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Research Article

Correlating the Immunohistochemical Expression of β-Catenin, Fascin and Survivin with the Pathogenetic Paradigm Observed in Pleomorphic Adenoma, Muco-Epidermoid Carcinoma, and Adenoid Cystic Carcinoma

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Abstract

Context: β-catenin, fascin and survivin are molecular markers which govern distinct biological processes. However, they have shown substantial overlap in pathogenesis of neoplastic processes. Studying the expression of these markers in benign and malignant salivary gland tumors (SGT) may help in understanding the biologic behavior of these neoplasms.

Aims: To compare the immunohistochemical expression of β-catenin, fascin and Survivin in Pleomorphic adenoma (PA), Mucoepidermoid carcinoma (MEC) and Adenoid cystic carcinoma (AdCC) and correlate it with the pathogenic, histogenic and morphogenic processes in the neoplasms.

Settings and Design: The present study was Cross sectional descriptive study. This study was executed in the Department of Oral Pathology and Microbiology of the Institute.

Materials and Methods: PA (n = 10), MEC(n = 10) and AdCC (n = 10) were subjected for immunohistochemical evaluation of β-catenin, fascin and Survivin. The scoring for immunoreactivity was assesed using Bittinger's method.

Statistical Analysis Used: Statistical analysis was performed using Kramer-Tukey test.

Results: Fascin and survivin expression in MEC was significantly higher as compared to PA. Raised expression of β-catenin, fascin and Survivin was observed in AdCC as compared to PA. A non-significant difference was observed in the immuno-reactivity of β-catenin between PA and MEC; MEC and AdCC and also with respect to survivin and fascin expression in MEC and AdCC.

Conclusion: The expression pattern of these markers in Salivary gland tumors can elude a possible role of interactions among these markers in the salivary gland tumorigenesis.

Keywords: *β-Catenin; Fascin; Survivin, Salivary Gland Tumors; Pleomorphic Adenoma; Mucoepidermoid Carcinoma and Adenoid Cystic Carcinoma*

Abbreviations

SGT: Salivary Gland Tumors; PA: Pleomorphic Adenoma; MEC: Mucoepidermoid Carcinoma; AdCC: Adenoid Cystic Carcinoma

Introduction

The hallmarks of cancer described by Hanahan and Weinber in 2011 have conceptualized and rationalized the complexities of neoplastic diseases. Different biologic processes such as sustained proliferative signalling, evading growth suppressors, resisting apoptosis, allowing replicative immortality, inducing angiogenesis, and activating invasion and metastasis via altered cytoskeletal rearrangement etc. have marked themselves as the perpetrators of carcinogenesis [1].

All these processes are associated with biochemical indicators which are found in abnormal concentrations in presence of the neoplasm. These are the tumor markers that credibly reflect the underlying tumor biology [2]. β-catenin, fascin and survivin are few such biomarkers that mark for cell proliferation, cell motility and cytoskeletal rearrangement and inhibition of apoptosis respectively [3-5].

Wnt/β-catenin signalling regulates transcription and gene expression via Wnt response elements (WRE) that alter the chromatin structure thus contributes to proliferation of tumor propagating cells [6]. Fascin, on the other hand is an actin bundling protein that has an important role in cell migration and alteration of cytoskeletal organization [4]. Survivin is a member of the Inhibitor of apoptosis family that interfaces at not only the blockade of effector caspases but also through loss of wild-type p53 [7].

Salivary gland tumors (SGTs) are reported to represent between 1% and 5% of all head-and-neck tumors with annual incidence reported to be between 0.4 and 13.5 cases/100,000 [8]. The biologic behaviour of salivary gland tumors has been under investigation due to their vast variation in terms of recurrences, malignant progression, metastasis and yet having concurring histogenetic and morphogenetic events during pathogenesis. Ackerman and del Regato succinctly described it as "The usual tumor of salivary glands is a tumor in which the benign variant is less benign than the usual benign tumor and the malignant variant is less malignant than the usual malignant tumors" [9].

Although numerous studies have been conducted in the past to assess the immunoreactivity of SGTs with respect to various biomarkers and linking them to its aggressiveness, the focus was usually on one of the underlying biological mechanisms. Pleomorphic adenoma (PA) and mucoepidermoid carcinoma (MEC) are the most common benign and malignant salivary gland tumors [8]. Also, of interest is the erratic behaviour expressed by these two neoplasms along with adenoid cystic carcinoma. Exceptionally few studies have attempted to correlate multiple sub-structural disease processes. Furthermore, studies have correlated the behaviour of fascin with CTNNB1. It has been implied that activated Wnt-signalling acts as a promoter of the epithelial migration machinery by controlling fascin in tumour cells [10]. Along the same lines, upregulation of survivin by β-catenin has been noted [11]. Thus, studying the three markers together is expected to better reflect the tumor behaviour.

Aim of the Study

The aim of the current study was to comprehend the biologic behaviour of Pleomorphic adenoma, mucoepidermoid carcinoma (MEC) and adenoid cystic carcinoma (AdCC) by assessing their immunohistochemical expression for three biomarkers that each represent a biologic machinery that forms an integral component of the hallmarks of tumorigenesis.

Materials and Methods

This study included 10 cases each (n = 30) of pleomorphic adenoma, mucoepidermoid carcinoma and adenoid cystic carcinoma retrieved from the department archives of the Institution. The study was undertaken after obtaining necessary permissions and clearances.

Immunohistochemistry

Sections of 4 µm of Paraffin-embedded tissues of each case were taken on Sialane coated slides, deparaffinized, and rehydrated through xylene and descending grades of alcohol. Antigen retrieval was carried out in EZ Retriver™ (Biogenex; USA) in EZ-AR II Elegance™ (Biogenex; USA) in three cycles of 5 minutes at 90˚, 10 minutes at 95˚ (2 cycles).

Polymer detection was performed as instructed by the manufacturer. Incubation with primary monoclonal antibodies for anti-β-catenin, anti-fascin and anti-survivin (Biogenex; USA) was done for 60 minutes. Subsequent treatment with secondary antibody was done. Visualization of immunoreactivity was performed using freshly prepared di-amino-benzidine (DAB) chromogen for 10 minutes. The slides were counterstained with Harris hematoxylin stain and mounted. For each batch, positive and negative controls were run to eliminate any bias due to procedural discrepancy.

Each slide was assessed by reviewing 10 fields/slide by three independent observers. Assessment of immunoreactivity was done by multiplying the percentage of stained cells and intensity of staining. The resultant value was considered as the integrated IHC score.

Results obtained were subjected to statistical analysis by Kramer-Tukey test and Kruskal-Wallis test.

Results and Discussion

The mean IHC score, the degree of positivity and the localization of markers - β-catenin, fascin and survivin in PA, MEC and AdCC is tabulated in table 1.

	PA	MEC	AdCC	"q" value for Kramer -Tukey test		
β-catenin				**PA - MEC**	**PA-AdCC**	**MEC-AdCC**
Mean IHC score	3.1	4	4.5	3.499#	5.442*	1.944#
Standard Deviation	0.5676	1.4714	0.8498			
Localization	Membranous and cytoplasmic	Cytoplasmic	Cytoplasmic and occasional nuclear			
Fascin						
Mean IHC score	2.3	4.3	4.7	7.775*	9.330*	1.555 #
Standard Deviation	1.494	0.8233	0.4830			
Localization	Cytoplasmic	Cytoplasmic	Cytoplasmic			
Survivin						
Mean IHC score	2.7	3.9	4.9	4.665*	8.552*	3.887#
Standard Deviation	1.059	0.5678	0.3162			
Localization	Cytoplasmic and nuclear	Cytoplasmic and nuclear	Nuclear			

Table 1: *Results discussing the Integrated Immunohistochemical scores and localization of β-catenin, fascin and survivin in Pleomorphic adenoma, Muco-epidermoid carcinoma and Adenoid cystic carcinoma. The test of significance for difference between each group. If the value of q is greater than 4.521 then the P value is less than 0.05 i.e. statistically significant (*) less than 4.521 is non-significant (#).*

In PA, positive staining for all three markers is exhibited by the proliferating neoplastic luminal and myoepithelial cells. The immunoreactivity was less in low-grade MECs as compared to high grade cases as the positivity for all the markers was expressed only by the myoepithelial or epidermoid cells. In AdCC cases, the luminal and basal cells showed strong positivity for β-catenin (4.5 ± 0.8498), fascin (4.7 ± 0.4830) and survivin (4.9 ± 0.3162). This group exhibited the least variation among the scoring in all three markers.

A shift in the localization was also observed with β-catenin and survivin among benign and malignant SGTs. The expression of β-catenin in the control (normal mucosal epithelium) was localized exclusively to the cell membranes. However, among SGTs, PA showed membranous as well as cytoplasmic expression and the malignant SGTs i.e. the MEC and AdCC presented cytoplasmic localization with immensely diminished membranous localization. Survivin expression in PA was cytoplasmic with occasional nuclear positivity which increased in MEC whereas in AdCC extensive nuclear expression was noted.

Salivary gland tumors represent the most heterogeneous histologic group of human tumors [12]. Among the SGTs, pleomorphic adenoma is the most frequently encountered tumor and mucoepidermoid carcinoma and adenoid cystic carcinoma are the most common malignant tumors. Together the SGTs represent 8% of all head and neck cancers [13]. These tumors not only have perplexing behaviour but also lead to unpredictable outcomes.

The Wnt/β-catenin signalling pathway participates in regulation of cell adhesion, proliferation, differentiation, and epithelial-mesenchymal transition [14]. Its proto-oncogenic effects on salivary gland tumors were first documented in 1988 by Tsukamoto., *et al* [15]. Various studies have proven overexpression of β-catenin to trigger proliferation and dedifferentiation of salivary gland epithelial cells, causing benign and malignant SGTs [13]. Studies targeting this gene have been conducted to identify and elaborate a probable role of cell adhesion, cytodifferentiation and sustained proliferation in oncogenesis of PA, MEC and AdCC. Among these, Orford K., *et al*. have shown that β-catenin directly interacts with members of the Tcf/Lef family of architectural transcription factors involved in the activation of specific target genes that regulates contact inhibition, anchorage-independent growth, anoikis and radiation-induced cell cycle arrest [16].

In the current study, membranous expression of β-catenin was evident in PA that was gradually lost in MEC and AdCC while cytoplasmic expression increased. along with an increased cytoplasmic immunoreactivity. The membranous loss of the E-cadherin-catenin complex and nuclear translocation of β-catenin were said to be the early events of gastric carcinogenesis in adenocarcinomas of stomach by Kim HS., *et al* [17]. Findings like ours were reported by Prado., *et al*. wherein they observed membranous and cytoplasmic immunostaining of β-catenin in benign and malignant SGTs. They further concluded that the loss of β-catenin adhesion molecule may be one of the pathogenetic mechanisms in pleomorphic adenoma, and that the cytoplasmic accumulation of the molecule may facilitate the malignant transformation of the pleomorphic adenoma [18].

MC da Costa Miguel., *et al*. observed that the reduced expression β-catenin observed in all high-grade MECs was probably due to the loss of adhesion function of the neoplastic cells, which may impart invasive potential to these tumors. Also, the redistribution of the β-catenin resulting in predominance of cytoplasmic expression has been associated with its transactivation potential mediated by the Wnt signalling pathway [19,20]. Nuclear/cytoplasmic accumulation of β-catenin was correlated with the adverse outcome of patients by Shiratsuchi H [21].

We observed, a similar aberrant expression pattern of β-catenin in AdCC cases where the membranous localization was altered to cytoplasmic and nuclear staining. This finding was analogous to that observed by Zhou CX and Gao Y and Ferrazzo KL., *et al* [22,23]. They also correlated reduced membranous expression of β-catenin with tumor metastasis [22].

Recurrence of tumors due to increased cell motility resulting in their spread away from the primary tumor is a major problem in cancer treatment. This is especially true in case of pleomorphic adenomas. Fascin is one such actin-bundling protein that has emerging roles in diverse forms of cell protrusions resulting in reorganization of cytoskeletal filaments that facilitates the epithelial mesenchymal transi-

tion resulting in exacerbated cell motility [24]. We observed moderate immunopositivity for fascin by Pleomorphic adenoma which was less as compared to MEC and AdCC. Fascin was studied by Brieger J., *et al*. as a marker for the likelihood of recurrence in pleomorphic adenoma of the parotid gland [25]. Rahrotaban S, Azmoudeh F and Kiyani SM had contrasting observations where they found no significant difference between fascin expression in PA and MEC cases [26]. Some authors have stated that this could be because of fascin's role in the formation of cellular dendrite and pseudopodia that develop beyond the tumor's capsule and help the recurrence of the tumor [27-29]. Ebrahim AE, Radi NA, Abo Hager EA evaluated fascin expression among PA, MEC and AdCC and had similar observations pertaining to its immunoreactivity which was significantly higher in malignant SGTs as compared to the PA [30].

The third biologic process studied in this paper is the evasion of apoptotic regulation. Cell cycle progression and apoptotic regulation are thought to be inter-dependent processes that are crucial for homeostasis and developmental morphogenesis [31,32]. Survivin is an Inhibitor of apoptosis family member expressed in the G2/M phase of the cell cycle in a cycle-regulated manner. Disruption of survivin–microtubule interactions results in loss of its anti-apoptosis function and increasescaspase-3 activity, which is an effector caspase, leading to apoptosis. Overexpression of survivin in cancers may overcome this checkpoint and support aberrant progression of transformed cells [33].

Positive immunohistochemical expression of survivin was observed in all three SGTs. The expression was highest for AdCC followed by MEC and even lesser in PA. Nikitakis., *et al*. also observed immunopositivity for survivin in all benign and malignant salivary gland carcinomas, however they did not observe any significant difference among benign and malignant tumors [34]. Jaemyoung Cho., *et al*. studied survivin expression in various salivary gland tumors such and reported findings similar to ours. They also studied its expression in other benign and malignant SGTs such as oncocytoma, adenocarcinomas, acinic cell carcinomas etc. and found significant immunopositivity in them [35].

Also noteworthy is the observation that, there was no significant difference among the expressivity of markers within each tumor. Also, there was no significant difference in the immunopositivity of all three markers among MEC and AdCC, signifying that the mechanisms underlying any malignant disease process are analogous.

Conclusion

Considering the paucity of literature regarding the role of fascin and survivin in pathogenesis and aggressiveness of benign and malignant SGTs, this study was an attempt to delve deeper into these under-explored pathologic processes. We observed a significantly higher expression of all three markers in malignant SGTs (MEC and AdCC) as compared to PA. While, β-catenin may be linked with cellular proliferation, loss of adhesion and lack of differentiation, fascin is regulating the cellular migrations that may increase the possible recurrences and survivin by inhibiting apoptosis is imparting cellular immortality.

Thus, the aggressive and rather elusive behaviour of the SGTs may be partway attributed to these mechanisms. This study had certain limitations in terms of a small sample size and being unicentric. Further studies along the same lines with a broader sampling will help in substantiating the roles of these biomolecules in the pathogenesis of salivary gland neoplasia.

Conflict of Interest

Nil.

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