Immunohistochemical expression of Basic fibroblast growth factor-2 and Heparanase in oral squamous cell carcinoma

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ABSTRACT

Back ground: The aim of this study was to evaluate the expression of fibroblast growth factor-2 and Heparanase in oral squamous cell carcinoma, and to correlate the two studied marker with each other and with clinicopathologicalfinding including grade, stage.

Methods: Sections of 30 formalin-fixed paraffin embedded blocks specimens of oral squamous cell carcinoma were immunostained to assess the expression of fibroblast growth factor-2 and Heparanse in oral squamous cell carcinoma cases.

Results: The expression of fibroblast growth factor-2 and Heparanase were positive in all oral squamous cell carcinoma cases (100%). The positive expression of fibroblast growth factor-2 was significantly correlated with tumor site (p=0.016),and clinical presentation(p-value =0.003).The positive expression of Heparanse was significantly correlated with tumor grade(p-value =0.002) .On other hand there was non-significant correlation between fibroblast growth factor-2 ,Heparanase and other clinicopathological parameters .Statistically significant correlation was found between the expressions of fibroblast growth factor-2 and Heparanase(p-value= 0.021).

Conclusion: The fibroblast growth factor-2 and Heparanase positive expression was noted in all cases of oral squamous cell carcinomasignifying their important role in the angiogenesis and lymph node metastasis in oral squamous cell carcinoma, furthermore they cooperate in promoting vascularization, suggesting that fibroblast growth factor-2 and heparanase are promising targets for the development of anticancer therapeutics for head and neck malignancies.

Key words: Oral squamous cell carcinoma, FGF-2, Heparanase. (J Bagh Coll Dentistry 2013; 25(1):94-98).

INTRODUCTION

Oral cancer is a major public health issue worldwide; it remains a highly lethal and disfiguring disease. It makes the whole dental team with important obligations, challenges, and a real opportunity to save lives [(1)]. Oral Squamous cell carcinoma account for about 95% of all malignant neoplasm's in the mouth [(2)]. It remains a lethal disease in over 50% of the cases diagnosed annually, due mostly to late detection of advanced stage cancer. OSCC characterized by a high degree of local invasiveness and a high rate of metastasis to cervical lymph nodes, but a low rate of metastasis to distant organs. Death as a result of cancer is often the result of local recurrence or regional and/or systemic metastasis [(3)]. The expansion or extension of existing vasculature, is necessary to deliver oxygen and nutrients to ischemic area in the wounds and solid tumors [(4)] . Angiogenesis is a crucial step in the successful growth, invasion and metastasis of tumors, without which tumors will not grow more than 1-2 mm^3 in size [(5,6)].

Tumor angiogenesis' plays an important role in the growth, invasion and metastasis of (OSCC) [(7,8)]. It's regulated by numerous pro angiogenetic factors and antiangiogenetic factors by interstitial cell and tumor cell itself [(9)]. Fibroblast growth factor-2(FGF-2) is a powerful pro angiogenetic factor [(10)]. It's the prototypic member of a family containing at least 23 structurally-related polypeptide growth factors. The expression of FGF-2 augmented at sites of chronic inflammation, after tissue injury, and in different types of human cancer [(11)]. FGF2 over expression plays a key role in the progression of OSCC, correlated with lymph node metastasis [(12)]. The activity of FGF-2 is mediated by binding to heparan sulfate proteoglycans (HSPG) and to high affinity, cell surface receptor tyrosine kinases.The role of HSPG in modulating FGF-2 activity has been described at many levels. The generation of stable, high affinity FGF-2/FGFR complexes is probably the major mechanism leading to HSPG-dependent FGF-2 activity. In addition, FGF-2 has been localized to the extracellular matrix (ECM) associated with HSPG [(6,7)]. Heparanase is an end glycosidase which cleaves heparan sulfate (HS) and hence participates in degradation and remodeling of the (ECM). Heparanase is preferentially expressed in human tumors and its over-expression in tumor cells confers an invasive phenotype in experimental animals. This enzyme also releases

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angiogenic factors from the ECM and thereby induces an angiogenic response in vivo.Many evidences suggest that the expression of heparanase in the tumor closely relates with thepotential for tumor invasion, angiogenesis and metastasisin most tumors examined [13].

MATERIALS AND METHODS

The Sample:

TheSample of this study included thirty formalin-fixed, paraffin-embedded tissue blocks, which have been diagnosed as OSCC, dated from (2000 till 2012). The study samples were obtained from Al-Shaheed Ghazi Hospital/ Medical City /Baghdad; the archives of the department of Oral and Maxillofacial Pathology/ College of Dentistry/ University of Baghdad; and private laboratories in Baghdad.Demographic and Clinicopathological datain regard topatient's age, sex, clinical presentation, site of the tumor, grading and stagingobtained from the case sheets.AllHematoxylin and Eosin stained tissue sections were reviewed by two specialized pathologists,andthe best sections and those representing the original tumor site from each specimen were selected. Another 4µm thick sections for each case were cut and mounted on positively charged slides for immunohistochemical staining with monoclonal antibodiesFibroblast growth factor-2(US. Biological)andHeparanase(US.Biological).Positi ve and negative tissue controls were obtained according antibodies manufacturer's datasheet and added to each test run.

Evaluation of Immunohistochemistry Results:

Immunohistochemical signal specificity was demonstrated by the absence of immunostaining in the negative control slides and its presence in recommended positive controls.For FGF-2tumor cells with clear brown cytoplasmic staining pattern wereconsideredpositive, and membranous or membranous and cytoplasmic immunoreactivities were considered positive for Heparanase. Immunohistochemical stained OSCC sections were studied by light microscope under 10Xobjective. In each tissue section, five representative fields (areas showed well preserved OSCC islands in which the reaction was clearly positive) were selected for FGF-2 and Heparanse monoclonal antibodies immunostaining evaluation, with an average of 1000 tumor cell per case and 200 tumor cells per field. Only the number of cells that were positive for FGF-2 and positive for Heparanse was quantified by counting at least one thousand cells in representative five fields at 40X objective in each case. The extent of staining was scored using the following scale: 0 = no staining (negative), 1 =staining of 1–25% of cells (weak positive), 2 = staining of 26–75% of tumor cells(moderate positive), 3 =staining of 76–100% of tumor cells(strong positive)[14].

Statistical Analysis:

The studied parameters were scored and considered as categorical data thus theypresented as count and percentage. The relationship between categories was tested byChi-square test. Spearman's rho correlation was applied to assess the linear association between FGF-2 and Heparanse. The level of significance was 0.05 (two-sided) in allstatistical testing.

RESULTS

Immunohistochemical staining with FGF-2 monoclonal antibody showed that FGF-2 expression was positive in all examined OSCC specimens.Positive FGF-2 immunostaining was detected as brown cytoplasmic staining of the tumor cells as shown inFigure(1 a, b, c).Regarding degree of expression as illustrated in table (1) which reveals that 3 cases (10.0%) showed weak positive expression, 9 cases (30.0%)showedmoderate positive expressionand 18 cases (60.0%) showed strong positive \ expression. Immunohistochemical staining with Heparanase monoclonal antibody showed that Heparanase expression was positive in all examined OSCC specimens.Positive Heparanase immunostaining was detected as brown for both cytoplasm and cell membrane in tumor cellsshown in Figure (2 a, b, c).Regarding degree of expression as illustrated in table (4) which reveals that 4 cases (13.3%) showed weak positive expression, 11cases (36.7%)showed moderate positiveexpression and 15 cases (50.0%)showed strong positiveexpression.The positive expression of fibroblast growth factor-2 was significantly correlated with tumor site (p=0.016),and clinical presentation (p value=0.003).The positive expression of Heparanse was significantly correlated with tumor grade(p-value =0.002) table (6).On other hand there was non-significant correlation between fibroblast growth factor-2,Heparanase and other clinicopathological parameters tables (2, 3, 5).Statistically significant correlation was found between the expressions of fibroblast growth factor-2 and Heparanase (p-value= 0.021) table (7).

Table 1: FGF-2 IHC expression in OSCC cases

FGF-2 score*	No.	%
1	3	10.0%
2	9	30.0%
3	18	60.0%
Total	30	100%

***1(weak expression),2 (moderate expression), 3 (strong expression).**

Table 2: Correlation of FGF-2 with tumor stage

		Stage				Total
		I	II	III	IV	
FGF-2 Score*	1	1	0	2	0	3
		33.4%	.0%	66.6%	.0%	100.0%
	2	2	1	2	4	9
		22.2%	11.1%	22.2%	44.5%	100.0%
	3	6	2	4	6	18
		33.3%	11.2%	22.2%	33.3%	100.0%
Total		9	3	8	10	30
		30.0%	10.0%	26.7%	33.3%	100.0%
		Value	Df		p.value	
Pearson Chi-Square		5.106	9		.825	

Table 3: Correlation of FGF-2 with tumor grade

		Grade			Total
		Well	Moderate	Poor	
FGF-2 Score*	1	0	2	1	3
		.0%	66.6%	33.4%	100.0%
	2	0	8	1	9
		.0%	88.9%	11.1%	100.0%
	3	7	9	2	18
		38.9%	50.0%	11.1%	100.0%
Total		7	19	4	30
		23.3%	63.3%	13.3%	100.0%
		Value	Df		p.value
Pearson Chi-Square		8.618	6		.196

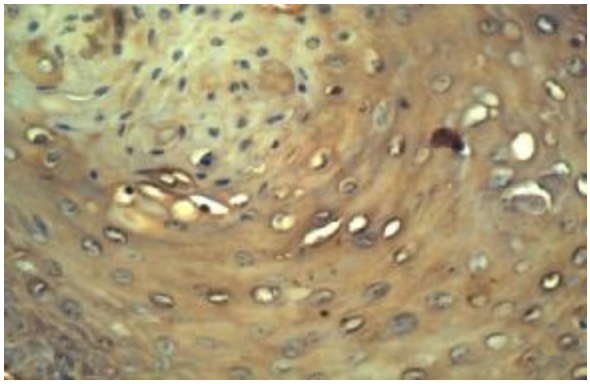
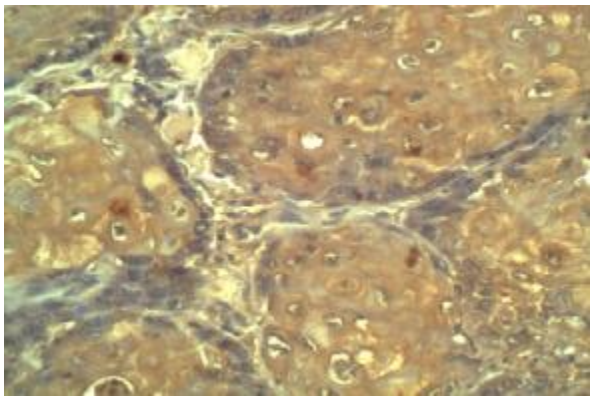
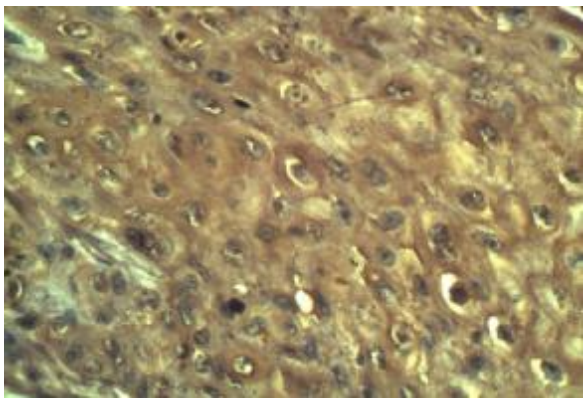


Figure 1: (a) Positive immunostaining of FGF-2in well differentiated OSCC(40X)



(b) Positiveimmunostaining of FGF-2 in moderate differentiated OSCC(40X)



(c)Positive immunostaining of FGF-2 in poorly differentiated OSCC(40X)

Table 4: Heparanase IHC expression in OSCC cases

Heparanasescore*	No.	%
1	4	13.3%
2	11	36.7%
3	15	50.0%
Total	30	100%

***1(weak expression),2 (moderate expression), 3 (strong expression).**

Table 5: Correlation of Heparanase with tumor stage

		Stage				Total
		I	II	III	IV	
Heparanase Score*	1	1	0	1	2	4
		25.0%	.0%	25.0%	50.0%	100.0%
	2	3	1	3	4	11
		27.3%	9.1%	27.3%	36.3%	100.0%
	3	5	2	4	4	15
		33.3%	13.3%	26.7%	26.7%	100.0%
Total		9	3	8	10	30
		30.0%	10.0%	26.7%	33.3%	100.0%
		Value	Df		p.value	
Pearson Chi-Square		3.036	9		.963	

Table 6: Correlation of Heparanase with tumor grade

		Grade			Total
		Well	Moderate	Poor	
Heparanase Score*	1	0	2	2	4
		.0%	50.0%	50.0%	100.0%
	2	1	10	0	11
		9.1%	90.9%	.0%	100.0%
	3	6	7	2	15
		40.0%	46.7%	13.3%	100.0%
Total		7	19	4	30
		23.3%	63.4%	13.3%	100.0%
		Value	Df		p.value
Pearson Chi-Square		20.345	6		.002

Table 7: The correlation of FGF-2 &Heparanase IHC expressions

		FGF-2	Heparanase
FGF-2	Pearson Correlation	1	19.591
	Sig. (2-tailed)		.021 *
Heparanase	N	30	30
	Pearson Correlation	19.591	1
	Sig. (2-tailed)	.021 *	
	N	30	30

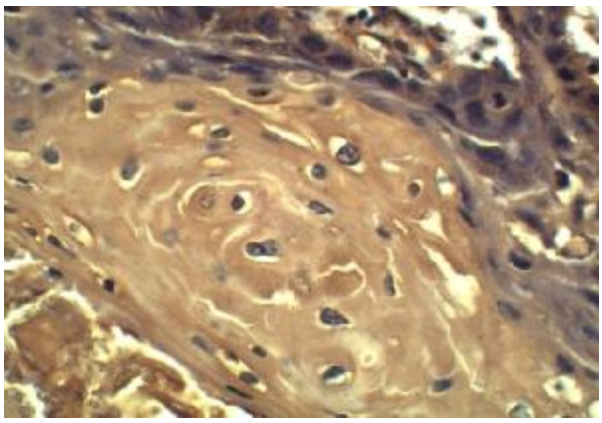
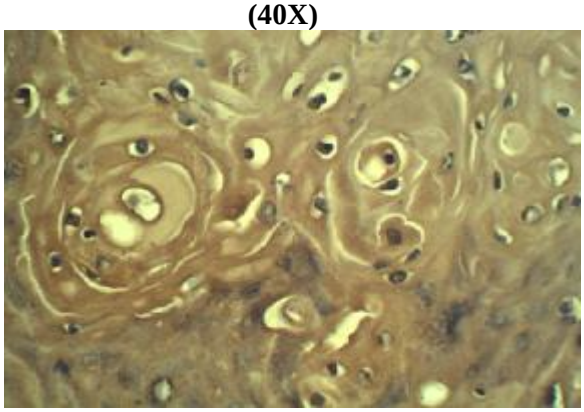
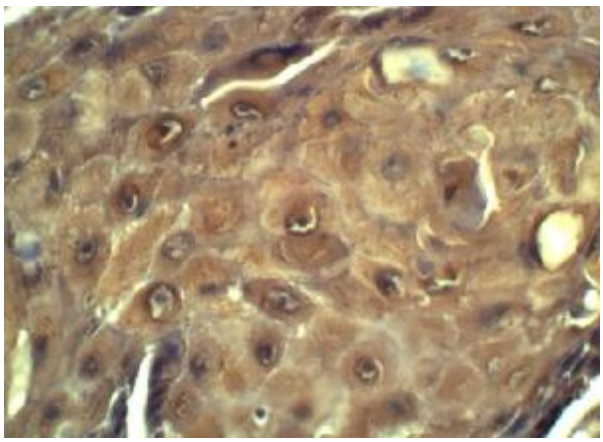


Figure 2: (a) Positiveimmunostaining of Heparanasein well differentiated OSCC (40X)



(b) Positive immunostaining of Heparanasein moderate differentiated OSCC (40X)



(c) Positive immunostaining of Heparanase in poorly differentiated OSCC (40X)

DISCUSSION

The results of this study showed positive FGF-2 expression in all OSCC cases with (60.0%) of cases showed strong positive score. The present finding was in agreement with previous reports in OSCC **(15-17)**. This suggest that FGF-2 may be involved in mitoses seen in squamous cells of oral squamous cell carcinoma **(15)**. It has been demonstrated that FGF-2 promotes the production of cancer cell proteinases and enhances their invasive ability, this explain that FGF-2 produced by cancer cells,and could activates the cancer cells themselves and/or the fibroblasts for the invasion and growth of the cancer **(17)**.The present study showed positive Heparanase expression in all OSCC cases, which also revealed that (50.0%) showed strong positive score ,these finding was in agreement withprevious reports in OSCC (18) .The key role of heparanase in tumorigenesis and the existing evidence for only one endogenous mammalian heparansulphate degrading endoglycosidase (19), as well as the expression of Heparanase even by a few tumor cells may be sufficient to promote dissemination of single tumor cells into adjacent tissues and lead to formation of local metastases (20) .In agreement with the role of the Heparanase in releasing FGF-2 from the ECM ,the results of the present study revealed that both FGF-2 and Heparanase showed similar pattern of expression, they were highly correlated by Pearson chi square with significant correlation between either proteins expression was found (p-value=.021). This result agree withprevious reports (21) that found Heparanase mRNA and FGF-2 mRNA are associated with higher tumor MVD in OSCC.It have revealed that Heparanase degradation of cell surface HS can augment FGF-2 activity,

depending on the Heparanase concentrations used to alter cell surface HS. FGF-2 binding and signaling require HS sequence-specific interactions. Depending on the extent of HS degradation, HS sequences, which bind to either FGF-2 or FGFR, could be removed or cryptic sites could be revealed, angiogenesis is dependent multiple components that can be affected by Heparanase in the ECM provide binding sites for angiogenic factors such as FGF-2 and vascular endothelial growth factor. Cell surface HSPG acts as growth factor and adhesion receptors on tumor cells and vascular endothelial cells. Modifying the HS may affect tumorigenicityby modifying the responsiveness of multiple receptors to the extracellular environment [(22),(23)].

In conclusion both Heparanase and FGF-2 might contribute in angiogenesis and metastasis in OSCC and they cooperate in promoting vascularization. These findings are contribute to our understanding of head and neck tumor biology, suggesting that FGF-2 and heparanase are the promising target for the development of anticancer therapeutics for head and neck malignancies.

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