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Signaling Dynamics in Cell Differentiation of Pleomorphic Adenomas

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Abstract

Salivary pleomorphic adenoma is a typical benign tumor of variable capsulation characterized by architectural rather than cellular pleomorphism. The differentiation of parenchymal neoplastic cells due to molecular signaling in the neoplasm. Therefore, the review describes at first the histopathology, and the cytological metaplasia, especially by molecular signalings: Wnt, Notch and HSP27. Recent literatures suggest that Wnt signaling is famous and involved in the suppression and differentiation of cells. Wnt signaling via β-catenin may be involved in the pleomorphic adenomas. Next, Notch expression was determined as localized in the nucleus of ductal cells and basal cells in solid neoplastic cells of squamous metaplasia. Therefore, Notch is a possible cell differentiation factor in the neoplasms, especially in the squamous metaplasia. Furthermore, HSP27 sometimes act as a molecular chaperone in cell-signaling. The immunofluorescent staining positive pattern of Wnt1 and HSP27 was consistent with previous results. These suggest that Wnt1 and HSP27 are both act in tumor cell differentiation of basaloid cells in solid nests and small cuboidal cells in duct-like structures. Moreover, HSP27 is highly involved in cell differentiation such as the formation of squamous metaplasia in solid tumor nests. Since HSP27 expression was similar to Wnt1, it can be inferred that HSP27 works as possible molecular chaperone in Wnt1 signaling. Furthermore, HSP27 may have function as a molecular chaperone of Notch in pleomorphic adenomas.

Keywords: Pleomorphic adenoma, Wnt signaling, β-catenin, Notch signaling, Cell differentiation, HSP27, Molecular chaperone, Immunohistochemistry

Introduction

Pleomorphic adenoma is the typical benign epithelial neoplasm of the salivary gland [1]. It has a wide histopathological spectrum even in the same neoplasm depending on the location [2-4]. Histopathologically it is characterized by being diverse, composed polygonal epithelial cells of ductal structures surrounded by neoplastic myoepithelial cells. Typically, a transition from epithelial to mesenchymal component creates a third type of so-called tumoral stroma, called cartilaginous or myxomatous tissues among the growth of the glandular structure [2-4]. However, the small cuboidal cells in tumor nests undergoing advanced differentiation, squamous epithelioid cells, plasmacytoid cells and other cell types involved in epithelial-mesenchymal transition have not been elucidated in previous researches. Consequently, the review focused on the differentiation of parenchymal tumor cells in pleomorphic adenoma, using our research group published literatures [2,5,6].

It is currently known that Wnt signaling is implicated in cell proliferation and differentiation. Wnt is activated by the canonical β-catenin and non-canonical Wnt pathways. In the β-catenin pathway, Wnt, secreted as a glycoprotein binds to receptors on the cell membrane, known to be involved in the suppression of differentiation and cellular response [7]. Because the signaling is also involved for the promotion in the suppression and differentiation of cells, it was hypothesized that a pathway other than the canonical Wnt signaling via β-catenin may be involved in the 'mixed' differentiation characteristic. Therefore, immunohistochemistry was performed to include CK7 and CK13 based on the study of other literatures using as the cell differentiation makers. Okuda, et al. [5] performed immunohistochemistry on pleomorphic adenomas to determine the role of Wnt in cell differentiation. The results suggested that Wnt is involved in cell differentiation in pleomorphic adenomas. We thought that Notch also might be involved in cell proliferation and differentiation in the same manner [5]. Thus, in this review, secondary, we focused on Notch expression in pleomorphic adenomas.

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Next, HSP27 is a protein that belongs to the small family of heat shock proteins. Although it is generally expressions in the cytoplasm, it shifts to the nucleus in response to some stress [8]. It acts as molecular chaperone to other proteins. Hence, we hypothesized the possibility of HSP27 functioning as a molecular chaperone during cell differentiation. Immunohistochemistry was described in the review.

Histopathology of Pleomorphic Adenomas

Pleomorphic adenoma is the most common salivary gland tumor classified as benign epithelial tumors [1]. Various cell types can be seen in the tumor indicating a high occurrence of cell differentiation. In general, histopathologically, the tumor tissue is composed of nodular compartments almost lined with relatively thin fibrous tissues. On the other hand, there are cases with tumor tissue extending into the normal glandular parenchyma beyond the fibrous tissues lining, creating small alveolar or cord-like tumor tissues (Figure 1). Inside the tumor cell nests, the characteristic biphasic appearance is noticeable consisting of epithelial cell nests and sparse array of relatively loose cells. Numerous cells grouped together formed solid cell nests. Within the tumor cell nests, duct-like structures are evident. A double layer of basaloid cells and relatively large cuboidal cells formed the duct-like structures (Figure 1 a,c). Some of the lumen of duct-like structures extends into the cystic cavity convening their secretions. The neoplastic myoepithelial cells are scattered in the surrounding so-called stromal tissues creating the 'mixed' appearance of the tumor, which is a characteristic of pleomorphic adenoma (Figure 1b). Other parts of the so-called stromal tissues consist of relatively dense growing spindle-shaped cells producing the neoplastic myxomatous tissue. Others consist of colorful substrates and glass-like substance creating an encapsulated cartilaginous stroma (Figure 1b). The so-called stromal tissue at the region corresponding to the outermost layer of tumor nests is not filled with small cuboidal cells but rather, numerous spindle-shaped cells arranged in fence-like structures were detected. Adjacent to the small cuboidal cells, squamous-like tumor cells which are caused by neoplastic metaplasia are evident (Figure 1d). Cartilage-like tissues and/or bone tissues can be seen in a wide range possibly due to the presence of cartilage-like matrix and cartilage-like cells. Neoplastic myoepithelial cells are dissociated from the mucous and/or cartilage-like substrate by their own secretions. The tumor parenchyma might have been formed by myxomatous cells, which migrated to seep into the structure of mesenchymal and cartilage-like cells and/or bone formation. Thus, the epithelial component is dissociated and mixed into the mesenchymal-like tissue, giving rise to a 'mixed appearance'. Moreover, tumor cells undergo remarkable squamous metaplasia.

Various researches regarding tumor development, cell differentiation and progression have been carried out producing clinical impact. In recent years, studies regarding the expressions of various cytological signaling factors in neoplastic tissues responsible for cell differentiation have been pursued and many have been reported [8-14]. Furthermore, there are some literatures regarding the cytological differentiation and related gene alterations, as PLGA1 and other related genes [15,16]. In the oral cavity, studies in odontogenic tumors including ameloblastoma in particular have been carried out [17-19].

Wnt Signaling in Pleomorphic Adenomas

Wnt signaling is responsible for cell-proliferation, differentiation and homeostasis although numerous researchers have also shown that Wnt signaling is associated with suppression [20]. Wnt is an extracellular secretory protein that is variously and widely expressed. However, gene expression, regulation, cell proliferation, cell motility, cell polarity and maintenance of homeostasis in regenerating organs are its inherent roles, when the Wnt play important roles in pathological aspects such as malignant transformation and invasion in neoplasms as well as various physiological transitions [21]. Physiologically typically in β-catenin pathway, the proteins accumulate and move into the

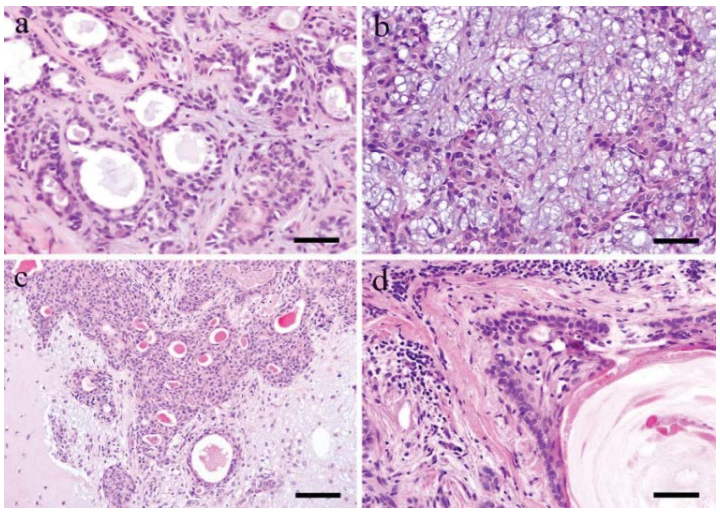

Figure 1: Histopathological appearances. a: Ductal area view a 73-year-old, male, palate case (scale bar= 50μm); b: Mucous appearance areas of a 68-year-old, female, palate case (scale bar=50μm); c: Comparative large tumor cell nests with some ductal sturucture of a 58-year-old, male, mandibular gland case (scale bar=100μm); d: View of squamous metaplasia of a 40-year-old, male, palate case (scale bar= 50μm). Quotation alteration of literature #5.

nucleus due to Wnt signaling. The protein later on combines with Tcf/Lef transcription factors and promotes the of gene expression [22,23]. β-catenin is held low if there is no stimulation of Wnt. Considering its relation to the neoplasm cell differentiation, the advent of hard keratin expression in ghost cells in tumors has been reported in Wnt signaling pathway, which deeply related squamous metaplasia and keratinization [24,25].

In β-catenin pathway, cytoplasmic protein adjusts its weight to suppress expression of the gene. By the way, two pathways have at least been identified in non-β-catenin pathway namely PCP and Ca2+ pathways [26]. Suppression of β-catenin pathway is known in these pathways. These literatures lead us to believe Wnt signaling is involved in malignant salivary gland tumors [27].

Currently, there are only few researches on cytological differentiation of pleomorphic adenoma particularly in Wnt [5]. In the manuscript, the neoplastic cells that form duct-like structures, the cell membrane and cytoplasm of polygonal cells and of spindle-shaped cells in the outer layer of the duct-like structure showing positively (Figure 2a). The cell membrane of spindle-shaped neoplastic cells, which proliferate in interstitial trabeculea is moderately positively while the scattered tumor cells show strong positively. In the typical mesenchymal component of the neoplasms, the neoplastic chondrocytes in mucous and/or hyalinised areas are almost negative. But, the spindle-shaped cells in myxomatous so-called stromal tissue show positively in the cytoplasm (Figure 2 a,b). Many of the squamous metaplastic cells in the nests exhibit positively on the cell membrane. In the squamous metaplasia areas, the basaloid cells strongly express Wnt although the same intensity is not observed in the keratinocytes (Figure 2b). Regarding to β-catenin, the small cuboidal cells in the outer layer of the duct-like structures in the nests showed strongly positive both of the cytoplasm and nucleus. The cytoplasm and nucleus of small cuboidal cells distributed in the outer part of the duct-like structures exhibited positive reaction (Figure 2c). Further, the nuclei of the spindle-shaped cells in the mesenchymal component are positive but comparing to the tumor cells in duct-like structures, the nuclei are weakly positive. The neoplastic chondrocytes in hialinized stromal tissue are almost negative.

Many squamous neoplastic cells inside solid nests and plasmacytoid cells on the outside show strong positive reaction. The basaloid cells in the mesenchymal component in particular show strong positive reaction, but the keratinocytes exhibited weaker reaction (Figure 2d). The nuclei of the neoplastic cells with advanced growth showing strong positive reaction. In particular, the nuclei of small cuboidal cells and polygonal cells in the solid nests show positive. The tumor cells of the duct-like structures in solid nests are positive (Figure 2c inset) but the nuclei of the cells showing squamous metaplasia show stronger positively (Figure 2d inset). CK7 react positively in the cytoplasm of cuboidal cells forming duct-like structures in solid tumor nests. A similar immunofluorescent reaction in the cytoplasm of cuboidal tumor cells forming duct-like structures is detected. CK13 is strongly positive in the squamous metaplastic and basaloid cells in the area displaying squamous metaplasia but no reaction is detected in other sites. According to the data [4], most neoplastic cells forming solid nests react positive to Wnt. The expression is localized in the cytoplasm of small cuboidal cells in tumor nests and strong positive reaction is detected especially on cell membrane.

Regarding to Wnt and β-catenin, Wnt shows strongly positive to react mainly in small cuboidal cells in the nests. β-catenin is also positive in almost all the same regions. Further, nuclear translocation of β-catenin is detected in cuboidal cells forming

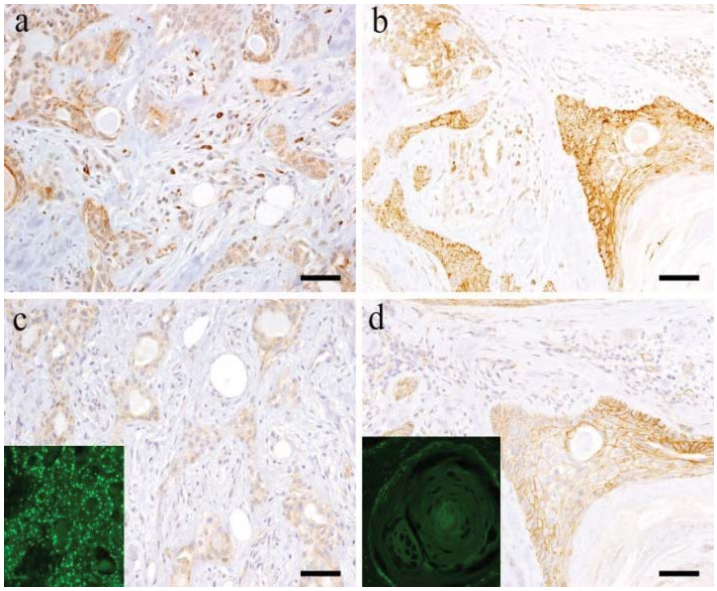

Figure 2: Immunoshitochemical features of Wnt signaling. a: Wnt1 of a 62-year-old, female, upper lip case (scale bar=50μm); b: Wnt1 of a 40-year-old, male, palate case (scale bar=50μm); c: β-catenin of a 62-year-old, female, upper lip case (scale bar=50μm), Inset immunofluorescence image is half magnification of the background image; d: β-catenin in the squamous metaplasia area of a 40-year-old, male, palate case (scale bar=50μm). Inset immunofluorescence image is half magnification of the background image. Quotation alteration of literature #5.

duct-like structures in particular. But, the positive reaction become weak in areas of squamous metaplastic area in the tumor cell nests. Localization in the nucleus of basaloid cells located at the margin of the nests is also strong. However, nuclear translocation was not seen in squamous cells. In the literature of Okuda, et al. [5], double staining and merging of Wnt and CK7, Wnt shows strong positive reaction in the area with small cuboidal cells within the solid nests. On the other hand, in the region with polygonal and spindle cells constituting the myxomatous parts, a positive reaction is observed in some cells but compared to the site with solid tumor cells. Wnt staining is stronger in the cytoplasm and nucleus of spindle-shaped cells. In the cells forming duct-like structures, Wnt and CK7 are both observed simultaneously. The maergination of the specimens is very clear. In the double staining and merging of Wnt and CK13, Wnt is localized in the cytoplasm in a wide variety of cells forming tumor cells. Wnt shows strongly positive in tumor nests. A comparative strong positive reaction is also observed in polygonal cells and in small cuboidal cells forming solid nests. Furthermore, cells forming duct-like structures in solid nests are also strongly positive (Figure 4a). However, CK13 is positive mainly in the cytoplasm of squamous epithelioid cells found in squamous metaplasia. Therefore, in Okuda's experiment [5], Wnt signaling localization as well as various factors involved in cell differentiation in pleomorphic adenomas are examined.

Regarding the 'mixed appearances' in pleomorphic adenomas, the formation of mesenchymal-like component, BMPs in the formation of cartilaginous or distinct bone tissues have been implicated. In hyaline cartilaginous tissue of major salivary gland tumors, BMPs related to cartilage formation like BMP-2, BMP-4, BMP-6, FGF-2 and so on are well known to be strongly expressed. From these phenomenons, the involvement of neoplastic myoepithelial cells in the formation of cartilaginous tissue has been suggested [28]. The type of collagen in cartilage tissue as well as the differentiation of cells in the cartilaginous tissue in pleomorphic adenoma has been found to be almost similar to the normal hyaline cartilage [29].

Moreover, analysis of the cytokeratin focused on the biphasic cell differentiation of myoepithelial cell in pleomorphic adenoma has been conducted hence CK7 and CK13 as using cell differentiation markers for squamous cells were selected this time [30,31]. In these researches, especially in the Okuda, et al. [4] the small cuboidal cells in solid tumor cell nests showed strong positive reaction to Wnt as well as to β-catenin with nuclear reaction recognized in the latter protein. This suggests that Wnt act through the β-catenin pathway. In the study of neural stem cells, Wnt may require the activation of Notch signaling known to suppress cell differentiation. β-catenin binds to Hes1 gene in the promoter region due to the inhibitory effect of the cell [32]. CK7-positive cells formed ductal structures. Wnt showed a strong positive reaction in the area where small cuboidal cells accumulated to form solid tumor cell nests. The site strongly both positive to Wnt and CK7 is consistent with the CK7 alone wherein numerous duct-like structures were formed [33]. In the cells that form duct-like structures, strong expression of both Wnt and CK7 was confirmed. At these sites of β-catenin localization, nuclear migration was observed strongly in small cuboidal cells distributed outside the duct-like structures. In spindle-shaped cells at the periphery of duct-like structures, Wnt had similar to β-catenin. Thus, in neoplastic cells that form duct-like especially the small cuboidal cells, Wnt signaling acts though β-catenin pathway while in the spindle-shaped cells arranged in cord-like

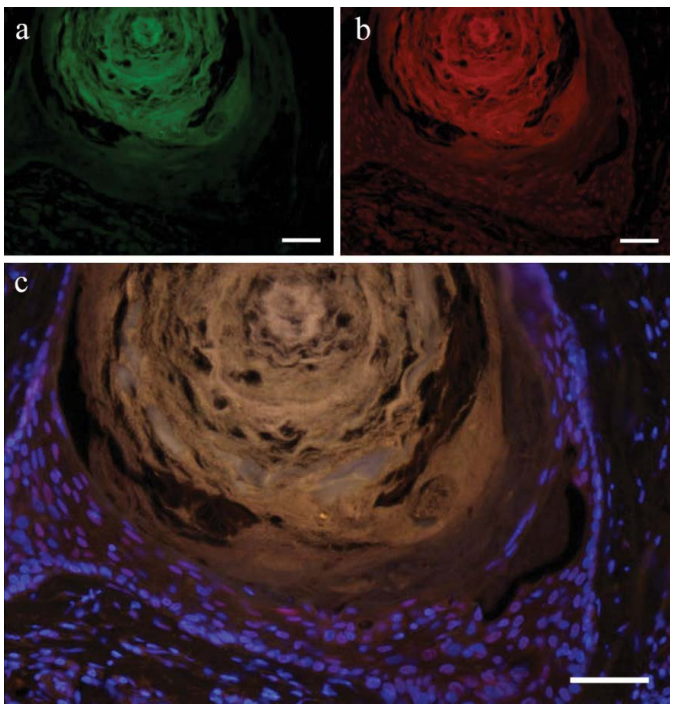

Figure 3: Immunofluorescent staining images of suquamous metaplasia area with keratin pearl (a: CK13; b: Notch1; c: marged image of CK13 and Notch1 and DAPI (a 69 year-old, female, right buccal mucosa case; scale bar=100μm). Quotation alteration of literature #35.

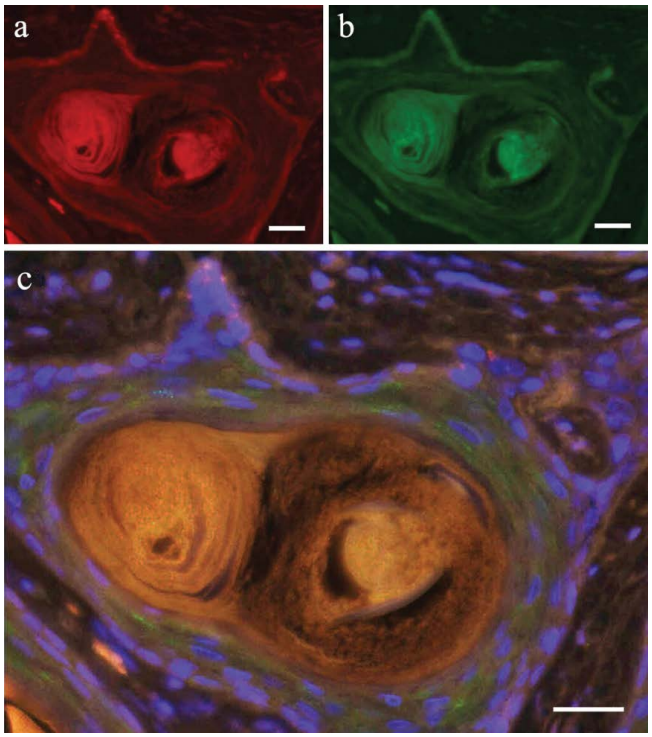

Figure 4: Immunofluorescent staining images of suquamous metaplasia area of a. a: Wnt1; b: HSP27; c: Wnt1 + HSP27 + DAPI (a 69 year-old, female, right buccal mucosa case; scale bar=25 μm). Quotation alteration of literature # 6.

structures, the expression of Wnt and β-catenin was not nearly recognized. Wnt expression in small cuboidal cells forming duct-like structures in solid nests was considered to be associated with cell differentiation.

In the area staining with CK13, the squamous metaplastic cells indicating the site of squamous metaplasia shows Wnt-positive. Furthermore, Wnt exhibited strongly positive in basaloid cells at the margin of tumor nests in particular. Although, positive reaction in cells with advanced differentiation shows weak. In similar, β-catenin shows positively in squamous metaplasia. The strongly staining around cell membrane is recognized in many cells but no cytoplasmic and nuclear translocation is detected. This trend is seen in basaloid cells especially around the tumor nest only in a small portion but there is also a site of nuclear translocation. It is suggested that Wnt act through β-catenin pathway in squamous epithelioid cells that form tumor nests and partly in basaloid cells in surrounding nests [34]. Although Wnt expressed in many cells, nuclear translocation of β-catenin did not occur suggesting that a different pathway for Wnt signaling might have occurred. Moreover, in myxomatous and cartilaginous cells, Wnt and β-catenin expressions are weak and the expression is considered for the performance of Wnt function.

Notch Signaling in Pleomorphic Adenomas

Okuda, et al. performed immunohistochemistry on pleomorphic adenoma to determine the role of Wnt in cell differentiation [5]. The above mentioned results suggest that Wnt involved in cell differentiation in the neoplasms. In the related examinations, according to our data [35], it is consider Notch might also be involved in cell proliferation and differentiation in the same manner. Therefore in this section, it is focused on Notch action (localization) in pleomorphic adenomas since it has been hypothesized to be strongly associated with neoplastic cell differentiation [31]. Furthermore, studies on the expression of Notch-signaling have been tremendously increasing.

According to the literature [35], Notch expression is seen in the cytoplasm of ductal epithelial cells as well as in some of the nuclei. Notch is also observed in the nucleus of tumor cells surrounding solid tumor nests. Notch express in the cytoplasm of plasmacytoid myoepithelial cells but not in the nucleus. In areas undergoing squamous metaplasia, Notch signaling is strongly expressed in the cytoplasm and nucleus of basal cells. However, the expression became weak towards the outer squamous cells. Ductal epithelial cells expressed CK7. Notch is also observed in the nucleus of some ductal epithelial cells. Double immunofluorescent staining revealed that Notch and CK7 co-expressed by tumor cells in ductal structures. Notch is appeared in most of the nucleus of tumor cells in solid nests as well as those in ductal structures. CK13 expression in neoplastic cells undergoing squamous metaplasia is also seen. CK13 showed positive reaction on prickle cell layer (Figure 3a). On the other hand, Notch is seen in the nucleus from basal cell to squamous cells (Figure 3b). Double immunofluorescent staining of Notch and CK13 revealed that most basal cells expressed Notch although expression decreased towards the squamous cells. Furthermore, nuclear expression was observed in basal cells and the expression became weak on the prickle cell layer (Figure 3c). Pleomorphic adenoma is the most common benign epithelial salivary glands neoplasm based on WHO classification [1]. The tumor shows a wide variety of tumor development brought about by cell proliferation and

differentiation as mentioned in previous studies [36-38].

Regarding to our examination data of the expression of Wnt in 30 cases of pleomorphic adenomas, the results showed that neoplastic cells in ductal structure and in solid tumor nests expressed Wnt. It was inferred that β-catenin pathway was involved in cell differentiation indicated by Wnt in cells undergoing squamous metaplasia [5]. For this reason, it is believed that Notch would have an important role in tumor cell differentiation considering its association with other tumors [39,40]. Notch appears as a typical signal that controls the growth of tissues responsible for the fate of cells [41]. Notch is a single-pass transmembrane receptor with a domain outside and inside the cell membrane. Notch intracellular domain (NICD) is cleaved and binds to a ligand such as Jagged. Furthermore, to the role of transmitting instructions for various morphogenesis and tissue differentiation during development, Notch plays an important role in cell-to-cell signaling and is involved in stem cell maintenance, differentiation and neuronal function in adult.

When there is a disturbance in the activity of Notch signaling pathway, it becomes oncogenic detected in several cancers such as esophageal cancer, breast cancer and lymphoblastic acute leukemia (T-ALL) [42,43]. Furthermore, Notch has been the focus of researches on the metastasis of malignant tumors such as adenoid cystic carcinoma and malignant ameloblastoma [44,45], believed to be a huge factor in the progress of malignant neoplasms. In contrast, during binary cell fate determination, Numb acts an inhibitor of Notch signaling cycle causing asymmetric distribution of the Notch pathway. This led to overexpression suggesting that Notch-Jagged1 is the primary mechanism in determining the fate of ghost cell which is one of special keratinization [46].

Squamous metaplasia depicted as having a variety of cytological differentiation is commonly seen in odontogenic tumors such as ameloblastomas. Squamous metaplasia can also be observed in pleomorphic adenomas. Muraki, et al. [47] suggested a close relationship between Notch and ameloblastoma shown by the localization of Notch in peripheral tumor nests. From this aspect, we suspected that Notch might also be involved in cell differentiation in pleomorphic adenomas.

To compared the results of our two examinatuons with previous reports, from the partial CK7 expression and substantial Notch expression in ductal epithelial cells as well as the Notch expression in solid tumor nests, it can be inferred that Notch is involved in cell differentiation [5,47]. CK13 expression was seen in cells undergoing squamous metaplasia and Notch expression was also seen in the nucleus of basal and squamous cells [48]. The intense Notch in basal cells and weakly expression in squamous cells suggests that Notch is involved in the differentiation from basal to squamous cell. Moreover, the loss of nuclear expression on the surface layer would signify that differentiation is about to end or has been terminated. Notch involvement is suspected in cell differentiation in areas showing ductal structures and squamous metaplasia. In the study by Okuda et al, small cuboidal cells forming ductal structure expressed Wnt and the expression was also confirmed in the basal cells surrounding those cells undergoing squamous metaplasia. The results coincided with our present study implicating the function of Notch in the same sites causing cell differentiation [5,6].

HSP27 as a Molecular Chaperone in Pleomorphic Adenomas

HSPs are one of major protein group expressed in various organs and tissues in response to cytotoxic stimuli and mechanical stress. HSPs are not only related to heat shock but are also stimulated by other various pathological conditions like radiation, enzyme, heavy metals, arsenic, ethanol and stress caused by active enzymes and amino acid derivatives [49].

Furthermore, HSP27 belongs to the small molecular family of heat shock proteins. Although it is generally present in the cytoplasm, the expression shifts to the nucleus in response to some stress [8]. Especially, HSP27 has been involved in various cellular differentiation [50]. Many kinds of HSPs expressed in response to cellular stress. HSPs have been associated with suppression of protein breakdown and are known as action in the repair of degraded protein. The low molecular weight HSPs have been regarded as molecular chaperones associated in cell growth and differentiation [20, 51-54]. Fujita, et al. mentioned that HSP27 is highly involved in cell differentiation in ameloblastoma [17].

According to the above section, Wnt express in many neoplastic lesions, strongly positive expression was primarily seen in solid tumor nests. Moreover, increased expression is observed in areas were cells differentiated into squamous epithelial-like cells. Wnt localize in small cuboidal cells in the outer lumen forming duct-like structures. The expression decrease in polygonal cells. Wnt1 is strongly expression in basaloid cells showing squamous metaplasia however the expression decreased in squamous-like cells (Figure 4a). Regarding to the HSP27, it is expressed in many tumor cells. Tumor cells that form duct-like structures express intense HSP27 in the cytoplasm of tumor cells outside the lumen. Positive nuclear reactions were also noted. Positive reaction is seen in the cytoplasm of many squamous-like tumor cells inside the solid nests. These neoplastic cells also show partially positive reaction in the nucleus. Particularly, intense expression was observed in basaloid cells during their transition into keratinocytes (Figure 4b). The expressions of Wnt and HSP27 tend to be similar and this was evident in the superimposition of the localization of Wnt and HSP27 as shown by double immunofluorescence staining (Figure 4c). As described above, Wnt is a typical signal that controls the differentiation of cell and tissue growth. In addition, to homeostasis and cell proliferation, researches are on its way to determine its association in suppressing tissue differentiation in tumors [12].

Our results showed that Wnt involved in cell differentiation in pleomorphic adenoma. Furthermore, HSP27 is also localized in the same sites with Wnt1. Fujita, et al. [17] previously mentioned that HSP27 express during the differentiation of cells into squamous metaplasia and said to be deeply involve in cell differentiation. Accordingly, the same expression in squamous metaplasia was also seen in pleomorphic adenomas. Since this is consistent with Wnt expression, HSP27 is presumed to have worked as a molecular chaperone of Wnt. Furthermore, since Wnt and HSP27 were both expressed by cells forming duct-like structures and those that underwent squamous metaplasia, it is possible that HSP27 worked as a molecular chaperone of Wnt.

Conclusion

In pleomorphic adenomas, there are known as typically

characteristics by variety characteristic histopathological features. These features are caused by the cell signaling such as Wnt and Notch. Wnt signaling is involved in cytological differentiation of neoplastic cells that form solid tumor cell nests and cuboidal cells that form duct-like structures, and particularly the basaloid cells into squamous metaplasia. In the 'mixed' characteristic of pleomorphic adenoma, although Wnt through β-catenin pathway is greatly involved in cell differentiation, another pathway is considered responsible for the change in cell morphology. Notch signaling also acts as cell differentiation of ductal cells in pleomorphic adenomas. Nuclear expression was shown in tumor cells in solid nests and surrounding structures. Moreover, Notch expresse by basal cells undergoing squamous metaplasia suggesting the participation of Notch in cell differentiation. Regarding the cell differentiation due to Wnt signaling, HSP27 works as molecular chaperone during cell differentiation. The literatures' data suggest that Wnt1 and HSP27 are involved in cell differentiation in squamous metaplasia and duct-like structures. Thus, HSP27 may have function as a molecular chaperone of Wnt1, furthermore may be Notch in pleomorphic adenomas.

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Conflicts of interest

The authors have declared that no competing interest exists.

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