RESEARCH

TENASCIN EXPRESSION PATTERNS OF SALIVARY GLAND TUMORS: AN IMMUNOHISTOCHEMICAL AND COMPARATIVE STUDY

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SUMMARY

Purpose: Tenascin is a multifunctional large glycoprotein component of extracellular matrix. Salivary gland neoplasms present a most diverse group of tumors some of which are rich in extracellular matrix. The study was set out to evaluate the expression of tenascin in a series of salivary gland neoplasms. Materials and Methods: 32 salivary gland tumors including; 18 pleomorphic adenomas, 8 Warthin’s tumors, 2 basal cell adenoma, 1 acinic cell carcinoma, 2 adenoid cystic carcinoma, 1 malignant pleomorphic adenoma were included in this study. All of the cases were stained with tenascin with immunohistochemical method. The staining intensities and localizations were evaluated. Results: In pleomorphic adenomas tenascin expression was intensive in tumoral stromal component especially around chondroid islands. In Warthin’s tumor staining was usually observed beneath epithelial regions and in the epithelial component. Conclusions: The main source of tenascin in salivary gland tumors is tumor’s stromal component or stroma. The major role of tenascin in pleomorphic adenoma may be proliferation and chondroid differentiation of stromal component. In Warthin’s tumor, tenascin observed under the epithelial component may be responsible of epithelial and mesenchymal interaction and high ratio of intracytoplasmic epithelial tenasin expression. This could be related with the cytokine secretion from the lymphoid stroma.

Keywords: Salivary gland secretion, tenasin, immunohistochemistry, pleomorphic adenoma, Warthin’s tumor

TÜKRÜK BEZI TÜMÖRLERİNDE TENASKIN EKSPRESYONU: İMMÜNOHİSTOKİMYASAL VE KARŞILAŞTIRMALI BİR ÇALIŞMA

ÖZET

examined including 18 cases of pleomorphic adenoma, 8 cases of Warthin’s tumor, 2 cases of basal cell adenoma, 1 case of acinic cell carcinoma, 2 cases of adenoid cystic carcinoma, 1 cases of malignant pleomorphic adenoma.

One representative tissue block was selected from each tumor for immunohistochemical evaluation. 4µm sections were cut from each paraffin-embedded tissue block. The sections were deparaffinized washed with tap water and boiled in citrate buffer saline for 20 min. for antigen retrieval. This procedure was followed by application of the primary antibody to tenascin (TN2 DAKO Carpenteria CA) in 1/50 dilution. Antibody detection was performed by adding biotin layered secondary antibodies, avidin-biotin complex and 3, 3’-diaminobenzidine. Negative controls were run in non immune serum and positive controls with appropriate tissue.

Immunoreactivity was evaluated in reference to the expression site, and intensity of tenascin. The evaluation according to the immunoreactivity localization showed some differences in different types of tumors. Epithelial, myoepithelial, stromal and intraluminal immunoreactivity were evaluated in pleomorphic adenomas Warthin’s tumors, adenoid cystic carcinomas, and malignant pleomorphic adenomas. The outer layer of the tumoral islands was accepted as myoepithelial, gland forming inner component accepted as epithelial. In basal cell adenomas epithelial and myoepithelial component identification was not possible. Only epithelial component was observed and evaluated in acinic cell carcinomas. The staining intensity was graded as; (+): focal and faint staining, (+++): immunoreactivity present in more than 75% of the slide, and (++) staining between these two intensity.

RESULTS

Localizations and mean diameters of different salivary gland tumor are summarized in Table 1. Percentages associated with tenascin expression intensity and localization was summarized in Table 2.

Pleomorphic adenomas were from 8 females and 10 males, the age range was 14 to 68 years. Tumor diameters were ranged between 1.8 and 5.0 cm. All tumors except the one from submandibular gland were originated from the parotid gland. All Warthin’s tumors were in males with an age range between 38 and 72. In one case there were two tumors in the same gland, and in another there were separate tumors in each parotid gland. Adenoid cystic carcinomas originated from hard palate and from the base of tongue. Acinic cell carcinoma originated from the submandibular gland of a 21 year old woman. The malignant pleomorphic adenoma tumor was of a 62 year old male, who had tumor recurrence.

Figure 1. Strong and diffuse stromal immunoreactivity in pleomorphic adenoma. (immunohistochemistry tenascin X40)

Figure 2. Glandular intracytoplasmic tenascin immunoreactivity in the form of clusters in pleomorphic adenoma. (immunohistochemistry tenascin X400)

Figure 3. Diffuse immunoreactivity in condroid matrix and intensive immunoreactivity in condrocyte cytoplasms in pleomorphic adenoma. (immunohistochemistry tenascin X200)
Figure 4. Intensive tenascin immunoreactivity around condroid islands in pleomorphic adenoma. (immunohistochemistry tenascin X40)

Figure 5. Tenascin immunoreactivity around myoepithelial cells made a pattern like chicken wire in pleomorphic adenoma. (immunohistochemistry tenascin X400)

Figure 6. Tenascin immunoreactivity in the forms of intraepithelial globules, and diffuse myoepithelial staining in Warthin’s tumour. (immunohistochemistry tenascin X200)

Figure 7. Weak stromal and intraluminal tenascin immunoreactivity in basal cell adenoma. (immunohistochemistry tenascin X100)

Figure 8. Intensive and diffuse intraluminal tenascin expression in adenoid cystic carcinoma. (immunohistochemistry tenascin X400)

Table 1. Percentages of anatomical Localization and tumor diameters of Pleomorphic adenoma, Warthin’s tumor, Basal cell adenoma, Acinic cell carcinoma, Adenoid cystic carcinoma and Malignant mixed tumor

<table>
<thead>
<tr>
<th>LOCALISATION</th>
<th>No</th>
<th>Parotid (%)</th>
<th>Non-Parotid (%)</th>
<th>DIA. (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>● Pleo.</td>
<td>18</td>
<td>17 (94.4)</td>
<td>1 (5.6)</td>
<td>2.54</td>
</tr>
<tr>
<td>▲ Wt</td>
<td>8</td>
<td>8 (100)</td>
<td>0 (0)</td>
<td>2.55</td>
</tr>
<tr>
<td>▼ BCA</td>
<td>2</td>
<td>2 (100)</td>
<td>0 (0)</td>
<td>3</td>
</tr>
<tr>
<td>▲ AsCC</td>
<td>1</td>
<td>0 (0)</td>
<td>1 (100)</td>
<td>3</td>
</tr>
<tr>
<td>▲ ACC</td>
<td>2</td>
<td>0 (0)</td>
<td>2 (100)</td>
<td>2.25</td>
</tr>
<tr>
<td>■ MMT</td>
<td>1</td>
<td>1 (100)</td>
<td>0 (0)</td>
<td>3.5</td>
</tr>
</tbody>
</table>

● Pleomorphic adenoma. ▲ Warthin’s tumor, ▼ Basal cell adenoma, ▲ Asinus cell carcinoma, ▲ Adenoid cystic carcinoma, ■ Malignant mixed tumor, † Tumor diameter
Tenascin expression was observed in all tumors and in nontumoral salivary gland. Tenascin expression was observed in epithelial and stromal interactions of acinar and ductal structures in normal salivary ducts.

Intensity of tenascin expression showed differences in tumor groups. The most intensive expression was observed in pleomorphic adenomas. Vascular structures and tumor capsules were also immunoreactive to tenasin in most of the cases.

Majority of the pleomorphic adenomas had strong tenasin immunostaining. Tumors rich in stromal component had more intensive and diffuse staining and immunoreactivity was more prominent in stroma than the other components (Fig. 1). Intracytoplasmic clustures were often observed in the epithelial component (Fig. 2). Stromal immunoreactivity was stronger in myxoid areas and weaker in hyalinised areas and chondroid matrix was always immunoreactive. Chondrocytes stained strongly and an intensive immunoreactive halo was observed around some of them. A strongly staining region in the periphery of chondroid islands was observed in some of cases (Fig. 3, 4). Tenasin immunoreactivity made a chicken wire like pattern around the myoepithelial cell clustures (Fig. 5).

In Warthin’s tumor tenasin expression was focal and faint. Staining was observed usually in basal membrane. Intracytoplasmic epithelial globules were observed in oxyphilic cells (Fig. 6). Immunoreactivity of myoepithelial layer was also observed in only one case.

In the basal cell adenomas, immunoreactivity was weak and detected only in tumor stroma, but not in tumor cells (Fig. 7). In acinic cell carcinoma, immunoreactivity was weak and limited to the stroma. In adenoid cystic carcinomas, focal and faint epithelial and stromal immunoreactivity and prominent intraluminal expression was observed (Fig. 8). In malignant mixed tumor, focal stromal and chondroid immunoreactivity was observed.

**DISCUSSION**

We analyzed the distribution of tenasin in normal salivary gland, different kinds of benign and malignant salivary gland tumors and found differences in staining intensity and localisation. In normal salivary gland tenasin was expressed in the same intensity and localization of epithelial mesengial interactions of ductal and aciner system. Tenasin expression in pleomorphic adenoma is significantly higher than that of other salivary gland tumors. Intraepithelial tenasin expression observed in pleomorphic adenoma, Warthin’s tumor, and adenoid cystic carcinoma but it was not present in basal cell adenomas and acinic cell carcinomas. We think that, tenasin may play different roles in tumoral growth in different salivary gland tumors.

The effect of tenasin in tumor growth is a well known phenomenon. In tumoral tissues neoplastic cells secret cytokines such as transforming growth factor-beta which stimulates stromal fibroblasts to produce tenasin. Tenasin contains epidermal growth factor like repeats and might induce tumor growth by an autocrine mechanism. It seems likely that there is an interaction between stroma and tumoral cells in tenasin expression. This interaction may have specialties in tumors with stromal or lymphoid component.

Pleomorphic adenomas has a complex histopathological morphology with epithelial component containing luminal tumor cells and outer tumor cells termed as modified myoepithelial cells. Myoepithelial cells are responsible for the production of excessive amounts of basal lamina-associated proteins.
proteins and glycosaminoglycans that can form a variety of stromal components ranging from fibrous, hyaline, myxoid and chondroid. Myoepithelial cells that have capacity to differentiate in various stromal components are the cause of the pleomorphic appearance of this neoplasia.

Strong immunoreactivity of tenascin in stromal component suggests a major role of this protein in the progress of in pleomorphic adenoma. The tenascin secretion from the stromal component modified from myoepithelial cells may cause proliferation of myoepithelial cells and myoepithelial cells produce more stroma. This positive feed-back mechanism may cause excessive tenascin accumulation of pleomorphic adenoma. Intensive immunoreactivity of tenascin in malignant and benign pleomorphic adenomas was also reported previously.

The chondroid changes in pleomorphic adenoma similar to normal hyaline cartilage at the immunohistochemical and ultrastructural level. Tenascin has a major role in chondrogenesis and osteogenesis during embryogenesis and may have a similar function in the stromal component of pleomorphic adenoma. It is present diffusely in embryologic chondroid tissue and observed mainly in the adult pericondrial tissue, which seems to be associated with chondroid change. We observed more intense immunoreactivity in myxoid rims surrounding the chondroid islets and strongly immunoreactive halos around the chondrocytes. This also confirms that tenascin is more intensive during the process of chondroid differentiation and seems to decrease progressively during the maturation process.

We observed a clear difference between the intensity of tenascin immunoreactivity of pleomorphic adenomas and other salivary gland tumors. Except asinus cell carcinoma all the other salivary gland tumors may show S-100 and smooth muscle actin immunoreactivity and known to have myoepithelial differentiation. The stromal component originating from myoepithelial cells is the main difference of pleomorphic adenoma. We also observed less tenascin immunoreactivity in myoepithelial component compared with the stromal component. As a result we strongly suggest that the major source of tenascin in salivary gland tumors were not myoepithelial cells but stromal cells.

On the other hand epithelial tenascin immunoreactivity was observed in Warthin’s tumor, pleomorphic adenoma and faintly in adenoid cystic carcinoma. As it is reported before the tenascin immunoreactivity observed in epithelial and stromal interaction in Warthin’s tumor but intraepithelial immunoreactivity is also reported before. The tumors rich in epithelial component with scanty stroma such as acinic cell carcinoma and basal cell adenoma do not have epithelial tenascin expression. In Warthin’s tumor, tenascin expression beneath the epithelial and stromal component may be responsible for epithelial and mesengial remodeling. The epithelial tenascin secretion may be related with the excessive expression of cytokins in the lymphoid stroma of Warthin’s tumor.

The tenascin expression of salivary gland tumors is dominantly located in the stromal component but may be observed in the intraepithelial region of stromal rich tumors. The amount of the tenascin expression is directly related with the amount of the stromal component and most intensive in the pleomorphic adenoma that has a tumoral stromal component. The major role of the tenascin expression in the pleomorphic adenoma may be the organization of the chondroid matrix. In Warthin’s tumor, the tenascin expression observed beneath epithelial stromal component may be responsible for the epithelial-mesengial organization. Lastly, intraepithelial tenascin expression can be observed in salivary gland tumors with prominent stroma and lymphoid stroma. This may be related with the cytokins secreted from stroma and lymphoid component.

REFERENCES


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