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Review

Immunohistochemical Characteristics of Odontogenic Neoplasms and Their Physiological Counterparts

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Abstract : Development of the tooth is a complex and fascinating set of processes which require a sequential integration of numerous biological steps. For dental doctors, interest is particularly high, because the tooth is mainly composed of surface ectodermal epithelium and neural crest derived neuroectodermal mesenchyma, and formed by epithelial-mesenchymal interactions. There are many different types of odontogenic neoplasms. In general, proliferation, development and cytological differentiation of the neoplastic cells reflect the normal physiological development of the outbreak mother cells and/or tissues. There would appear to be a relationship between the cytological differentiation of odontogenic neoplastic cells and the physiological development and differentiation of tooth germ. We describe some morphogenesis regulation factors, such as Notch signaling, in the odontogenic neoplastic cells, in both well-differentiated and poorly-differentiated neoplasms. Our results suggest that these factors play some role in cytological differentiation or acquisition of tissue-specific characteristics in neoplastic cells.

Key words: Odontogenic neoplasm, Tooth, Development, Immunohistochemistry

Introduction

A neoplasm is an abnormal growth mass of cells and/or tissue of which exceeds and is uncoordinated with the physiological cells and/or tissues. Thus, neoplasms are said to be transformed because they continue to replicate, apparently oblivious to the regulatory influences that control physiological original cell and/or tissue growth. The words “differentiation and anaplasia” are applied to the originating parenchymal cells that constitute the transformed neoplastic components of neoplasms. The word “differentiation” of parenchymal cells refers to the extent to which they resemble their physiological features, both morphological and functional in findings.

Biology of neoplasms

In benign neoplasms, the parenchymal cell components consist of well-differentiated cells that quietly resemble their originating mother cell and/or tissue. In benign neoplasms, mitoses are extremely rare in number and are of normal physiological features. In malignant neoplasms, they are characterized by a wide variety of parenchymal cell differentiation, from completely well- to un-differentiated. Malignant neoplasms are composed of poorly- to un-differentiated cells when said to be anaplastic. Anaplasia, a lack of differentiation, is thought to be a standard of malignancy. Anaplasia means literally to “form backward”. In fact, it is well accepted that a malignant neoplasm originates from stem cells in tissues. Therefore, a failure of differentiation, rather than de-differentiation of physiologically specialized cells, accounts for un-differentiated neoplasms. In general, the more malignant and the more un-differentiated (anaplastic) a neoplasm, the less likely it is to have specialized functional activity. The cells in benign neoplasms are almost always well-differentiated and resemble the physiological normal cells of the originating mother cells and tissue. The cells in malignant neoplasms are more or less differentiated, but some loss of differentiation is always present. The differentiation in these neoplastic lesions is controlled by, in part, the normal physiological regulation system. The regulation mechanism of specialization growth of a physiological

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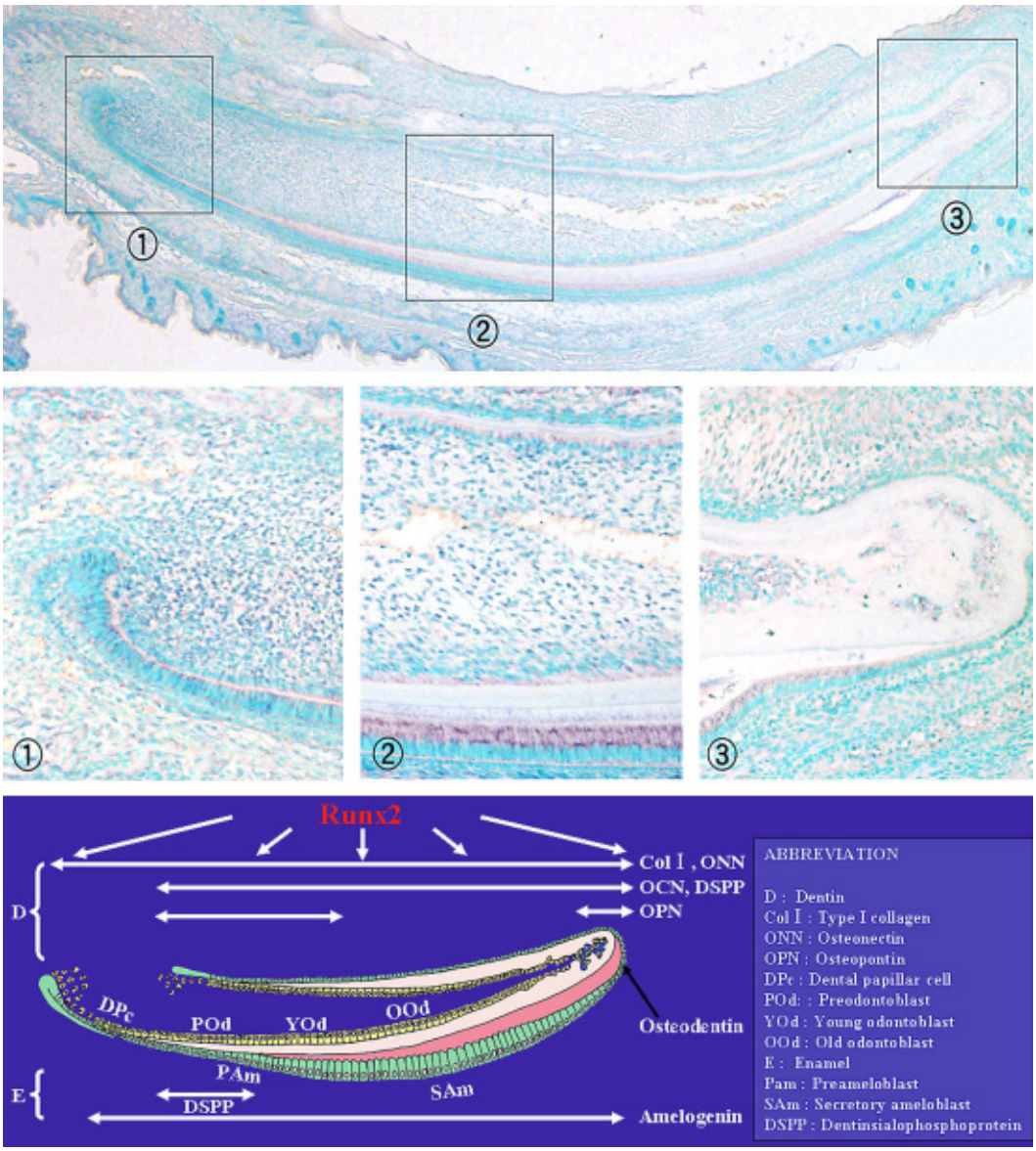

Figure 1. Runx2 regulatory control of the signaling pathway of odontogenesis. The gene expression is visible in various stages of ameloblasts (PAm, SAm, and maturation stage).

organization functions on a neoplasm, and the specialized growth of neoplasms is also regulated. Therefore, it is important to examine the normal physiological cell differentiation mechanisms for understanding the regulation system of neoplastic conditions.

Developmental biology of tooth

Teeth develop physiologically, as deciduous teeth and permanent teeth, from oral ectodermal epithelium and neural crest derived neuroectodermal mesenchymal cells[23]. The enamel is derived from ectoderm of the oral cavity, and all other tissues differentiate from the mesenchyme derived from mesoderm; neural crest cells are imprinted with morphogenetic information before or shortly after they migrate from the neural crest. Tooth development is initiated by the inductive influence of the neural crest mesenchyme on the covering ectoderm. Tooth development is a continuous step. However, it is usually divided into a bud stage, cap stage, and bell stage.

Odontogenesis, or tooth development, is a complex and highly-regulated process characterized by sequential epithelial-mesenchymal interactions leading to tooth initiation, morphogenesis and cell-differentiation with eventual formation of enamel, dentin and cementum matrices[18, 20]. Osteogenesis or bone formation is also a tightly-coordinated process involving many different tissues that interact with each other via a matrix-mediated inductive mechanism, and ending in the formation of a specialized tissue, bone. Both of these processes, though distinct, are closely related in that they share common signaling pathways in terms of morphological differentiation of their cells and

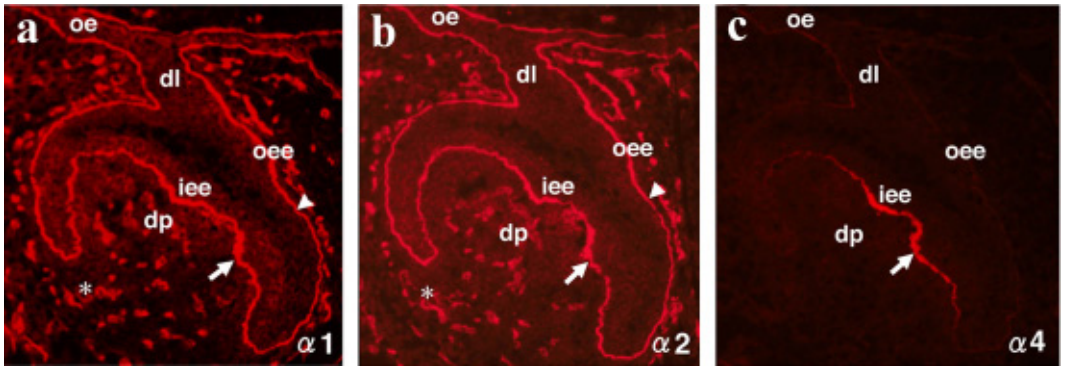

Figure 2. Distribution of α chains of type IV collagen in early bell stage of E15 mice. The basal membrane of inner enamel epithelium (iee, arrow) stains for α1(a), α2(b), and α4(c) chains. oee: outer enamel epithelium, dp: dental pulp, oe: oral epithelium, dl: dental lamina, *: vascular basal membrane [quoted from Nagai N, et al.[31]]

functional differentiation of their matrix proteins. The ameloblasts and odontoblasts, which are exclusive enamel matrix-producing and dentin matrix-producing cells, respectively, share several molecular characteristics with the osteoblast, which is the bone matrix-forming cell. Runx2, a transcription factor, is essential for osteoblast differentiation (Figure 1). In the course of odontogensis, the Runx2-knockout mice experiments results suggest the following: Runx2 is associated with morphogenesis of teeth and matrix protein gene expression[33]. Next, compared to the incisor tooth germ, the molar tooth germ is more strongly subjected to control by Runx2, suggesting the presence of factors involved in odontogenesis of the incisor tooth germ which are different from those present in osteoblasts. Furthermore, in Runx2-knockout mice differences in expression of osteopontin and osteocarcin, matrix proteins common for teeth and bone, suggest different mechanisms of cellular differentiation or transcription regulation pathways in incisor odontoblast and bone forming cells, or osteoblasts[34].

The tooth germ basal membrane interposed between the odontogenic epithelium and mesenchyme mediates the sequential and reciprocal epithelial-mesenchymal interactions essential for morphogenesis and cell-differentiation for tooth formation (Figure 2). It composes some isoforms of type IV collagen, laminin, nidogen/entacin, heparan sulfate, proteoglycan, fibronectin, and other component molecules[30,37,61]. The molecules of type IV collagen, a major framework-forming peptide of basal membrane, are heterotrimers composed of three α chains that exist in six genetically-distinct forms (α1 to α6) and with at least three molecular forms[41,46,47]. The expression patterns of type IV collagen molecular forms in tooth germ organogenesis and the marked stage-specific changes in the type IV collagen distribution during the odontogenesis are limited[21]. According to the examination results using mouse developing molar tooth germ at the dental placode and bud stage in the course of odontogenesis, the basal membrane of the oral cavity epithelium expresses α1, α2, α5 and α6 chains while the gubernaculums dentis, in addition to the above 4 chains, also expresses α4 chain. An asymmetrical distribution of α4, α5 and α6 chains has been observed at the bud stage in the odontogenesis. At the early bell stage, the basal membrane associated with the inner enamel epithelium of molar germ expresses α1, α2 and α4 chains while the basal membrane of the outer enamel epithelium only expresses α1 and α2 chains. With the onset of dentin formation, the collagen α chain profile of the basal membrane of inner enamel epithelium gradually disappeared. From the bell stage, however, the gubernaculums dentis consistently expressed α1, α2, α5 and α6 chains, and this distribution pattern resembles the one of the fetal oral cavity epithelium. These features suggest that the odontogenic stage- and the position-specific type IV collagen α subunit distribution is according to the tooth germ odontogenesis, and its changes are essential for the morphogenesis and cell-differentiation for tooth development[7,30,58].

Tenascin is an extra-cellular matrix glycoprotein which appears to regulate cell morphology[31]. It is more restricted to tissue distribution than fibronectin and is able to interface with the cell-binding function of fibronecion. Tenascin is most typically expressed in epithelial-mesenchymal interactions during physiological development and in the stromal tissue of malignant neoplasms. Extracellular matrix protein is shown to play important roles in cellular growth and differentiation, in complex cell matrix interactions, in physiological organ development and in neoplastic transformation course[31].

At first, in dental lamina of the bell stage of human tooth germ, tenascin is present only on the submucosal connective tissue side, not on the dental follicle tissue side[5]. At this stage there are no morphological differentiations in the odontogenic epithelium on either side. Concerning fibronectin, a weak or negative localization is seen in the condensed mesenchyme surrounding the dental lamina. In the cap stage, different patterns of the distribution between tenascin and fibronectin ares evident in the human tooth germ. Strong tenascin accumulation is present in the dental papilla under the basal membrane, preodontogenic layer

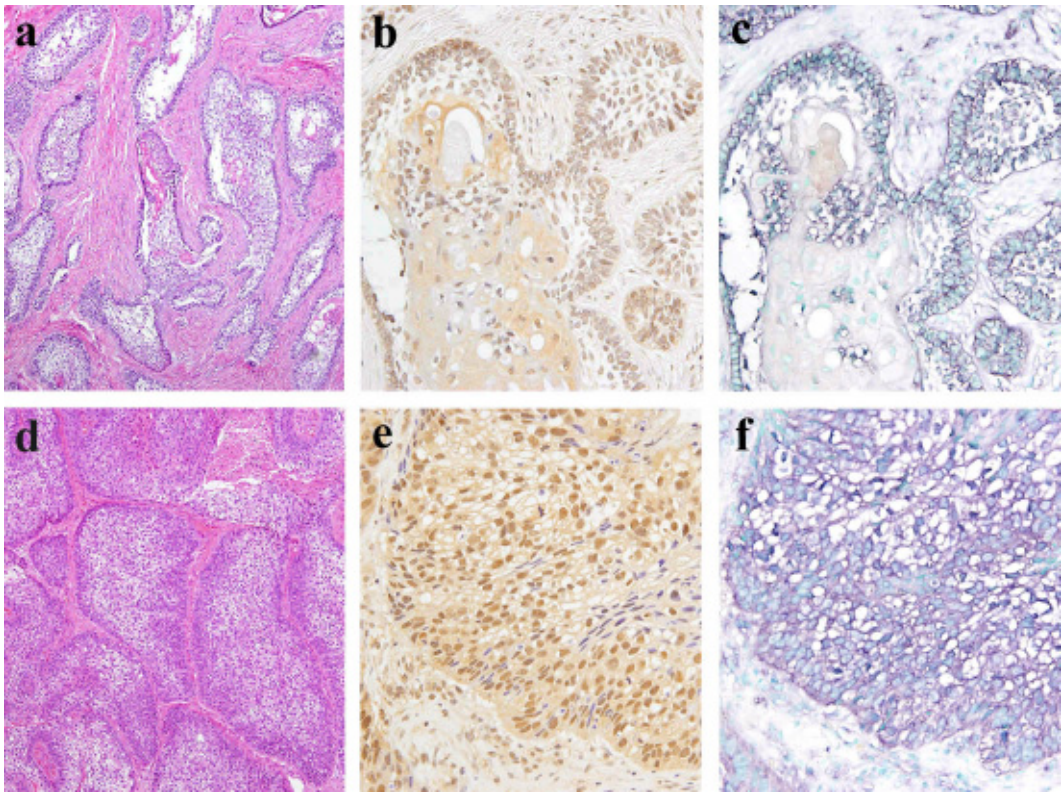

Figure 3. Proliferating follicular nests of ameloblastoma are visible (a). Immunohistochemically Notch peptides are observed in the cells at the peripheral layer (b) and gene signals are detected in the cytoplasm (c). Polyhedral neoplastic cells are visible in proliferating ameloblastic carcinoma cell nests (d). The Notch positive products are observed uniformly in these cells (e) and the gene expressions are also detected in the cytoplasm (f).

and osteogenic tissue of alveolar bone. However, tenascin is immunohistochemically negative in the dental follicle, the fibroblastic layer developing into the periodontium[31]. Intense fibronectin is evident in the alveolar bone. The epithelial components of the tooth germ are imunohistochemically negative for both tenascin and fibronectin.

Notch signaling in cell differentiation and development

In general, Notch signaling plays an important role in the regulation of cell fate, morphogenesis and/or development[4,15]. Regarding tooth development, there are some published data on how the expression of Notch1, 2, and 3 is regulated by epithelial-mesenchymal interactions in the developing mouse tooth and associated with determination of ameloblast cell fate. Jagged1 is also expressed as a ligand of Notch in the developing tooth[28]. Notch signaling is an evolutionarily-conserved cell-to-cell transmembrane interaction mechanism. Furthermore, asymmetric distribution of Notch has been observed in immature cells prior to cell division, suggesting a role in the regulation of daughter cell fate, including whether the cells remain stem cells or give rise to differentiated progeny[36]. Regarding odontogenesis, Notch1 is expressed in stellate reticulum cells, and Jagged1 is expressed in differentiated ameloblasts in the course of tooth development. During tooth development, Notch expression has been associated with the differentiation of odontogenic epithelial and mesenchymal tissues. However, Notch expression is absent in epithelial cells in close contact with mesenchyme, a feature which may be important for ameloblast cell fate. These data suggest that mesenchymal tissue negatively regulates Notch expression in epithelium. In other words, Notch expression is downregulated in odontogenic epithelium juxtaposed to mesenchyme, indicating that odontogenic epithelium needs a mesenchyme-derived signal to maintain the down regulation of Notch[28].

Cell differentiation in pathological conditions

In the oral and craniofacial region, there are many types of neoplasms and pathological conditions, such as odontogenic neoplasms, bone and cartilage neoplasms, pathological bone and cartilage formation and/or proliferation. Odonotogenic neoplasms consist of a plural number of blastoderms. The neoplastic cell differentiation process is complicated, and it is thought that the cell differentiation and growth pattern are copied from a physiological system of the odontogenesis.

Odontogenic neoplasms

Ameloblastoma is classified as a benign, locally-infiltrative

odontogenic neoplasm, which is composed of proliferating odontogenic epithelial nests within a fibrous stromal tissue. Some variants have been sub-classified as follows: solid/multicystic, extraosseous/peripheral, desmoplastic, and unicystic[10,22,24]. Furthermore, other variants have been reported in the literature, and these include acanthomatous, ghost cell, and vacuolated or clear cell types[40,44,49-51,59]. Odontogenesis is a complex biological process, and this process is directly reflected in the development of odontogenic neoplasms[14,19], especially ameloblastomas[16,17]. It is thought that the above-mentioned variants are due to the developmental complex system[45].

Notch signaling in ameloblastomas and ameloblastic carcinomas

Regarding Notch signaling, the focus of our attention is on examining Notch1 and Jagged1 peptide expression, as well as their genes, in ameloblastomas and ameloblastic carcinomas (Figure 3). The speculation on their possible roles is in cytological differentiation and proliferation of ameloblastomas. In one examined case of ameloblastoma, histopathologically, the main specimens showed follicular nests consisting of islands of odontogenic epithelium within a fibrous stroma. Cells of the peripheral layer of these islands were columnar, with hyperchromatic nuclei, and lined up in a palisade fashion, whereas the central cells were stellate reticulum-like. Their cytoplasms were generally vacuolated. Some nests showed central cyst formation. In small parts, the odontogenic epithelium exhibited focal basal palisading. Furthermore, occasionally a large number of nests underwent squamous metaplasia with keratinizing pearl formation. In general, degeneration of the parenchymal cells and cyst formation occurred in these ameloblastoma nests. According to the immunohistochemistry examination results[25,26,38,39], NICD-positive products were detected in most proliferating odontogenic epithelial nests of ameloblastomas by IHC. The positive reactions existed in the cytoplasm and/or nucleus. Strong reactions were seen in the pre-ameloblast-like cells or some localized cells within the nests. In some ameloblastoma nests, there were no positive reactions to NICD. Jagged1 positive reactions were also observed in the cytoplasms of same cell types in the ameloblastoma nests. Strong reactions existed at the peripheral layers. The pattern of distribution and the intensity of expression of Jagged1 were closely similar to the pattern and intensity seen in NICD. Notch1 gene signaling was localized in the cytoplasm of IHC-positive neoplastic cells. These mRNA positive signals showed variable labeling intensity. Jagged1 mRNA signals were also detected in the cytoplasm of ameloblastoma cells, and the strength pattern was nearly the same as that of Notch1. These mRNA signal expressions were not consistent with those of the transcription factor peptides. Histopathologically, the follicular type of ameloblastoma is the most common, consisting of proliferating odontogenic epithelial islands and nests in the fibrous stromal tissues. Cellular modifications, such as squamous metaplasia, keratin pearl formation, parenchyma cell degeneration and cystic changes, may also occur. Morphogenesis is a complex biological process, and this process directly reflects the development and proliferation of neoplasms. Regarding the proliferation of ameloblastomas, some morphogenesis factors are overly-expressed in ameloblastoma tissues in comparison with tooth germs. According to the analysis of gene expression in ameloblastomas and human fetal tooth germs using a cDNA microarray, some results have been published. Aanalysis included tumor-necrosis-factor-receptor-1 (TNFRSF-1), sonic hedgehog (SHH), Cadherins 12 and 13 (CDH 12 and 13), and transforming growth-factor-1 (TGF-β1), the gene expression profile identified candidate genes that might be involved in the origination of ameloblastoma, as well as several genes previously unidentified in relation to human tooth development. The expression of SHH signaling in ameloblastomas, in comparison with human tooth germs, was also detected. The literature concluded that the SHH signaling might play a role in epithelial-mesenchymal interactions and cell proliferation in the growth of ameloblastomas[27].

On the transcription factors Notch1 and Jagged1 in ameloblastoma, the results demonstrate that Notch1 (NICD) and Jagged1 are both detected by IHC, and their expression patterns are very similar[62]. This phenomenon means that Notch signaling is activated in the neoplastic epithelium of ameloblastoma. It is likely that the signaling plays the role of daughter cell fate regulation. Positive Notch1 reactions suggest that proliferation and cytological differentiation are probably occurring in these neoplastic cells. This explains the variation in the strength of these signals and their distribution patterns in the ameloblastoma cell nests. Furthermore, the mRNA of Notch1 and Jagged are also expressed in the ameloblastoma cells, as determined by ISH. These mRNA signal expressions are consistent with those of the transcription factor peptides. The examination of larger case series of ameloblastoma and other odontogenic epithelial neoplasms, including epithelial, mesenchymal, benign and malignant entities such as calcifying epithelial odontogenic tumor, adenomatoid odontogenic tumor, keratocystic odontogenic tumor, ameloblastic fibroma, odontoma, and odontogenic carcinoma, would help to elucidate further the role of these genes in odontogenic tumorgenesis. The results suggest that Notch signaling plays a role in cytological differentiation or acquisition of tissue-specific characteristics in these neoplastic cells of ameloblastomas[50].

Histopathologically, in ameloblastic carcinoma, proliferating polyhedral neoplastic cells show strong cellular atypia, such as mitosis and pleomorphism, especially in peripheral layers of the nests. NICD-positive products are observed in most proliferating nests of benign ameloblastoma by IHC, and strong reactions are seen in the cells at the peripheral layer of the nests. In the case of ameloblastic carcinomas, positive products have also been detected, and strong reactions uniformly observed. The positive reactions are comparatively weaker in benign than in malignant

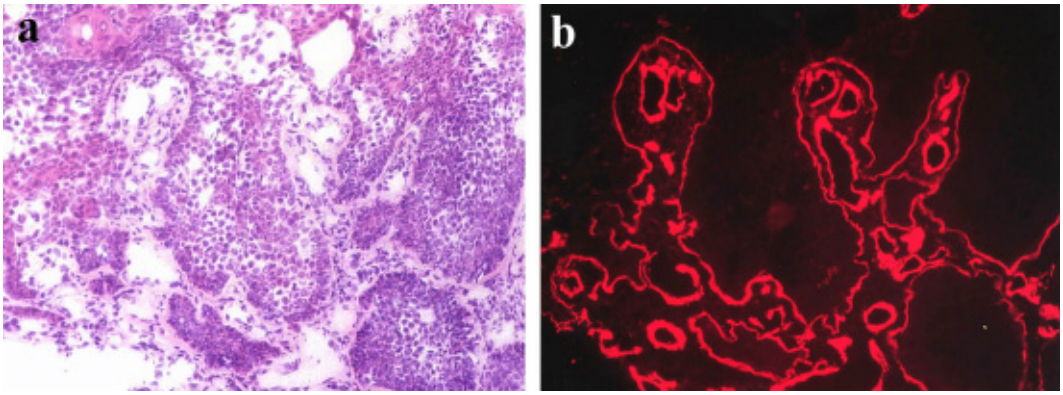

Figure 4: Histopathological feature of examined plexiform ameloblastoma (a) and immunofluoresence localization of type IV collagen α1 chain in the basal membrane zone (b).[quoted from Nakano K, et al.[36]]

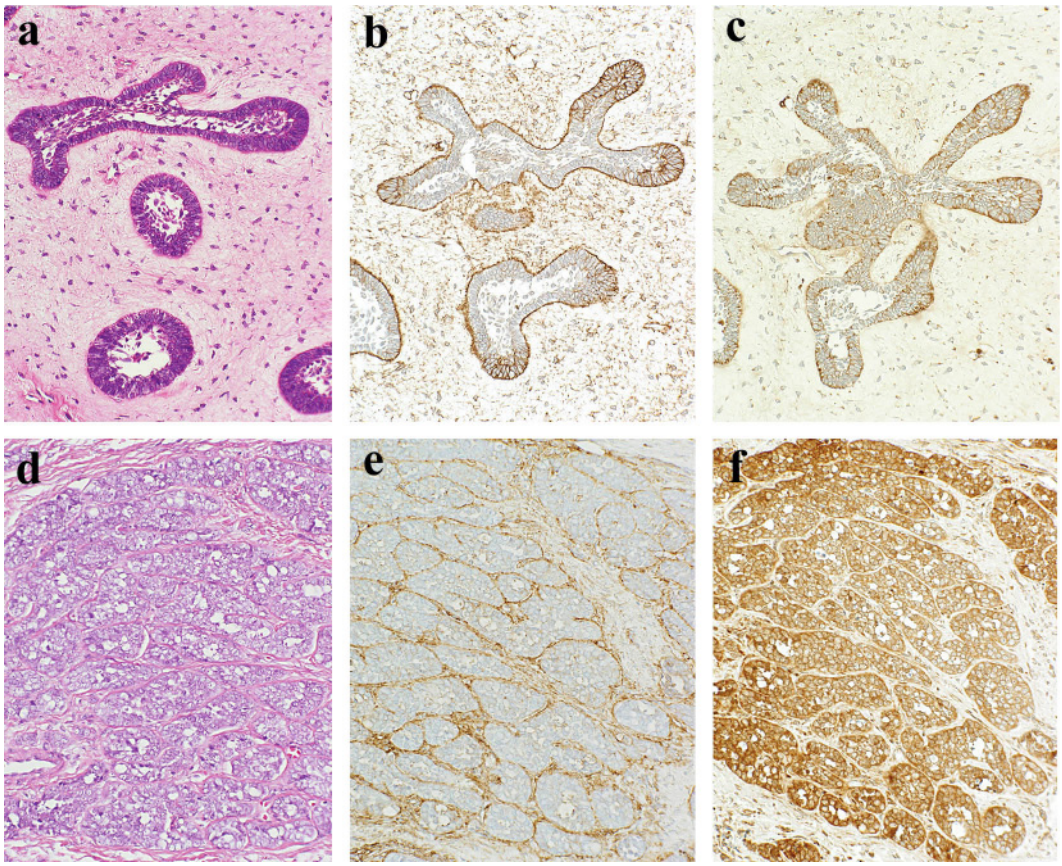

Figure 5. Ameloblastic epithelial islands within scattered dental papilla-like ectomesenchymal tissue (a). HS and heparanase immunohistochemical reactivity accentuates the cellular processes of dental papilla-like cells (b and c). Note the clear confinement and continuity of basal membrane despite the prominent localization of heparanase (b). Histopathologically, cancer cell nests of ameloblastic carcinoma increase nuclear-cytoplasmic ratio and prominent nuclei (d). HS is not detected in the cancer cells but is strongly localized in the stromal tissues adjacent to cancer nests (e). Intense and diffuse heparanase expression is observed in all cancer cells (f) in contrast to the strong staining limited in the basal cells of benign ameloblastoma (c).[quoted from Han PP, et al.[12]]

tumors. In both benign and malignant cases, gene (mRNA) expressions have been detected in the cytoplasms of IHC-positive cells by ISH. In general, Notch signaling is responsible for cytological regulation of cell fate, morphogenesis and/or development. In examinations conducted by the present authors, IHC and ISH examination results have suggested that Notch signaling plays some role in cytological differentiation or acquisition of tissue-specific characteristics in neoplastic cells of tooth enamel organ-derived neoplasms, including benign and malignant neoplasms, ameloblastoma and ameloblastic carcinoma.

Histopathologically, follicular nests proliferate in the fibrous connective tissue in benign neoplasms. In some nests, parenchymal cyst formations or squamous metaplasia are evident. The histopathological features of the malignant neoplasms are as follows: proliferating polyhedral neoplastic cells show strong cellular atypia, such as mitosis and pleomorphism, especially in peripheral layers of the nests. Notch intra-cellar domain positive products are observed in most proliferating nests of benign neoplasms by immunohistochemistry. Strong reactions are seen in the cells at the peripheral layer of the nests. In malignant ones, positive products are also detected, and strong reactions uniformly observed. The positive reactions in benign neoplasms are comparatively weaker than in malignant ones. In both the benign and malignant cases, the gene expressions are detected in the cytoplasm of immunohistochemistry-positive cells by ISH. Our examination results suggest that Notch signaling plays some role in cytological differentiation or acquisition of tissue specific characteristics in neoplastic cells. Furthermore, there would appear to be a relationship between the cytological differentiation in the oral and craniofacial neoplastic cells and the physiological development and differentiation of their originating mother cells and tissues of the oral and craniofacial region.

Cell differentiation in odontogenic neoplasms

Amelogenin is a typical enamel matrix protein. The expression pattern of amelogenin genes (AMGX, AMGY) has not yet been identified in ameloblastomas. In surgical materials, amelogenin gene is expressed in all ameloblastoma cells. The mRNA of AMGY expression increases, although that of AMGX does not. This is an interesting feature in physiological normal male tooth development, in which the expression of AMGY is considerably lower than that of AMGX. This finding suggests that epigenetic change of sex chromosomes may have some correlation with tumorigenesis of ameloblastoma[60].

Regarding the collagen subunits of basal membrane components of oral neoplasms, there are some published data[1,11,53,54,55]. Regarding ameloblastomas, co-expression of type IV collagen α1 and α2 chains appears as thin lines with limited areas of discontinuity along the basal membrane of neoplastic cell nests[35,36]. The expression staining is strong and in a linear continuous manner, in the periphery of the nests of the desmoplastic types. In the neoplasms, α5 and α6 chains are co-localized as continuous linear patterns demonstrating the tumoral nests from the surrounding connective tissue stroma. These collagen subunits also appear as random intracellular staining of the neoplastic cell nests[8,9]. There is no remarkable differentiation of the distribution pattern among the tumor growth patterns and various cellular subtypes within ameloblastomas (Figure 4). Distribution of α subunit of collagen in the basal membrane of ameloblastic fibromas is uniformly demonstrated in its pattern. Subunits of α1/a2, α4 and α5/α6 are distributed as liner continuous patterns that compartmentalize the neoplastic epithelial cell nests, islands and strands from the surrounding dental papilla-like ectomesenchymal cell proliferation. These α subunits of collagen are randomly expressed in the periphery preameloblast-like and central stellate reticulum-like cells. In adenomatoid odontogenic tumors, subunits of α1/α2 and α5/a6 are strongly expressed at the area of interface between tumor cells and stromal tissues, especially in the cribriform regions. Faint to non-positive expression of these collagen molecules is detected in the basal regions of conglomerated masses of solid epithelial whorls/rosettes/nests and duct-like structures. There are intensely-positive reactions to the amorphous deposits; however, there is little or no reaction to the mineralized bodies. In malignant neoplasms, at first in ameloblastic fibro-odontosarcomas, α1/α2 and α4 chains demonstrate moderate intensity along with the periphery of the epithelial components, while α5/α6 chains are strongly co-distributed as continuous linear patterns demarcating the benign neoplastic cell nests from the surrounding sarcoma tissues. In the inductive dental hard tissue regions, no reactivity is found. In the malignant neoplasm type of ameloblastic carcinomas, collagen IV α chains demonstrate an irregular and disrupted expression pattern with specific loss of α1/α2 chains. In those regions containing poorly-differentiated neoplasm cell nests, there is complete disappearance of α chain subunits. In α5/α6 subunits, there is a discontinuous and fragmented pattern. In primary intraosseous carcinomas, the expression pattern is similar to that of ameloblastic carcinomas. From the above-mentioned findings for cell differentiation in various benign and malignant odontogenic neoplasms, the basal membrane pattern of neoplastic epithelial cell nests yields three features: (1) the basal membrane of benign and malignant odontogenic neoplasms has distinct α chain subunits of collagen type IV; (2) modifications in the relative abundance of collagen type IV α chains in basal membrane of odontogenic neoplasms probably represent a host protective response; and (3) early specific loss of α1/α2 chains proceeds the loss affecting α5/α6 chains during odontogenic neoplasm progression. Therefore, these results suggest that modification and remodeling of basal membrane collagen type IV α chains are dynamic processes crucial for odontogenic neoplastic cell growth and progression[6,36].

Heparan sulphate (HS) and heparanase appearing in the odontogenic neoplasms are interesting molecules for these neoplastic transdifferentiations[12,13,43]. HS proteoglycans (HSPG) constitute a group of ubiquitous extracellular matrix macromolecules and are composed of a core protein and covalently linked HS sugar chains (Figure 5). Although HSPG plays critical functions in cell-to-cell and cell-to-matrix interactions through core proteins, their HS chains confer most of their biological functions[2]. The negativel-charged HS chains can bind and sequester numerous heparin/HS binding molecules, including growth factors, cytokines and cell adhesion molecules. HS chains

also take part in the important cellar events conferred by these tethered molecules and have an influence on various developmental and pathological processes, such as neoplastic transformation, its local invasiveness and transdifferentiation[2,3].

In the examination results on ameloblastomas, HS is clearly evident on the cell surface of peripheral basal cells and also in the intercellular region of some parabasal cells, while it is not present in the central stellate cells of ameloblastoma nests. Heparanase is expressed in peripheral epithelial cells of ameloblastoma nests. The strong expression is localized at the budding region of the strands mainly in the invasive fronts in histopathological specimens. In adenomatoid odontogenic tumors, the strongly localized and limited expression is present on the surfaces of dark cuboidal cells surrounding the whorls and solid tumor cell nests. HS is also evident in luminar surfaces of some duct-like structures. In the lumen and/or duct-like structures, eosinophilic materials are HS positive. The periphery of immature calcified materials are positive to both HS and heparanase; however, completely calcified materials are negative.

In ameloblastic fibromas, as one of typical epithelial and mesenchymal mixed odontogenic neoplasms, HS exists in nearly the same location as in ameloblastomas. HS is evident in the basal membrane, but is more defined. HS is also present in the ectomesenchymal cells, unlike the stromal cells in ameloblastomas. HS and heparanase are diffusely present both in epithelial and mesenchymal tissue of the neoplasms. In some parts, heparanase exists focally in nuclei of mesenchymal cells. In ameloblastic carcinomas as malignant odontogenic neoplasms, neoplastic cells are absent of HS in contrary in benign neoplasms, such as ameloblastomas. Instead, HS clearly and strongly is present in the stromal tissues, especially in the intercellular matrices within the vicinity of neoplastic cell nests. Regarding heparanase activity, positive reactions are intense and diffuse, and occur in intracellular spaces. The above-mentioned findings are compared with the data of physiological tooth development, in both experimental animals and human materials. Furthermore, when the results are examined using various types of oral squamous cell carcinoma, there are some differences between the types of histological and clinical malignancy grades. In summary, the general localization of HS and the heparanase activity in odontogenic neoplasms are temporally regulated in relation to cellular growth and function. Furthermore, heparanase over-expression is reported to promote hair follicle morphogenesis and its growth[13,62]. Both hair follicular morphogenesis and odontogenesis are governed by similar growth factors and signaling pathways. Heparanase may also have physiological function in tooth development through local modulation and release of HS-bound growth factors. Taken together, the facts suggest that heparanase may have physiological function in tooth development, and the increase in heparanase expression maybe an important initiating factor for odontogenic neoplastic transformation. The stromal HS sugar molecule localization and heparanase over-expression may represent the malignant progression of ameloblastoma to ameloblastic carcinoma[32].

In odontogenic neoplasms, both benign and malignant, the immunohistochemical distribution of tenascin and fibronectin is compared with that in human tooth germs[56,57]. In ameloblastomas, the extracellular matrix components of the stromal tissue of ameloblastomas exhibit considerable variety: dense and loose connective tissues, hyalinization regions, and stromal cystic spaces. In the hyalinised stroma, tenascin and fibronectin exhibit both positive and negative reactions. In cystic spaces, positive reactions of tenascin and fibronectin are seen. In follicular type of ameloblastomas, the basal membrane region reacts irregularly positive to tenascin. The fibronectin reactivity exhibits uniformly and weakly positive in the dense connective tissue of the stromal region in the follicular ameloblastomas. A partial accumulation of tenascin is found in the basal membrane. The tenascin-positive basal membrane shows fuzzy fibrillar materials, whereas the loose or myxomatous tissues of stromal region of follicular type of ameloblatoma exhibit no reaction to fibronectin. Regarding malignant odontogenic neoplasms, the data on ameloblastic carcinomas are as follows. The stromal tissue and basal membrane of ameloblastic carcinomas show an irregular and strong immunohistochemical-positive reaction to tenascin. In the epithelial cell islands of ameloblastic carcinomas, a scattered or granular positive reaction is evident. The connective stromal tissue of ameloblatic carcinomas shows an irregular and strong reaction to fibronectin. According to the localization pattern of tenascin and fibronectin in the varied types of odontogenic neoplasms, such as benign and malignant, the dental follicle of the tooth germ lacks tenascin but has fibronectin. The osteogenic tissues generally contain both tenascin and fibronectin. The ameloblastic fibromas show positive or negative distributions in the stromal tissues, which suggest a differentiation to the papilla of the tooth germ. It is also suggests that the stromal tissue cells of ameloblastic fibroma differentiate to dental follicular tissues. The relative distribution of menisci and fibronectin can be a marker in histological diagnosis of periodontal and osteogenic fibrous tissues. Furthermore, the findings also suggest that fibronectin and tenascin may be used as markers in cell differentiation of epithelial-mesenchymal interactions during tooth development and in odontogenic neoplasm for trans-differentiation[31].

Cytokeratins with intermediate filaments characteristic of epithelial cells are very stable and range in weight from 40 to 67kD[33]. Regarding the distribution patterns of cytokeratins in some types of ameloblastomas, such as follicular ameloblastomas, the peripheral columnar cells resembling preameloblasts react positively for NSE-K (52.5kD) and 19-K (40kD) in a linear pattern along the basal membrane, but not for the markers of squamous cells, SE-K (56, 56.5, 58 and 68kD). The reaction pattern of NSE-K and 19K shows a frame-like structure in cytoplasm in

peripheral columnar cells, while the central stellate reticulum-like cells show an immunohistochemical reaction with all markers of the cytketain SE-K, NSE-K and 19-K throughout the cytoplasm. In plexiform ameloblastomas, both the central spindle and peripheral cuboidal cells demonstrate a positive reaction with SE-K and 19-K, but not with NSE-K. Regarding the oral mucosa and developing tooth germ, there are differences for immunohistochemical reactivities of cytokeratins between the fetal oral mucosa and adult gingiva. The cells of both the fetal oral mucosa and adult gingiva demonstrate positive reactions to SE-K, and negative reactions to NSE-K. However, positive reactivity to 19-K is noted only in cells of the fetal mucosa and Merkel cells in adult gingiva. The dental lamina connecting with the basal cells of the oral mucosa have all these cytokeratins, especially 19-K. In the enamel organ, however, the immunoreactivity for SE-K, a maker of squamous differentiation, is different in each cell layer: positive in the outer enamel epithelium; slightly positive to negative in the stellate reticulum and stratum intermedium; and negative in the columnar inner enamel epithelium. NSE-K and 19-K are evident in all types of cells that compose the enamel organ proper. The inner columnar enamel epithelium especially expresses a diffuse positive reaction for NSE-K and 19-K throughout the cytoplasms. These features suggest that the characteristics of the cells of the plexiform ameloblastoma are similar to the fetal oral epithelium not odontogenic epithelium. This later feature suggests that the different expression patterns of cytokeratin in ameloblastoma depend on the follicular or plexiform types. As a coincidence of cytokeratin and functional pattern is not noted among the ameloblastomas and tooth germs, these data suggest that the columnar cells of the follicular ameloblastoma have little resemblance to ameloblast-like cells in cytokeratin structure or in cellular functions[33].

Conclusion

Tooth development is a complex and fascinating set of processes which require a sequential integration of numerous biological steps, and the differentiation in cells of odontogenic neoplasms is also a complex and fascinating compound process. Therefore, there are many different types of odontogenic neoplasms. It is known that proliferation, development and cytological-differentiation of the neoplastic cells reflect the normal physiological development of the outbreak mother cells and/or tissues. Some cell-differentiation, development and proliferation factors may also play roles in the neoplastic cells, and therefore, their behavior is closely related in cytological differentiation and clinical behavior and/or grade.

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