Insulin-like growth factor-I receptor in human oral cancer

Reszeć J, Duraj E, Koda M, Musiatowicz B, Sulkowska M

Department of Pathology, Medical University of Bialystok

Abstract

The purpose of the study was to evaluate the expression of IGF-IR in primary tumours and lymph node metastases of oral cancers and the correlation between expression of IGF-IR and some clinicopathological features. Fifty-seven (57) oral cancers were examined by immunohistochemical studies, using the avidin-biotin-peroxidase method. Our study included only oral cancers, classified histopathologically as squamous cell carcinoma (7 cases in G1 grade, 44 (G2) and 6 (G3); 23/pT1 stage, 18/pT2, 7/pT3 and 9/pT4). Positive immunostaining for IGF-IR was noted in 32, out of 57 (56.1%) of oral tumours. We found a tendency (p=0.081) toward an association between IGF-IR expression in the primary tumours and their stage (pT3 and pT4). A comparison between the primary tumours and matching lymph node metastases revealed that 13, out of 20, (65%) cases showed a convergence between primary tumours and matching lymph node metastases with regard to either negative or positive staining.

Introduction

The IGF family of peptide ligands (IGF-1 and IGF-2) the IGF-1 and IGF-2 receptors are fundamentally involved in the regulation of somatic growth, cell proliferation, transformation and apoptosis. IGF-1 stimulates growth and metabolism by binding to the IGF-1R receptor, thereby activating, by protein tyrosine phosphorylation, a signal transduction cascade that is similar to the one, involved in insulin action. The IGF-IR, a tyrosine kinase receptor, is the major receptor for both IGF-I and IGF-II and consists of two α two ß subunits. The α subunits are entirely extracellular chains, containing a cysteine-rich domain responsible for ligand binding, and the ß subunits display a highly hydrophobic transmembrane domain, which divides the subunit into an extracellular and intracellular region, containing a tyrosine kinase domain [1]. Recently, lots of data suggested a role of IGF-1 and IGF-1R receptor in neoplastic transformation. A variety of tumour systems, including colon cancers, demonstrate an altered expression of IGF-I and IGF-II and their principle receptor IGF-IR [2]. However, the biological effect of IGF-I in malignant progression has not - so far - been fully elucidated. Some studies have shown that IGF-IR may prevent apoptosis in numerous tumour cell systems; in addition, it may play a role in induction of cell proliferation. Several studies have shown that IGF-IR is crucial for maintaining normal growth and development, as well as mitogenic activity, cell survival, and insulin-like actions are essential for embryogenesis, post-natal growth physiology, and breast development [3].

The purpose of the study was to evaluate the expression of IGF-IR in primary tumours and lymph node metastases of oral cancers. Moreover, we assessed the correlation between the expression of IGF-IR and some clinicopathological features.

Material and methods

We examined a series of 57 oral squamous cell cancers and 20 cases of lymph node metastases. The obtained biopsy specimens were fixed in a 10% buffered formalin solution, embedded in paraffin at 56°C, then cut into 5µm slices and stained with haematoxylin and eosin (H+E). The evaluation of IGF-IR expression was performed by immunohistochemical reaction, using monoclonal antibodies from Santa Cruz Biochemicals, USA. In order to visualize the antigen-antibody reaction, the LSAB technique was applied, using DAB (diaminobensidine)

ADDRESS FOR CORRESPONDENCE:
Joanna Reszeć
Department of Pathology
Medical University of Białystok
Waszyngtona 13; 15-269 Białystok, Poland

Table 1. Ki-67 and PCNA expression in the epithelial component of fibroepithelial tumours

Variable		IGF-IR (-)		IGF-IR (+)	
		No	%	No	%
Age	< 50	5	20,8	11	33,3
	> 50	19	79,2	22	66,7
PT	pT1	13	52,0	10	31,25
	pT2	8	32,0	10	31,25
	pT3	1	4,0	6	18,75
	pT4	3	12,0	6	18,75
G	G1	4	16,0	3	9,4
	G2	19	76,0	25	78,1
	G3	2	8,0	4	12,5

(DAKO Cytomation, Denmark). The IgG1 kappa (DAKO Cytomation, Denmark) antibody was used as a negative control, whereas samples of breast cancer tissue, which showed a strong positive IGF-IR immunoreactions, were used as a positive control. The estimation of immunostaining was done under a light microscope in representative fields, using a lens with a magnification of 20x. The criteria for a positive reaction were as follows: (+) above 10% cancer cells showing positive immunostaining, and (-) below 10% of cells with positive IGF-IR immunostaining. We analysed IGF-IR expression in relation to the patients' age, sex, grading and staging and lymph node metastases. The obtained results were statistically analyzed, using the Fisher and Chi-square tests, as well as the Students-test for paired samples, employing the SPSS software package v.8.0 for Windows (SPSS Inc., Chicago, IL). The values at $p<0.05$ were considered statistically significant.

Results

Our study included only oral cancers, classified histopathologically as squamous cell carcinoma: 7 cases in G1, 44 in G2 and 6 in G3. There were 23 tumours in pT1, 18 in pT2, 7 in pT3 and 9 in pT4. Twenty-three, out of fifty-seven (23/57) (40.4%) patients had lymph nodes involved at the time of diagnosis. The results are shown in Table 1. Thirty-two (32), out of 57 tumours (56,1%) showed IGF-IR positive immunoreaction; in 13, out of 20 (65%) lymph node metastases, IGF-IR positive expression was observed. Comparing the IGF-IR expressions in the primary tumours and metastases, the results are as shown: in 9, out of 13 pairs (69,2%) IGF-IR expression was positive; in 3, out of 13 pairs (23,1%) there was no positive expression of IGF-IR observed; in 4, out of 7 pairs (57,2%) IGF-IR was positively noted in lymph node metastases with no expression in the primary oral cancer; in 4, out of 7 pairs (57,2%) IGF-IR positive immunoreaction was observed in the oral squamous cell cancer, while no IGF-IR expression was found in lymph node metastases (Table 2). Above all, in our study, we observed a high tendency towards the association between tumour size and IGF-IR immunopositivity (p= 0,081), the higher percentage with IGF-IR expression was observed in pT3 and pT4 tumours. There were no statistically significant relationships between grading, the occurrence of lymph node metastases, the age and IGF-IR expression in primary oral squamous cell cancer, although IGF-IR positive lymph node metastases were observed in statistically older patients- after 50 years old (p= 0,017).

Table 1. Clinical characteristics, anthropometric and densitometric parameters in relation to COLIA1 gene alleles.

IGF-IR in primary tumours	Expression level	IGF-IR in metastases		
		(-)	(+)	Total number of cases
	(-)	3	4	7
	(+)	4	9	13
	Total number of cases	7	13	20

Discussion

There is no data in the literature, associated with IGF-IR expression in oral squamous cell cancer. Some studies have shown a strong correlation between IGF-IR receptor expression and colorectal, breast, prostate cancer development, but the evidence is unclear [4, 5, 6]. The studies found an association between raised plasma IGF-I levels and an increased prostate risk. Serum concentrations of IGF-II were higher in patients with colorectal adenomas, compared to those in normal controls [2]. Also other studies show IGF-I as a factor that determines a lifelong risk for breast cancer [7]. Freier at al [8] observed 40 times higher IGF-II expression in malignant colon tissue than in tissue, adjacent to the tumour. The expression of IGF-I receptor was 2.5 times higher in the malignant tissue than in tissue adjacent to the tumour. Weber et al [9] observed an overexpression of IGF-IR in colon carcinomas; colon carcinoma cells exhibited a positive staining for IGF-IR in 91% of all tumours (30, out of 33), whereas the adjacent normal colonic epithelial cells showed only either a very faint or no significant IGF-IR immunoreactivity. Reinmuth et al [10] showed IGF-I positive tumours with decreased tumour cell proliferation; furthermore, IGF-IR DN-transfected cells yielded significantly decreased tumorigenicity and growth in the liver.

In our study, 13, out of 20 (65%) tumour-metastases were IGF-IR-positive, what might suggest an association between IGF-IR expression in primary tumours and metastatic ability,

but the data must be investigated more carefully. IGF-IR expression in oral squamous cell cancer might be a predictive marker in tumour progression and metastases ability. We would like to emphasise the importance of studies, concerning the proteins involved in the proliferation in lymph node metastases because the knowledge about heterogeneity between primary tumours and lymph node metastases could shed some light on tumour biology and lead to a development of more effective anti-cancer therapies.

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