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Tetranectin expression in gastric adenocarcinomas

D.L. Arvanitis[1], E.F. Kamper[2], L. Kopeikina[2], A. Stavridou[1], M.N. Sgantzos[1], V. Kallioras[1], E. Athanasiou[3] and P. Kanavaros[4]

[1]Departments of Anatomy and Pathology, University of Thessaly, Medical School, Larissa, Greece,

[2]Department of Experimental Physiology, University of Athens, Medical School, Athens, Greece,

[3]Department of Surgery, University of Thessaly, Medical School, Larissa, Greece and

[4]Department of Anatomy and Histology, University of Ioannina, Medical School, Ioannina, Greece

Summary. *Aims:* The aim was to analyze the immunohistochemical localization of tetranectin in gastric adenocarcinomas and the adjacent tissues of the wall of the stomach. *Methods and results:* Forty cases of gastric adenocarcinomas were stained by the indirect immunoperoxidase method. Of the ten cases of mucinous signet ring cell carcinomas 5 showed high, 3 moderate and 2 low tetranectin expression. Of the ten cases of well-differentiated intestinal type adenocarcinomas (ITA) 4 showed moderate regional, 3 low regional and 3 negative tetranectin expression. Of the ten cases of moderately-differentiated ITA 3 showed moderate regional, 4 low regional and 3 negative tetranectin expression. Of the ten cases of poorly-differentiated ITA 4 showed focal low and 6 negative tetranectin expression. Overall, the mucinous signet ring carcinomas showed significantly higher tetranectin expression compared to ITA (χ^2=3.95, $p<0.05$). In contrast, no significant relationship was found between tetranectin expression and the degree of differentiation in ITA (χ^2=2.5, $p>0.05$). In all cases, the perineoplastic desmoplastic reactive stroma showed high expression of tetranectin intra- and extracellularly. The mast cells and goblet cells in the areas of intestinal metaplasia showed high tetranectin expression. *Conclusions:* This study shows that: a) tetranectin is produced and deposited extracellularly in the desmoplastic peritumoral stroma of infiltrating gastric adenocarcinomas; b) tetranectin is more highly expressed by the mucinous signet ring cell carcinomas compared to ITA; and c) the amount of tetranectin produced by the ITA is unrelated with the degree of tumor differentiation.

Key words: Tetranectin, adenocarcinoma, stomach, immunohistochemistry

Offprint requests to: Prof. Dimitrios L. Arvanitis, Department of Anatomy, University of Thessaly, Medical School, Papakyriazi 22, 41222 Larissa, Greece. Fax: 041 565085

Introduction

Tetranectin, a plasma protein which binds specifically to the kringle 4 region of plasminogen (Clemmensen et al., 1986) has been found immunohistochemically to be present in the cytoplasm of many normal cells (Christensen et al., 1987; Christensen and Clemmensen, 1989). Concerning neoplasia, tetranectin has been found to be deposited abundantly in the extracellular matrix of malignant tumors such as breast carcinomas (Christensen and Clemmensen, 1991; Christensen, 1992) and colonic carcinomas (Verspaget et al., 1994) and shows reduced plasma concentration in patients with cancer (Jensen and Clemmensen, 1988; Hogdall et al., 1991). The exact functional role of tetranectin remains to be clarified, although the structure of the protein and its gene has been established (Fuhlendorff et al., 1987; Berglund and Petersen, 1992; Wewer and Albrechtsen, 1992). Tetranectin is considered to play a role in fibrinolysis because it binds to plasminogen and participates in the plasminogen activation cascade (Clemmensen et al., 1986). These proteolytic processes are also considered to be involved in tumor growth, invasion and metastasis (Markus, 1984) since the plasminogen activators and their inhibitors play an active role in tumor development (Dano et al., 1985; Markus, 1988; Hart and Rehemtulla, 1988; Verspaget et al., 1989). To the best of our knowledge, the immunohistochemical expression of tetranectin has not been investigated in gastric adenocarcinomas. Thus, in the present study we evaluated tetranectin expression in 40 cases of gastric adenocarcinomas and we compared it with tetranectin expression occurring in the adjacent gastric tissues.

Materials and methods

Materials

Paraffin-embedded tissues from forty cases of gastric adenocarcinomas were studied including ten cases of

mucinous signet ring cell carcinomas and thirty cases of intestinal type adenocarcinomas divided into ten cases of well-, ten cases of moderately- and ten cases of poorly-differentiated carcinomas.

Methods

The specimens were fixed in 10% buffered neutral formalin and embedded in paraffin. Four micron sections were cut and deparaffinized. Then, proteolytic digestion of the sections was carried out by incubating the sections with pronase in buffer for 6 minutes at room temperature. After washing, the endogenous peroxidase activity was inhibited by incubating the sections with 3% H_2O_2 in buffer for 30 min. After washing, the non-specific binding of the antibodies was inhibited by incubating the sections in 10% normal swine serum in tris-buffer for 30 min. Then, the primary anti-human tetranectin polyclonal rabbit antibody purchased from DAKO (Lot no. A0371) was applied in 1:100 dilution in tris-buffer, for 24 hours, 22 hours at 4 °C and 2 hours at room temperature. Then, after washing in tris-buffer for 5x6', the secondary swine anti-rabbit peroxidase-conjugated antibody purchased from DAKO (Lot no. 033) was applied in 1:50 dilution in tris-buffer for 60 min. Then, after washing in buffer for 5x6 min, the DAB system was used as substrate. Then, the sections were washed and counterstained with hematoxyline. Negative controls by omitting the anti-tetranectin antibody were included in every experiment. The immunohistochemical staining for stromal tetranectin was evaluated according to its intensity using the following scores: negative, low, moderate, and high. The expression of tetranectin in the tumors was evaluated according to the intensity of the staining and the percentage of the positive tumor cells due to the heterogeneity of the staining even within the same case. In the present study 10 tumor areas in x40 magnification were evaluated. The grade was evaluated as follows: a) well-differentiated: gland-like spaces predominate, b) moderately-differentiated: gland-like spaces with diffuse areas; and c) poorly-differentiated: diffuse areas predominate. Statistical analysis was performed using the chi-square test. The results were considered significant for $p<0.05$.

Results

Normal tissues

The normal epithelial and mesenchymal cells of the gastric wall showed either negative or low tetranectin expression, with the exception of the mast cells which showed high expression (Fig. 1A) and the parietal cells which showed in some cases moderate expression. In fifteen cases, which comprised areas of intestinal metaplasia, the goblet cells showed high expression (Fig. 1B), whereas the intervening columnar epithelial cells showed negative expression. The normal stroma of the lamina propria and submucosa remained negative in all cases.

Neoplastic tissues

The mucinous signet ring cell carcinomas showed heterogeneity of the staining even within the same case, since intensely stained tumor cells and negative tumor cells were found in every case. Five cases which showed more than 60% stained cells were recorded as high tetranectin expression cases (Fig. 1C), three cases which showed between 30% and 60% stained cells were recorded as moderate expression and two cases which showed less than 30% stained cells were recorded as low expression cases (Tables 1 and 2). The intestinal type of adenocarcinomas (ITA) revealed the same heterogeneity of the staining. This type of adenocarcinoma revealed staining limited either to small regions, or to foci or to individual cells. Four cases of well-differentiated ITA showed moderate regional staining (Fig. 1D), three cases showed low regional staining and three cases showed negative staining. The moderately-differentiated ITA showed three cases with moderate regional staining, four cases with low regional staining and three cases with negative staining. The poorly-differentiated ITA showed

Table 1. Tetranectin expression in relation to histological type.

HISTOLOGICAL TYPE	TETRANECTIN EXPRESSION	
	Positive	Negative
Mucinous adenocarcinomas	10	0
ITA	28	12

ITA: Intestinal type adenocarcinomas. $\chi^2 = 3.95$ and $p < 0.05$

Table 2. Tetranectin expression in relation to histological type and grade.

HISTOLOGICAL TYPE	TETRANECTIN EXPRESSION			
	High	Moderate	Low	Negative
Mucinous adenocarcinomas	5	3	2	-
Well-differentiated ITA	-	4	3	3
Moderately-differentiated ITA	-	3	4	3
Poorly-differentiated ITA	-	-	4	6

ITA: Intestinal type adenocarcinomas

Table 3. Tetranectin expression in relation to the grade of ITA.

GRADE OF ITA	TETRANECTIN EXPRESSION	
	Positive	Negative
Well-differentiated ITA	7	3
Moderately-differentiated ITA	7	3
Poorly-differentiated ITA	4	6

ITA: Intestinal type adenocarcinomas. $\chi 2= 2.5$ and $p>0.05$

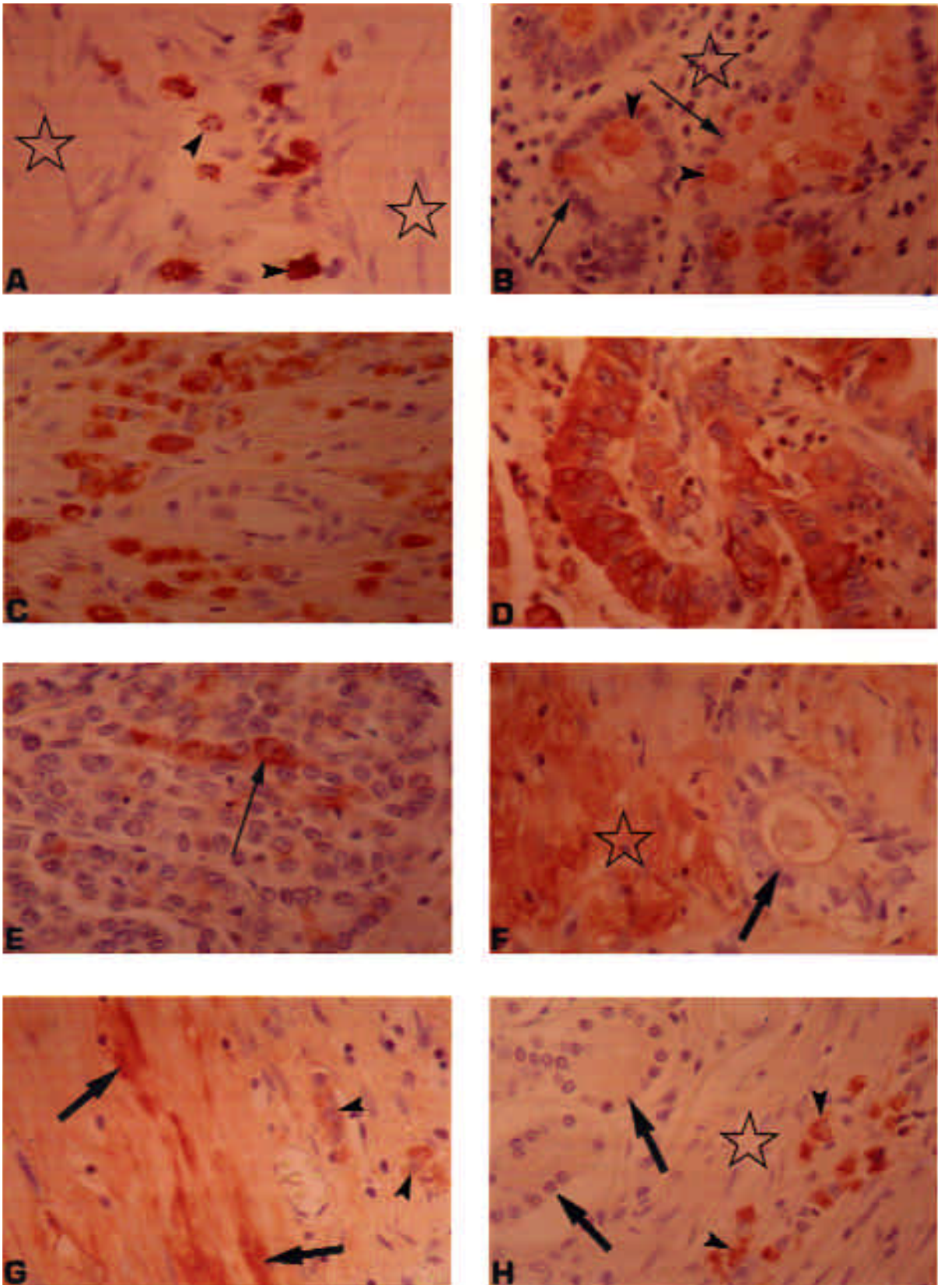

Fig. 1. A. Mast cells (arrow heads) in the muscularis propria of the stomach, stained intensely for tetranectin. Muscle cells (asterisks) are negative. x 400. **B.** Goblet cells (arrow heads) showing high expression of tetranectin, in an area of intestinal metaplasia. Intervening normal glandular cells (arrows) and adjacent stromal elements (asterisk) are negative. x 400. **C.** Mucinous signet-ring cell adenocarcinoma showing high expression of tetranectin. x 400. **D**. Well-differentiated intestinal type adenocarcinoma with moderate regional expression of tetranectin. x 400. **E**. Poorly-differentiated adenocarcinoma with focal staining for tetranectin (arrow). x 400. **F**. Infiltrating well-differentiated intestinal-type adenocarcinoma. The desmoplastic reactive stroma shows intense diffuse deposition of tetranectin (asterisk). The adjacent carcinomatous gland is negative (arrow). x 400. **G.** Infiltrating signet-ring cell adenocarcinoma cells (arrow heads) with fibrillated pattern of extracellular stromal tetranectin deposition (arrows). x 400. **H.** A small group of signet-ring carcinoma cells (arrow heads) with high expression of tetranectin in the gastric submucosa. The adjacent unreactive stroma of the submucosa (asterisk) is negative for tetranectin. Adjacent pyloric glands (arrows) are negative for tetranectin. x 250

four cases with focally or individually dispersed (Fig. 1E) stained tumor cells and six cases with negative staining (Tables 1 and 2). The above-mentioned results indicate that there is no significant relationship between the degree of tetranectin expression and the degree of differentiation of intestinal type of carcinomas (Table 3) (χ^2=2.5 and $p>0.05$). The mucinous signet ring cell carcinomas showed higher expression of tetranectin compared to intestinal type of adenocarcinomas (χ^2=3.95 and $p<0.05$). The reactive stroma, which surrounded the infiltrating tumor islands, showed high staining in all cases. The stained stroma showed either a diffuse (Fig. 1F) or a fibrillated (Fig. 1G) pattern of staining with extracellular deposition of the stain in both patterns. It is interesting to note that only the reactive peritumoral stroma showed positive staining, whereas normal stromal elements surrounding small foci of either stained or unstained tumor cells remained unstained (Fig. 1H).

The smooth muscle fibers of the muscularis propria which were adjacent to infiltrating stained or unstained tumor cells did not reveal any elevation of the staining compared to that of the remaining muscularis propria lying away from the infiltrating tumor islands.

Discussion

The results of the present study in gastric adenocarcinomas are in keeping with previous research data in breast, ovarian and colorectal adenocarcinomas, which showed that tetranectin is deposited extracellularly in the reactive stroma which surrounds the infiltrating islands of carcinomas (Christensen and Clemmensen, 1991; Christensen, 1992; Verspaget et al., 1994; Hogdall, 1998). In addition, the findings confirm previous work in colon carcinomas that the tetranectin which is deposited in the tumor stroma is produced by the stroma cells and not by the tumor cells (Wewer and Albrechtsen, 1992) since abundant tetranectin was present in the stroma which surrounded tumor islands, even in the cases where the tumors did not produce tetranectin. It has to be noted that the data show that the mere presence of infiltrating tumor cells is not sufficient to produce deposition of tetranectin in the surrounding tissues. The tetranectin is deposited extracellularly in the areas where desmoplastic reaction takes place around the infiltrating tumor islands. This is obvious in the areas where small groups or isolated tumor cells are present without surrounding desmoplastic reaction, which do not show deposition of stroma tetranectin, although the tumor cells are intensely stained for tetranectin. The high expression and extracellular deposition of tetranectin is a property of the peritumoral desmoplastic stroma since other tissues, such as the muscularis propria did not show increased expression of tetranectin, even in the areas which were heavily infiltrated by large tumor islands. Concerning the other tissue elements, the study confirms previous findings in normal tissues, which showed that the mast cells produce high amounts of tetranectin (Christensen and Clemmensen, 1989). Interesting is the finding that the goblet cells in the areas of intestinal metaplasia showed high expression of tetranectin compared to that of normal gastric epithelium. This finding agrees with one previous work, which showed that the goblet cells of normal colon mucosa show high expression of tetranectin (Verspaget et al., 1994). Concerning the tumor cells, our study shows that the mucin-producing signet-ring cell carcinomas show higher tetranectin expression compared to both the normal gastric epithelial cells and the intestinal type carcinomas. This relatively high expression of tetranectin by the mucin-producing signet-ring tumor cells reflects the ability of these cells to synthesize high amounts of tetranectin, as happens with normal mucin producing cells such as the goblet cells. Previous investigators (Markus, 1984, 1988; Dano et al., 1985; Hart and Rehemtulla, 1988; Verspaget et al., 1989) have suggested that the extracellular deposition of tetranectin in the desmoplastic peritumoral tissue stroma may facilitate the expansion, spread and metastasis of malignant tumor cells because tetranectin binds to plasminogen and participates in the extracellular protein and tissue matrix degradation. The well-known ability of mucin-producing signet-ring cell carcinomas for fast infiltration, spread and metastasis may be due in part to high tetranectin production by these cells, if an interplay can take place among intracellular tetranectin, cell surface receptors and extracellular matrix components. In this respect it is interesting to note that in ovarian cancer tissues heavy stromal tetranectin staining was associated with reduced survival (Hogdall, 1998) further supporting the idea that extracellular deposition of tetranectin may enhance tumor spread and metastasis.

Our study shows that tetranectin production of intestinal-type gastric adenocarcinomas is limited and is not associated with the degree of tumor differentiation. There are only two previous works which deal with the study of tetranectin immunohistochemical expression in colorectal tumors. One study showed that colon carcinomas do not produce tetranectin (Wewer and Albrechtsen, 1992), whereas the other study (Verspaget et al., 1994) showed that tetranectin can be produced by the tumor cells of colon adenocarcinomas as happens with the intestinal type of gastric adenocarcinomas in our study.

In conclusion, our study shows that: a) tetranectin is produced and deposited extracellularly in the desmoplastic peritumoral stroma of infiltrating gastric adenocarcinomas; b) tetranectin is more highly expressed by the mucinous signet ring cell carcinomas compared to ITA; and c) the amount of tetranectin produced by the ITA is unrelated with the degree of tumor differentiation.

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