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Syndecan-1 and Wingless-type protein-1 in human ameloblastomas

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BACKGROUND: Aberrant Wingless type 1 glycoprotein (Wnt) pathway in ameloblastomas and a role of syndecan-1 (SDC1) in activating Wnt signalling were perspected. SDC1 shifting from epithelium to stroma was reported in invasive non-odontogenic neoplasms. The aim of this study was to reveal the role of SDC1 and Wnt1 in intraosseous ameloblastomas (IA_s).
METHODS: SDC1 and Wnt1 expressions were investigated in 29 ameloblastoma subtypes and seven tooth buds.
RESULTS: SDC1 immunostaining strongly depicted stromal cells, extracellular matrix (ECM) and basement membranes of ameloblastomas. It also showed epithelial tumour cells in the acanthomatous and plexiform subtypes, and it often occurred in stellate reticulum cells and basal ameloblasts of tooth buds. Parallel Wnt1 expression occurred in ameloblastomatous epithelial cells, but it was common in basal cells of tooth buds too. Statistically, a significant correlation was found between the percentage of IA_s-bearing SDC1-positive stromal cells and ECM and the percentage of IA_s-bearing Wnt1-positive epithelial cells.
CONCLUSIONS: A role of SDC1 in stromal cells and ECM can be hypothesized as a critical factor for carcinogenesis and local invasiveness of IA_s.
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Keywords: ameloblastoma; signalling transducers; syndecan-1; Wingless-type protein-1

Introduction

Syndecan-1 (SDC1), a transmembrane heparan sulphate proteoglycan, is also known as CD138 and participates in odontogenesis (1). SDC1 has been localized in different cell lines, including epithelial, endothelial and stromal tissues (2). In mature epithelia, SDC1 binds proteins of extracellular matrix (ECM), modulating epithelial–stromal interactions and cell proliferation (3). It is transiently expressed by mesenchymal cells and ameloblasts of tooth buds, as a developmentally regulated matrix receptor for growth factors (1) and signalling transducers.

The Wingless type 1 glycoprotein (Wnt1), belonging to a large family of 19 secreted signalling transducers, besides promotes cell proliferation (4). Wnt1 assists embryogenesis and is normally involved in tooth development (5), together with β-catenin and adenomatous polyposis coli (APC) expressions (6). An aberrant Wnt pathway was suggested to occur in oncogenesis (7), leading to nuclear translocation of non-phosphorylated β-catenin (8–12), and a wide range of human neoplasms displays inappropriate activation of Wnt1, with subsequent proliferative and antiapoptotic effects (11, 13, 14). Recently, nuclear translocation of non-phosphorylated β-catenin has been regarded as a possible indicator of aberrant Wnt pathway and deregulation of tumour cell proliferation, but it has been found in no more than 20% of ameloblastomas (15–17).

Previous experimental data suggest a direct role of SDC1 in activating Wnt signalling, as a critical factor for Wnt1 induced carcinogenesis and tumour cell invasion (18, 19). SDC1 has been reported to be downregulated in epithelial tumour cells, while its expression shifts from epithelial to stromal tumour cells and ECM: these events have been considered a feature of tumour invasiveness (2, 20–24). In this way, an involvement of SDC1 is reliable in odontogenic tumours, so that it might be interesting to seek both SDC1 and Wnt1 expressions in slow-growing and locally invasive neoplasms, such as intraosseous ameloblastomas (IA_s).

As the SDC1 expression in IA_s has not been investigated to date, the present study was aimed at immunolocalizing both SDC1 proteoglycan and Wnt1 protein in different subtypes of human IA_s and tooth buds.

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Materials and methods

Tissue preparation

Archival paraffin blocks of 29 formalin-fixed IA samples were selected from the files of our Divisions of Oral Pathology. About 5-μm-thick paraffin sections were used for both histological and immunohistochemical purposes. Histology was carried out on haematoxylin–eosin slides, and diagnosis was made according to the WHO histological typing of odontogenic tumours (25). Seven tooth germs of the third mandibular molar, removed for orthodontic reasons, were also processed as normal controls. Histologically, 13 *follicular*, nine *plexiform*, three *acanthomatous* and four *desmoplastic* IA_s were classified depending on their prevalent growth pattern. Tooth germs, featuring the late bell stage or early stage of crown mineralization were parallelly processed and compared with the IA_s.

Immunohistochemistry

Deparaffinized tissue sections were treated with 0.3% hydrogen peroxide in methanol and microwave heated in 0.01 M citrate buffer (pH 6.0) for 10 min. After PBS washing and treatment with normal rabbit serum for 30 min, the sections were incubated with primary antibodies at 4°C overnight. Mouse anti-SDC1 monoclonal antibody (clone DL-101) and goat anti-Wnt1 polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) were applied, both diluted at 1:100. Immunoreactions were carried out using the Labelled StreptAvidin Biotin method (LSAB; Dako Cytomation, Carpinteria, CA, USA) and visualized by 0.03% diaminobenzidine solution (DAB substrate; Vector Laboratories, Burlingame, CA, USA), containing 2 mM hydrogen peroxide, for 2–4 min. At last, a faint haematoxylin nuclear counterstaining was carried out. Parallel negative controls were performed by omitting primary antibody incubations.

Evaluation of immunostaining

Immunohistochemical reactivity to SDC1 and Wnt1 antibodies, as a cell membrane or cytoplasmic pattern, was revealed in: (I) ameloblasts or ameloblast-like tumour cells (EC_s) and stellate reticulum cells (SR_s); (II) stromal cells (SC_s) and (III) ECM.

The intensity of positive immunoreactions was classified in two groups as weak (+) and strong (++) reactivity, in comparison with negative controls.

Statistical analyses

Statistical differences between total IA_s and tooth buds were evaluated by the chi-square test, as regards the expression rates of SDC1 and Wnt1 antigens in EC_s, SR_s, SC_s and ECM.

Separated standard regression line tests were also performed so as to evaluate possible relationships between the percentage of SDC1-positive SC_s or ECM and the percentage of Wnt1-positive EC_s in the studied IA subtypes (MICROCAL ORIGIN version 7.5 – Software, Inc., Northampton, MA, USA). As regards statistics, probability values (P) lower than 0.05 were considered to be significant.

Results

Syndecan-1 and Wnt1 immunohistology variously depicted epithelial and stromal components of the studied polycystic IA_s and tooth germs. Different immunostaining patterns were recognized at: (I) EC membrane or cytoplasm; (II) SC membrane or cytoplasm and (III) ECM.

In tooth germs, SDC1-positive SC_s and ECM were common findings, together with Wnt1-expressing EC_s. The latter mostly prevailed inside the inner layer of developing enamel epithelium, as a cell membrane/cytoplasmic pattern, in contrast to a weak Wnt1 reactivity of both SR_s and endothelial cells (Fig. 1). In five of seven tooth germs (71.4%), a weak to strong SDC1 immunostaining of ameloblasts and SR_s was different from an occasional, weak SDC1 expression of pre-odontoblasts.

As regards total IA_s, SDC1-positive SC_s were found in 14 of 29 cases (48.3%) and SDC1-positive EC_s were found in six of 29 cases (20.6%), while Wnt1-expressing EC_s occurred in 19 of 29 tumour samples (65.5%). Statistical analysis of these data showed that: (I) SDC1-positive EC_s and SC_s were more frequent in tooth germs than observed in IA_s, at a highly significant level

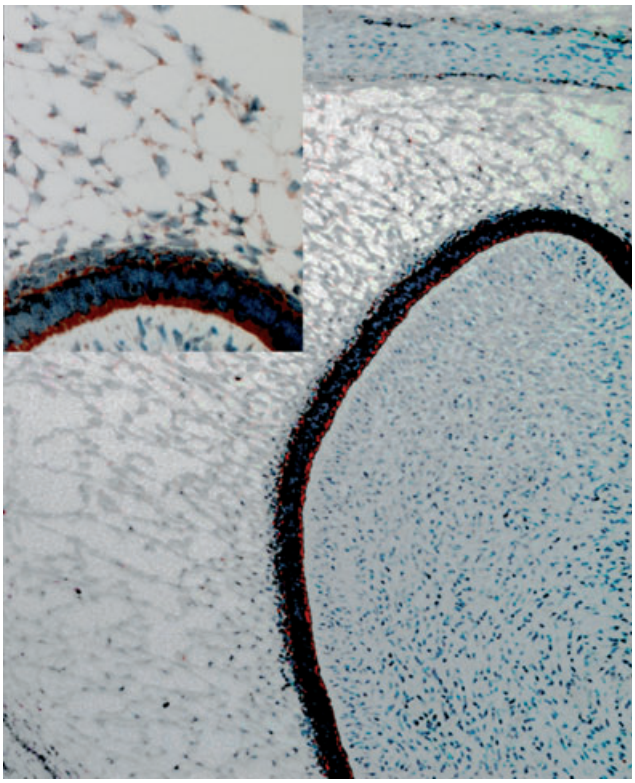

Figure 1 Tooth germ showing Wingless type 1 glycoprotein (Wnt1)-positive immunoreactions of both EC_s and SR_s. A strong Wnt1 immunostaining of EC_s is obvious in the inner layer of developing enamel epithelium (original magnification 75×; insert 300×).

Table 1 SDC1 and Wnt1 expression rates of ECs, SCs and ECM, as a frequency means in total IAs and tooth buds

Studied tissues	*Total samples*	*SDC1 labelling*			*Wnt1 labelling*		
		EC_s–SR_s	*SC_s*	*ECM*	*EC_s–SR_s*	*SC_s*	*ECM*
IAs	29	6/29	14/29	20/29	19/29	1/29	0/29
Tooth buds	7	5/7	7/7	7/7	7/7	0/7	0/7
χ^2		6.84 ($P < 0.01$)	6.20 ($P < 0.05$)	2.89 ($P > 0.05$)	3.34 ($P > 0.05$)	0.24 ($P > 0.05$)	–

Chi-square test (χ^2) and significance level (P) denote that in tooth buds SDC1-positive epithelial cells prevail at a highly significant level ($P < 0.01$), as SDC1-positive stromal cells prevail at a significant level ($P < 0.05$).
SDC, syndecan; ECM, extracellular matrix; EC, epithelial cells; SC, stromal cells; IA, intraosseous ameloblastoma; Wnt, Wingless type 1 glycoprotein.

Table 2 SDC1 and Wnt1 immunoreactions in the studied IAs and tooth germs are localized in ECs, SR cells or SR-like cells, SCs and in ECM

IA subtypes	*Cases (n)*	*SDC1 labelling expression rate (%)*			*Wnt1 labelling expression rate (%)*		
		EC_s–SR_s	*SC_s*	*ECM*	*EC_s–SR_s*	*SC_s*	*ECM*
Follicular	13	0/13 (0)	9/13 (69.23)	11/13 (84.61)	12/13 (92.30)	0/13 (0)	0/13 (0)
Plexiform	9	3/9 (33.3)	3/9 (33.33)	4/9 (44.44)	3/9 (33.33)	1/9 (11)	0/9 (0)
Acanthomatous	3	3/3 (100)	2/3 (66.6)	3/3 (100)	2/3 (66.6)	0/3 (0)	0/3 (0)
Desmoplastic	4	0/4 (0)	0/4 (0)	2/4 (50)	2/4 (50)	0/4 (0)	0/4 (0)
Tooth buds	7	5/7 (71.4)	7/7 (100)	7/7 (100)	7/7 (100)	0/7 (0)	0/7 (0)

Positive results are expressed as a ratio to the number of cases (n) for every IA subtype; values in parentheses indicate percentage of respective expression rates (%).
SDC, syndecan; Wnt, Wingless type 1 glycoprotein; ECM, extracellular matrix; IA, intraosseous ameloblastoma; EC, epithelial cells; SR, stellate reticulum; SC, stromal cells.

($P < 0.01$) and at a significant level ($P < 0.05$), respectively; (II) differences between total IA_s and tooth germs in their Wnt1-expression rates were non-significant ($P > 0.05$, Table 1).

Among the studied IA subtypes (Table 2), a weak SDC1 labelling of spindle-shaped SC_s was found in follicular, plexiform and acanthomatous IA_s, but a strong SDC1 immunostaining was recognized inside ECM and basement membranes surrounding neoplastic islands, as in 11 of 13 follicular IA_s (Fig. 2) as in all the acanthomatous ones. Similar SDC1 binding of ECM could also be seen in four of nine plexiform and in two of four desmoplastic IA subtypes, close to interdigiting cords of cuboidal tumour cells. Concerning the SDC1 expression in tumour EC_s, a strong membranous/cytoplasmic immunoreaction was observed in three of nine plexiform IA_s and it marked both tumour keratinizing cells and cuboidal cells of the acanthomatous IA_s (Fig. 3).

Parallelly, a strong cytoplasmic Wnt1 cell-binding diffusely characterized palisading tumour epithelial cells and stellate reticulum-like ones, if present, in 12 of 13 follicular (92.3%), in two of four desmoplastic (50.0%) and in three of nine plexiform (33.3%) IA subtypes. The above Wnt1 expression in tumour EC_s prevailed in solid areas and in cuboidal cells neighbouring the basal membrane of follicular, plexiform and desmoplastic IA_s (Fig. 4). It could also be seen in two of three acanthomatous IA_s (66.6%), as a strong Wnt1 membranous reaction of tumour EC_s, within the palisades and cohesive morule-like arrays.

Instead, SC_s and ECM were mostly Wnt1-negative in IA_s, except in a single case of plexiform IA, where a weak Wnt1 reactivity could be seen in rare spindle-shaped SC_s, close to the ameloblast-like tumour sheets.

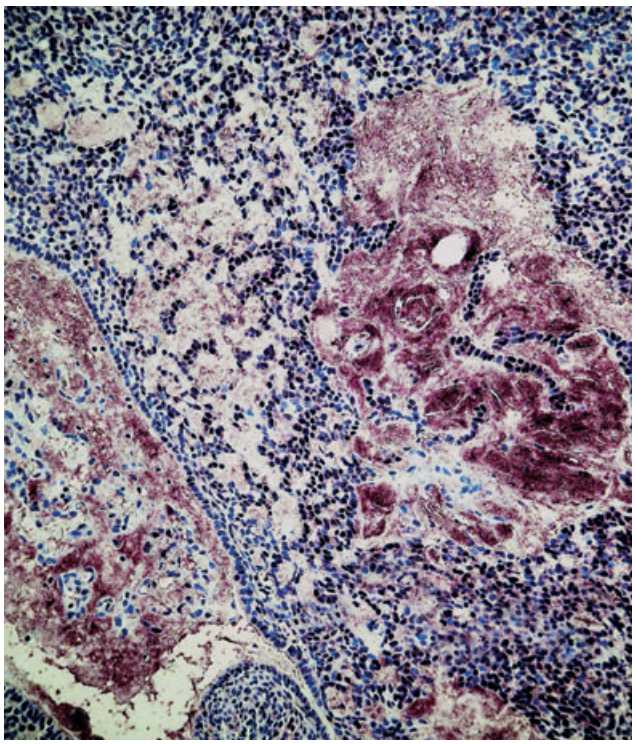

Figure 2 Follicular intraosseous ameloblastoma. Syndecan-1 immunostaining strongly depicts extracellular matrix, among the neoplastic islands and cords (original magnification 125×).

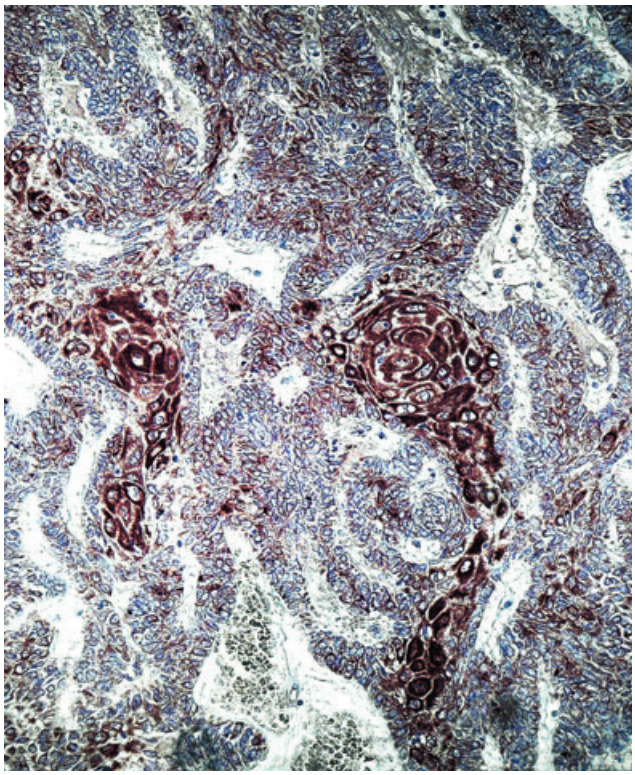

Figure 3 Acanthomatous intraosseous ameloblastoma showing a diffuse syndecan-1 positivity of ameloblast-like tumour cuboidal cells that strongly depicts a keratinizing cell array (original magnification 150×).

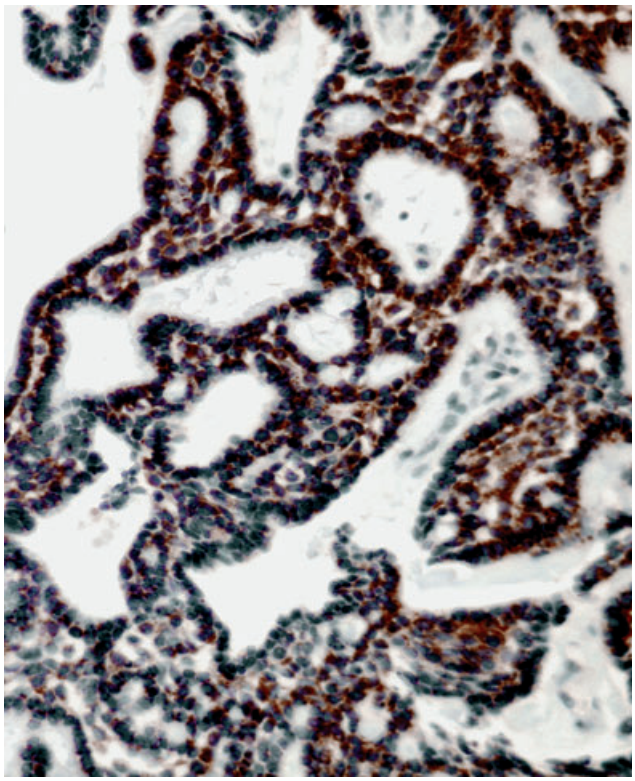

Figure 4 Plexiform intraosseous ameloblastoma in which an obvious Wingless type 1 glycoprotein cell positivity can be seen inside cord-forming cuboidal tumour cells (original magnification 150×).

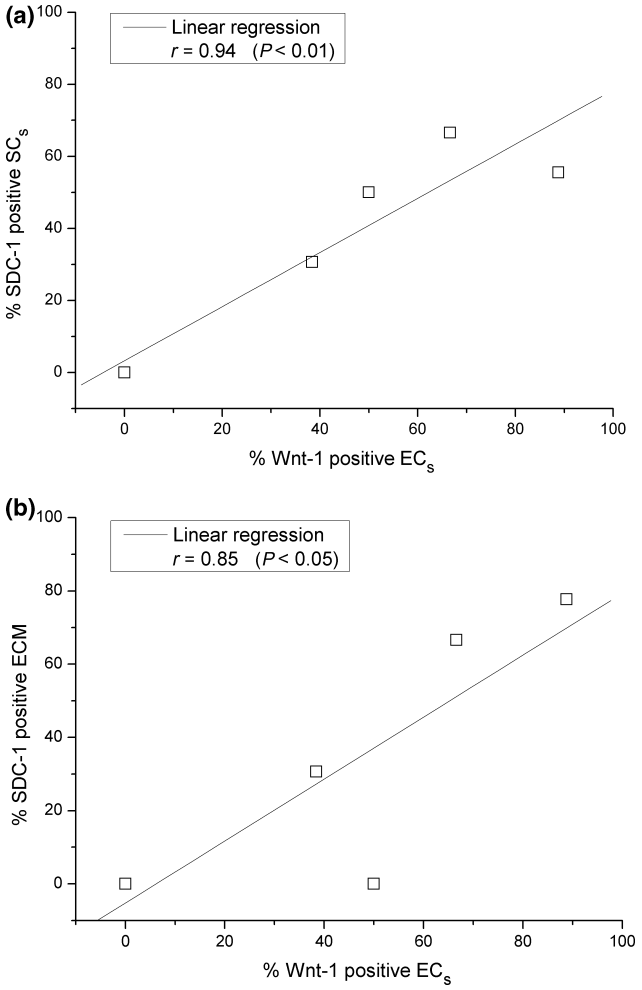

Figure 5 (a) Scatterplot for percentage of the intraosseous ameloblastoma (IA) subtypes bearing syndecan-1 (SDC1)-positive stromal cells (ordinate), when compared with percentage of the IA subtypes bearing Wingless type 1 glycoprotein (Wnt1)-positive epithelial cells (abscissa). Such linear relationship is highly significant ($r = 0.94$; $P < 0.01$). (b) Scatterplot for percentage of the IA subtypes bearing SDC1-positive extracellular matrix (ordinate) when compared with the percentage of the IA subtypes bearing Wnt1-positive epithelial cells (abscissa). Such linear relationship is significant ($r = 0.85$; $P < 0.05$).

In the studied IA subtypes, the proportion of Wnt1-positive EC_s statistically correlated with SDC1-positive SC_s, at a highly significant level ($r = 0.94$; $P < 0.01$), while it also did so with SDC1-positive ECM ($r = 0.85$; $P < 0.05$), at a significant level (Fig. 5a,b).

Discussion

The studied lesions are both slow-growing and locally invasive neoplasms, arising inside an ecto-mesenchymal compartment, where tumour EC_s interact with both SC_s and ECM (26).

With regard to the described SDC1 immunostaining in tooth buds, a positive reactivity is frequent in maturing enamel epithelium, and it is also a common feature of both SC_s and ECM. This corroborates the expected involvement of SDC1 heparan sulphate proteoglycan for ecto-mesenchymal interaction in tooth

buds, as a developmentally regulated epithelial cell product and a matrix receptor (1, 5).

Compared with tooth buds, SDC1 expression of total IA_s is reduced in the EC_s at a highly significant level, but it persists in SC_s, and especially in ECM. The above-mentioned SDC1 downregulation in EC_s might be regarded as an important event for the growth of some IA subtypes, predicting their invasive potential, as suggested for non-odontogenic malignancies (2, 18–21).

Cytoplasmic Wnt1 reactivity of tooth buds is common inside the inner epithelial layer of the maturing enamel organ, substantiating a direct role of Wnt1 glycoprotein for tooth development and differentiation, as previously pointed out (5). In the studied odontogenic tumours, Wnt1-positive epithelial cells mainly occur in follicular and acanthomatous IA_s, but they are also frequent in plexiform and desmoplastic ones. Follicular and acanthomatous IA_s have been previously reported to show nuclear β-catenin accumulation, low APC expression and an increased cell proliferation, possibly due to an aberrant reactivation of the Wnt signalling pathway (15), as otherwise hypothesized for basal cell carcinomas (10).

Wnt1-positive epithelial cells in some of our IA_s might denote that follicular and acanthomatous IA_s often tend towards an increased cell proliferation. On the other hand, the described Wnt1 expression in keratinizing cells of acanthomatous IA_s is coherent with the hypothesis that abnormal Wnt signalling can be responsible for morphogenesis of the morule-like structures in adamantinomatous craniopharyngiomas (27).

On account of the observed Wnt1-negative epithelial cells in 10 of 29 IA_s (34.4% of the tumour samples), it is conceivable that different signalling proteins or members of the Wnt family (other than the Wnt1 glycoprotein) may be variously involved in some ameloblastomatous lesions.

On the basis of SDC1 and Wnt1 expressions in the studied IA subtypes, different patterns can be noticed, supporting supposed relationships between the above cell products in human odontogenic (15) and in non-odontogenic neoplasms (18, 19, 28).

On the other hand, the SDC1 downregulation has been observed in dysplastic oral squamous cells (29), and the activated Wnt pathway in transformed epithelia implies an increased extracellular shedding of heparan sulphate proteoglycans, such as SDC1 (23).

Parallel evidence has been provided that Wnt-dependent cell proliferation is modulated by extracellular heparan sulphate proteoglycans such as SDC1, which is known to act as a co-receptor for Wnt ligands, in cell growth of both normal and cancer tissues (2, 19).

Syndecan-1 is not expressed in tumour epithelial cells of follicular and desmoplastic IA_s, while SDC1 reactivity variously occurs in tumour stromal cells and ECM of follicular, plexiform, acanthomatous and desmoplastic IA_s. However, SDC1-expressing stromal cells and ECM are often associated with Wnt1-positive epithelial islands or cords. Percentages of the IA subtypes showing SDC1-positive stromal cells and ECM significantly correlate with percentages of the IA subtypes with Wnt1-positive epithelial cells, as shown by the performed regression line tests.

In particular, the above statistical relationship is found to be highly significant between SDC1-expressing stromal cells and Wnt1-positive IA epithelial ones. This suggests that SDC1 and Wnt1 can be coherently involved in promoting proliferation and local invasiveness of transformed ameloblast-like cells, because of their respective expressions in stromal and epithelial components of the studied IA subtypes.

As regards the SDC1 immunoexpression in tumour stromal cells and ECM, it is interesting to note that the SDC1 downregulation often occurs in pre-malignant (29) and malignant epithelia (19), and the SDC1 shifting from epithelial to stromal cells and ECM may have dramatic effects on tumour progression and metastasis (2, 24) as may also occur in local invasiveness of some IA subtypes.

Most of the studied IA subtypes display both the SDC1 reactivity of stromal cells and ECM and the Wnt1 expression by tumour epithelial cells, as also observed in tooth buds. These features directly correlate one with the other, except for two of the four studied desmoplastic IA_s. This relationship suggests that cell proliferation and locally invasive potential of IA_s are more frequent in follicular and acanthomatous subtypes. Conversely, stromal SDC1 and epithelial Wnt1 reactions are infrequent in the studied plexiform and desmoplastic IA_s.

Wnt1 expression is not a common feature of IA epithelial cells, differently observed in the inner layer of the normal enamel epithelium. Moreover, it is obvious in 65% of the IA_s, where significant relationships are found between Wnt1-expressing tumour epithelial cells and both the SDC1-positive tumour stromal cells and ECM.

To the best of our knowledge, the SDC1 expression has not been previously investigated in IA_s and the present results, for the first time, suggest a role of the SDC1 in highly expansive IA_s.

In conclusion, SDC1 is conceivable as a critical factor for Wnt-induced carcinogenesis in the odontogenic epithelium. This heparan sulphate proteoglycan might be involved in promoting local invasiveness of some IA subtypes, depending on its expression by tumour epithelial cells and subsequent shifting to stromal cells and ECM.

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