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IGFBP3 in Patients with Advanced Stomach Carcinoma

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***Abstract*— IGF peptides family plays important role in cell growth and apoptosis regulation both in normal and neoplastic cells. The aim of the study was assessment of IGFBP3 expression in main mass of tumor and metastatic lymph node in patients with advanced stage stomach carcinoma and correlation with selected prognostic and clinico-pathological factors. The study group consisted of 76 patients with advanced stage stomach carcinoma (24 women and 52 men). Mean patients' age was 60,4 (30-78) yrs. Immunohistochemical staining with monoclonal anti IGFBP3 antibody (mouse IgG2B monoclonal anti-human IGFBP3 antibody) was used for IGF BP3 assessment. Statistical analysis revealed no correlation between chosen clinical and pathological parameters and expression of IGFBP3 protein in the lymph node with metastasis. No correlation was found between expression of IGFBP3 in the main mass of tumor and patient's gender, tumor location, depth of invasion in the wall, histological differentiation, Bormann's classification, Lauren's classification as well as expressions in the lymph node with metastasis. Moreover, there was no association between expression of IGFBP3 and overall postoperative survival time (data not published). Positive expression of IGFBP3 protein in main mass of tumor was observed mainly in poorly differentiated tumors located in 1/3 of middle and 1/3 lower part and in all stomach. Similarly, positive expression of IGFBP3 in lymph node metastasis was associated with diffuse type of cancers, type IV (according Bormann's classification) and low stage of histological differentiation G3.**

***Index Terms*— IGFBP3,IGF family, stomach carcinoma.**

I. INTRODUCTION

Stomach carcinoma is considered to be second most often (after lung cancer) neoplastic cause of death. Fulminant increase in number of stomach carcinoma cases was observed in the second half of the twentieth century. Populations of Japan, China and South America seem to be most affected in contrast with West Europe and North America [1]. In Poland, as in other countries, stomach carcinoma is considered to be second most prevalent cause of male death due to malignancies. Women more often suffer from neoplastic diseases of breast, lungs, colon, ovaries, and cervical cancer [2].

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Recently role of the growth factors in pathogenesis of different malignant problems seems to be intensively investigated. Especially their role in metastatic changes are being under thorough research.

Insulin-like growth factor (IGF-I, IGF-II) belongs to the family of proinsulin-like proteins. While acting paracrine and autocrine to the surrounding tissues and cells, they are involved in regulation of i.e. cells proliferation and apoptosis. IGF-I is mainly produced by liver as the response to growth hormone (GH), however IGF-II synthesis is not growth hormone dependent. IGF is mainly present in circulating blood, however its activation and function is closely related to the structure of binding protein. Over 90% of circulating IGF makes 150 kDa complexes with insulin-like growth binding protein 3 (IGFBP3) and acid-liable molecule (ALS). This complex form is responsible for prolongation of IGF half-time from 10 minutes to over 15 hours, protects from effect of hypoglycaemia induced by unbounded IGF molecules and prevents translocation of circulating IGF into target tissues [3,4].

Influence of IGFBP3 to uncontrolled cells growth (typical for neoplastic chanages) is not clear. Correlation between high IGFBP3 concentration and increased risk of breast cancer and increased risk of morality has been shown in several publications [5,6]. Some papers suggested protective function of IGFBP3 and link its higher concentration with lowered cancer prevalence [7-9]. IGFBP3 proapoptotic capabilities, its ability to inhibit carcinoma's cellular growth and to decrease proliferation, seem to be major factors validating such approach [11]. IGFBP3 expression has been shown to be significantly higher in neoplastic changes in stomach carcinoma. Such increased expression was more often observed in the diffuse type cancer (Lauren's classification) localised below antrum [10]. It is interesting that some papers discuss potential role of IGFBP3 as a marker for type of the stomach cancer more susceptible to anticancer therapy [12].

The aim of the study was to immunohistochemically identify IGFBP3 expression in tumor and lymph nodes tissues of patients with advanced stage stomach cancer. We also try to correlate IGFBP3 expression and selected anatomopathological factors and time of survival in affected patients.

II. MATERIAL AND METHODS

A. Study group

The study group consisted of 76 chosen patients (24 women and 52 men) who performed surgery due to gastric cancer from 1996 to 1998, in 2nd Department of General and

Gastroenterological Surgery, Medical University of Bialystok. The mean age was 60,4 years (range 30-78).

B. Immunohistochemistry assessment

Histopathological and immunohistochemical investigations were performed the Department of General Pathomorphology, Medical University of Bialystok.

In all cases, specimens were obtained from the main mass of tumor. They were fixed in 40g/L formaldehyde solution, embedded in paraffin and cut into 5 µm sections. The sections were deparaffinized in three changes of xylene and hydrated through an alcohol series of a decreasing concentration. For detection of IGFBP3 protein, the sections were incubated with 3 % hydrogen peroxide solution in methanol for 30 min followed by incubation with a mouse monoclonal IGFBP3 antibody (mouse IgG_{2B} monoclonal anti- human IGFBP3 antibody, no MAB305, R&D Systems) in dilution 1:80 for 45 minutes at room temperature. Novocastra Peroxidase Detection System (No RE7120-K, Novocastra Laboratories; visualization of mouse and rabbit primary antibody) was applied as a detection kit. The antigen-antibody complex was visualized by DAB chromogen (3'3-diamonobenzidine; DAB chromogen RE7105 Novocastra). After rinsing in distilled water, the sections were stained with hematoxylin and mounting on a Canadian balsam.

C. Evaluation of samples

Protein expression was assessed using a semi-quantitative method and defined as follows: low expression (0) – no reaction or less than 30% of cells IGFBP3 positive in main mass of tumor and lymph node metastasis, and high expression (1) – more than 30% of cancer cells in main mass of tumor and lymph node metastasis were IGFBP3 positive. The percentage of IGFBP3 positive cells was calculated in 500 cancer cells in each preparation, at magnification of 400x (by two independent pathologists).

D. Statistical analysis

Statistical analysis was based on Fisher's test. The $p<0,05$ was considered statistically significant. For statistical analysis we use Statistica 10 for Windows software.

III. RESULTS

Statistical analysis revealed no association between chosen chosen- pathological parameters and expression of IGFBP3 protein in the lymph node with metastasis (Table No.2). No statistical significant differences were found between expression of IGFBP3 in the main mass of tumor and patient's gender, tumor location, depth of invasion in the wall, histological differentiation, Bormann's classification, Lauren's classification as well as expressions in the lymph node with metastasis (Table No.1). Moreover, there was no association between expression of IGFBP3 and overall postoperative survival time (data not published). Positive expression of IGFBP3 protein in main mass of tumor was observed mainly in poorly differentiated tumors located in 1/3 of middle and 1/3 lower part and in all stomach (Table No.1). Similarly, positive expression of IGFBP3 in lymph node metastasis was associated with diffuse type of cancers, type IV according Bormann's classification and low stage of histological differentiation G3 (Table No. 2).

IV. DISCUSSION

IGFBP3 plays a role in cell growth control and apoptosis. IGFBP3 can inhibit growth and induce apoptosis in IGF-dependent and IGF-independent manners, its induction by DNA damage and hypoxia suggest IGFBP3 plays a role in the physiologic protection against aberrant cell growth [13]. IGFBP3 binding to a cell surface protein is indispensable for its antiproliferative action in human breast cancer cells, and the mid-region of IGFBP3, which is the least conserved region among the IGFBP-1-6 protein, is responsible for cell surface binding [14, 15]. Moreover, IGFBP3 induced apoptosis through the activation of specific caspases that are involved in the death receptor-mediated apoptotic pathways in breast cancer cells. These findings strongly suggest the existence of an IGFBP3-specific receptor participating in the direct proapoptotic effect of IGFBP3 in cancer cells [16].

Gigek et al. [10] have shown that 80% of evaluated stomach cancers showed IGFBP3 expression, however such expression was observed significantly more often in the intestinal type than in diffuse type cancer. IGFBP3 expression was more frequently (although not significant) observed in diffuse- type tumors located in the non-cardia region (fungus, body and pylorus regions of the stomach) than in the cardia region (gastroenterophageal transition). Zhang et al. [20] observed positive immunohistochemical reaction in 55,4% cases of tested stomach cancers, what stay in consistency with our results. Patients with well- or moderately- differentiated tumors also showed significantly higher percentage of positive staining of IGFBP3, than those with poorly-differentiated tumors. We observed positive IGFBP3 immunohistochemical reaction in the intestinal type the ratio was 74,1% of cases and in patients with diffuse type carcinoma this ratio reached 72%, mainly in poor differentiated tumors (G3), located in 1/3 middle and 1/3 down part of stomach.

Jeng et al. [17] have shown correlations between IGFBP3 expression and age of patients, diameter of the stomach carcinoma, depth of the neoplastic change and local lymph nodes malignant infiltration. Moreover, they have suggested positive correlation between IGFBP3 expression and shortened survival time and fatal prognosis in course of stomach carcinoma, correlation which was independent from the stage of malignancy. Results they published might suggest that IGFBP3 expression might play a role in stomach carcinoma as an independent prognostic factor. In our study, no correlations were found between expression of IGFBP3 in the main mass of tumor and depth of invasion in the wall, histological differentiation and expressions in the lymph node with metastasis. Moreover, there was no association between expression of IGFBP3 and overall postoperative survival time.

In contrast, Pharm et al. [18] observed lowered (but not reaching statistically significant level) risk of developing stomach cancer in patients with elevated IGFBP3 levels in blood serum. Strong support to those suggestions was given by Xue et al. [19], who noticed significant longer post-surgical survival time in patients with higher IGFBP3 levels. They revealed that IGFBP3 expression was significantly downregulated in 86 gastric adenocarcinomas tissues relative to their adjacent non- cancerous tissues. Also expression of IGFBP3 protein was significantly lower in gastric tumor with lymph node metastasis compared with that without lymph node metastasis. In our work we observed stronger expression of IGFBP3 in more advanced stages of

stomach carcinoma, of lowered differentiation level, fully infiltrating stomach wall, localised in middle and lowered part, with metastatic infiltration of local lymph nodes Such differences in IGFBP3 expression, however, has not reached level of significance.

IGF protein family play important role in cell growth regulation mechanism, both in healthy ones and neoplastic changed. IGFBP3 protein is considered to have the strongest bioactivity [20]. It has been proven that the patients with intestinal metaplastic infiltration in stomach have lowered IGFBP3 serum level in comparison with the patients without such changes [20]. Moreover, the patients with well differentiated tumors have significantly more cancer cells revealing strong positive IGFBP3 expression, than the patients with non-differentiated tumors [10,20]. IGFBP3 might potentially downregulate cellular growth [19] and induce apoptosis in many different tissues [21], presented results might indicate ability to reduce pre-malignant changes, thus indicate the possible protective function against stomach carcinoma development. It is possible that additional evaluation of proapoptotic protein expression in evaluated tissues might confirm protective IGFBP3 function against carcinogenesis [20]. Zheng et al. [20] observed significantly higher percentage of positive IGFBP3 staining in tumor tissue in patients with well or moderately differentiated tumors than in those poorly differentiated, indicating that IGFBP3 may be associated with better prognosis. Moreover, they showed that patients with antral or corpus intestinal metaplasia had significantly lower serum levels of IGFBP3 than those without those changes.

That results suggests that IGFBP3 may play protective role against the development of gastric cancer by preventing the formation of intestinal metaplasia and improve the prognosis of gastric cancer [20]. In our study group, nearly 80% of evaluated cases of stomach cancer had strong expression of IGFBP3. Strong expression of evaluated marker was more often observed in intestinal type tumors, changes localised in stomach 1/3 middle or lower, and of low-grade differentiation. Positive IGFBP3 expression in metastatic lymph nodes were more often seen in diffuse changes (according to Lauren classification), in type IV changes (according to Bormann classification) and low-grade differentiation changes (G3). Observed higher prevalence of observed IGFBP3 expression, however, did not reach the level of statistical significance.

Our work has several limitations: one of the most important is lack of serum IGFBP3 assessment during surgery, which was not performed due to bio-ethics committee restrictions. The second is the lack of prospective evaluation of IGFBP3 i.e. before surgery, after surgery and in the follow-up visits. However, we think that our work might contribute for growing evidence of the IGF-family growth factors, which might influence and possible change the time-course and/or prognosis of the patients with stomach carcinoma.

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Table 1. Association between expression of IGFBP3 in main mass of tumor and chosen clinico- pathological parameters

Parametrs		Expression IGFBP3-tumor		
		0	1	p
Gender	Female	4 (16,7%)	20 (83,3%)	0,249
	Male	14 (26,9%)	38 (73,1%)	
Location	1/3 up part	2 (33,3%)	4 (66,7%)	0,739
	1/3 middle part	8 (25,8%)	23 (74,2%)	
	1/3 down part, all stomach	8 (20,5%)	31 (79,5%)	
Depth of invasion	T1 (mucosa, submucosa)	2 (20,0%)	8 (80,0%)	0,441
	T2 (muscular layer)	6 (31,6%)	11 (68,4%)	
	T3 (serosa)	10 (20,4%)	39 (79,6%)	
Bormann's classification	I	1 (16,7%)	5 (83,3%)	0,789
	II	6 (31,6%)	13 (68,4%)	
	III	8 (20,5%)	31 (79,5%)	
	IV	3 (25,0%)	9 (75,0%)	
Lauren's classification	Intestinal type	14 (25,9%)	40 (74,1%)	0,343
	Diffue type	4 (18,2%)	18 (71,8%)	
Histological differentiation	G2	11 (21,6%)	40 (79,4%)	0,860
	G3	7 (20,0%)	28 (80,0%)	
pN	0	11 (21,6%)	41 (78,4%)	0,299
	1	3 (20,0%)	12 (80,0%)	
	2	4 (50,0%)	4 (50,0%)	
	3	0 (0%)	1 (100%)	

Table 2. Association between expression of IGFBP3 in lymph node with metastasis and chosen clinico-pathological parameters.

Parametrs		Expression IGFBP3-lymph node		
		0	1	p
Gender	Female	5 (50,0%)	5 (50,0%)	0,254
	Male	12 (70,6%)	5 (29,4%)	
Location	1/3 up part	2 (100%)	0 (0%)	0,370
	1/3 middle part	7 (70%)	3 (30%)	
	1/3 down part, all stomach	8 (53,3%)	7 (46,7%)	
Depth of invasion	T1 (mucosa, submucosa)	1 (50%)	1 (50%)	0,162
	T2 (muscular layer)	5 (100%)	0 (0%)	
	T3 (serosa)	11 (55%)	9 (45%)	
Bormann's classification	I	1 (100%)	0 (0%)	0,605
	II	4 (66,7%)	2 (33,3%)	
	III	10 (66,7%)	5 (33,3%)	
	IV	2 (40%)	3 (60%)	
Lauren's classification	Intestinal type	13 (72,2%)	5 (27,8%)	0,162
	Diffue type	4 (44,4%)	5 (55,6%)	
Histological differentiation	G2	11 (73,3%)	4 (26,7%)	0, 198
	G3	6 (50%)	6 (50%)	