining in calcifying cystic odontogenic tumor.

Figure 3 (a) pSmad1/-5/-8 predominantly nuclear and sporadically cytoplasmic immunostaining in follicular ameloblastoma. (b) pSmad1/-5/-8 predominantly nuclear and sporadically cytoplasmic immunostaining in adenomatoid odontogenic tumor. (c) pSmad1/-5/-8 predominantly nuclear and sporadically cytoplasmic immunostaining in calcifying cystic odontogenic tumor.
isoforms (58). In contrast, TGF-β1 is increased in terminal differentiation of mucosal keratinocytes (59), while decreased expression of TGF-β1 and its receptors has been inversely correlated to differentiation in oral squamous cell carcinomas (60) and other cancers (35,37). Thus, lower expression of TGF-β1 in AM may be associated to its more primitive phenotype, characterized by increased proliferation and decreased differentiation.

Smad expression in odontogenic tumors has been previously studied in spontaneous developing keratocystic odontogenic tumors (KCOT) in mice that have been associated with ablation of Smad-4 (28). In the present study, pSmads-2/-3, pSmads-1/-5/-8, and Smad-4 were expressed in AM, AOT, and CCOT. In parallel with TGF-β1, pSmads-2/3, and Smad-4 expression was generally higher in AOT and CCOT compared to AM, and this could be indirectly associated with reduced apoptosis and enhanced cell proliferation (61–63). In fact, previous studies have shown reduced expression of apoptotic factors in AM, compared to AOT and CCOT (61–63). It has been found that AM has two relatively distinct patterns, an outer anti-apoptotic layer that could be considered the oncogenic and proliferative frontal of the tumor as reduced apoptosis is one of the major events during tumorigenesis and an inner pro-apoptotic differentiating site (63). In total, AM demonstrates an increased oncogenic activity compared to AOT and CCOT (53) that could be attributed to disturbed TGF-β1/Smad-2/3 signaling. Therefore, it has been shown that TGF-β acts as a potent tumor suppressor in the early stages of tumor progression, while later functioning more to enhance the malignant phenotype (10).

Statistical analysis showed a lack of positive correlation between TGF-β1 and pSmads-2/3 expression although Smads-2/3 is a substrate of TGF-β1 (16). In contrast, there was a statistically significant correlation between TGF-β1 expression and Smad-4, as well as pSmads-2/3 and Smad-4. Smad-4 is a key point of the TGF-β/Smad pathway that could have been activated by other molecules as well; thus, correlation between TGF-β1 and Smad-4 could have been secondary to other molecular events. A disturbance of the TGF-β/Smad pathway at the pSmad-2/3 level could be attributed to disturbed TGF-β1 signaling (e.g., functional inactivation of TβRs) (10,31,41), enhanced inhibitory effect of Smad-7 (64) or Smads-2/3 disturbance such as mutation (31, 47, 50, 65). Smad-7 is an inhibitor of Smad-2 and Smad-3 that interferes with TβRI receptor, causing inhibition of phosphorylation of Smad-2 and Smad-3 and thus preventing TGF-β-mediated responses (64). Mutant Smad-3 may block the activation of wild-type Smad-2 and Smad-3 (66). Mutations or deletions of Smad-2, Smad-3, and Smad-4 have been described in aggressive metastatic colorectal cancer (50, 51, 65), while in head and neck cancer loss of Smad-2 is considered an important event (67). In oral squamous cell carcinomas, low expression of pSmad-2 and TβRII has been associated with increased tumor aggressiveness, possibly due to the loss of the protective anti-oncogenic signaling transduced by TGF-β through Smad-2 (68).

Moreover, various kinases such as the mitogen-activated protein kinase Erk (69) can also phosphorylate the linker region of Smad-2/3 thus preventing the R-Smads’ accumulation in the nucleus and suppressing their activity (69,70). In particular, kinase-induced phosphorylation of the Smad-2/3 linker region by Erk1/2 MAP kinase, cyclin-dependent kinase, and p38 MAP kinase has been found in ameloblastomas (54,61,71–73). Although that could explain the weak positive correlation between TGF-β1 and Smad-2/3, not in our study the antibody used detects exclusively

Figure 4 (a) Smad-4 nuclear and cytoplasmic immunostaining in (A) normal oral mucosa. (b) Smad-4 nuclear and cytoplasmic immunostaining in follicular ameloblastoma. (c) Smad-4 nuclear and cytoplasmic immunostaining in adenomatoid odontogenic tumor. (d) Smad-4 nuclear and cytoplasmic immunostaining in calcifying cystic odontogenic tumor.
the TGF-β-induced dually phosphorylated Smad-2 and Smad-3 at C-terminal Ser423 and Ser425 residues.

Overall, the decreased pSmads-2/-3 expression in AM compared to AOT and CCOT found in the present study may be associated with its aggressive behavior.

In the present study, Smad-4 immunoreactivity was mostly cytoplasmic and nuclear, as has been previously described in head and neck carcinomas (48,74). CCOT and AOT were more likely to demonstrate a strong reactivity for Smad-4 compared to AM. Low expression levels of Smad-4 were more likely to demonstrate a strong reactivity for desmin (48,74). CCOT and AOT were more likely to demonstrate a strong reactivity for Smad-4 compared to AM. Low expression levels of Smad-4 were more likely to demonstrate a strong reactivity for desmin (48,74).

In the present study, Smad-4 immunoreactivity was mostly cytoplasmic and nuclear, as has been previously described in head and neck carcinomas (48,74). CCOT and AOT were more likely to demonstrate a strong reactivity for Smad-4 compared to AM. Low expression levels of Smad-4 were more likely to demonstrate a strong reactivity for desmin (48,74).

In conclusion, the present study showed that TGF-β1/Smad signaling pathway is activated in AM, AOT, and CCOT. There was a statistically significant difference in the expression of TGF-β1 and Smads between AM and AOT/CCOT that could be associated with the proliferation and differentiation of the latter, but the effect of this difference on the pathogenesis and/or biological behavior of the tumors needs further investigation.

References

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