Characteristics of Gastroprotein Synthesis and Phosphorylation in Human Gastric Carcinoma Cells

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Characteristics of the synthesis and Mg-dependent phosphorylation of gastroproteins are studied in intact cells of the mucous coat of the stomach and in the cells of tumor nodes in the same patients. Comparison of polypeptide maps reveals 4 main types of mucous coats which contain p18 (phosphorylated or not), p20, or p22, or no characteristic feature at all. Two kinds of changes, namely, accessory proteins and proteins disappearing from the spectrum, are manifested mainly in extracts of primary tumors as compared to homologous mucous coats of the stomach. Three classes of gastric tumors are distinguished according to the difference between tumor and normal cell polypeptides: I) no difference, II) isolated differences, and III) numerous qualitative and quantitative differences in gastropolypeptides and their phosphoforms.

Key Words: *gastric carcinoma; mucous cells; gastroproteins; Mg-dependent phosphorylation*

Genes responsible for the malignant transformation of cells of the mucous coat of the stomach (MCS) have not jet been found in man, although the role of hereditary predisposition, environmental proto- and oncogenes as well as of other initiating and promoting factors is being discussed [6,10]. Some such genes may be identified by screening the corresponding cDNA-expressing libraries for poly(A)-mRNA from tumor cells of the stomach by monoclonal antibodies against tumor-associated proteins (TAP) [7,11].

The aim of the present investigation was to study the synthesis and phosphorylation of gastroproteins in human gastric tumor cells and to indentify some TAP. It seemed fruitful to compare the phenotypic diversity of patterns of cancer cells with different degrees of differentiation with MCS cells in the same patients.

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MATERIALS AND METHODS

Surgical material obtained from 131 patients with pathomorphologically confirmed gastric carcinomas of different TNM stages (according to the international classification of the World Health Organization T reflects the spread of the primary tumor, N the state of regional lymph nodes, and M the presence or absence of metastases) [1]. The samples of control and tumor tissues were thawed from the liquid nitrogen and destroyed by 3-5-trial freeze-thawing. The thawed tissue was homogenized in a porcelain mortar with the addition of 0.2 M phosphate buffer (pH 7.5) at a ratio of 1:4 (weight/volume). Homogenates were stored at -70°C. For electrophoretic analysis homogenates were mixed with equal volumes of the same buffer containing a 2% solution of the nonionic detergent Nonidet P-40 (NP-40) to liberate membrane-bound proteins, carefully

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suspended, and then mixed with equal volumes of the sample buffer [8], suspended, and kept for 20 min. The samples were then boiled for 3 min in polyethylene tubes and centrifuged at 11,000 g and 4°C for 10 min. Supernatants were deposited on gel plates. To inhibit autoproteolysis the homogenates were treated with cold 95% ethanol at a ratio of 1:20 (v/v), kept for 30 min at -10°C, centrifuged at 11,000 g for 10 min, and freed of supernatant [4]. The sediments were resuspended in sample buffer and used for electrophoresis. For comparative phosphorylation of proteins *in vitro* [5] tissue homogenates in 0.2 M phosphate buffer were combined with equal volumes of the same buffer containing 0.2% NP-40, 200 mM KCl, and 20 mM $MgCl_2$, suspended, and placed in polyethylene tubes with equal amounts (2-3 μCi) of gamma-[^{32}P]ATP (≥5000 Ci/mmol, St. Petersburg). The phosphorylation reaction was performed at 4°C and halted by noncontact freezing of the mixture in liquid nitrogen. Immediately after thawing, the samples were mixed with sample buffer and routinely processed (3 min at 100°C, 10 min at 11,000 g). Phoregrams were dried and exposed with PM X-ray film at -70°C.

Electrophoresis [8] in gradient 10-18% polyacrylamide gel with 0.1% sodium dodecyl sulfate was performed in a specially designed chamber [3] with a contact refrigerator and vertically mounted 10×17× ×0.12 cm gel plate. A layer of concentrating gel with pockets for the samples was formed prior to electrophoresis in the upper part of the separating gel. Electrophoresis was performed for 9-10 h with a current of 6 mA and electric field intensity of 2-3 V/cm, and then for 3-4 h at 10 mA and 5 V/cm, respectively. The electrophoregrams were fixed in 50% methanol for 30 min, stained with Coomassie Brilliant Blue G-250, and washed in three changes of 10% isopropanol. Protein molecular weights were determined by means of a calibration curve with 67, 45 и 12.4 kD markers.

RESULTS

Comparison of the polypeptide spectra revealed in intact MCS cells in different patients showed a high degree of similarity of their qualitative characteristics. Extract proteins proved to be quite resistant to autoproteolytic degradation by endogenous proteinases in the weakly alkaline conditions of solubilization used. Only in some cases, in particular in extracts of the upper region of the greater curvature of the stomach, did the nonspecific inhibition of proteinase activity used turn out to be very effective. Along with this, 3 out of the 37 usually found poly-

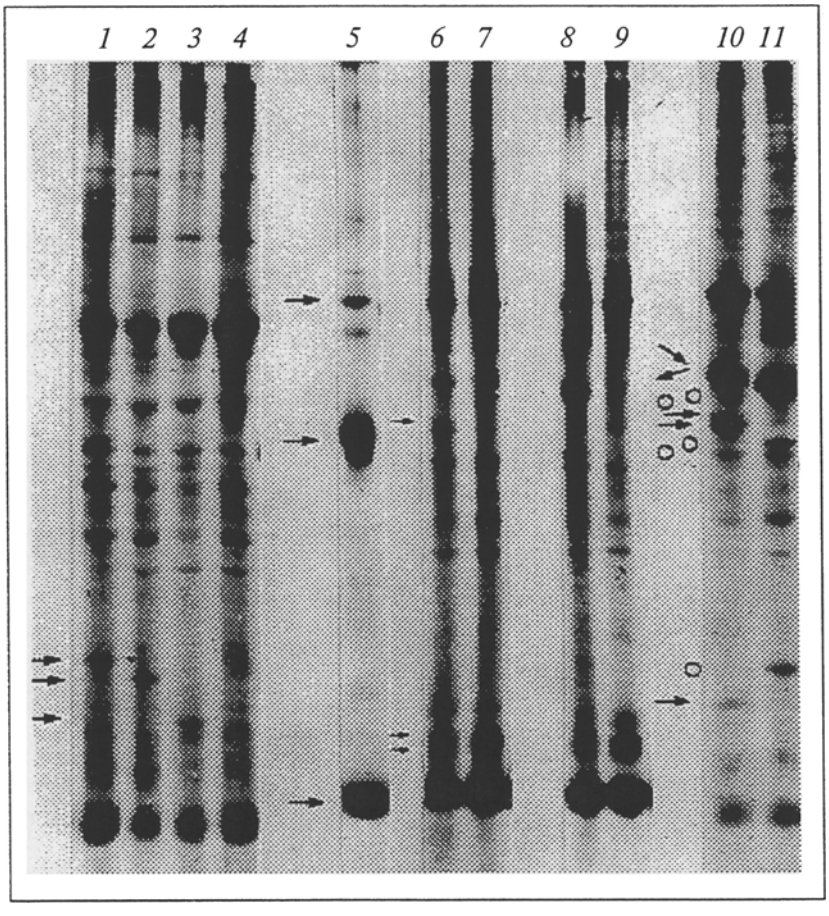

Fig. 1. Characteristics of gastropolypeptide synthesis in the cells of the mucous coat of the stomach in different patients (*1*: "p22" type, *2*: "p20" type, *3*: "p18" type, *4*: "—" type) and in tumor cells as compared to intact gastric mucous cell. *6*) transformed cells and *7*) nontransformed cells of patient Z.: additional polypeptide p46, coordinated decrease of p15 and p15.5 polypeptides in *6* (arrows); *8*) nontransformed cells and *9*) transformed cells of patient Ya.: additional p51 polypeptide (arrow), and disappearance of p50 and p42 polypeptides in tumor cells in *9* (circles); *10*) transformed cells and *11*) nontransformed cells of the patient P.: additional p18, p45, p46, and p51 polypeptides (arrows) and disappearance of p20, p42, and p50 polypeptides in tumor cells in *10* (circles); *5*) molecular weight scale, from bottom to top: cytochrome C 12.4 kD, ovalbumin 45 kD, bovine serum albumin 67 kD.

TABLE 1. Frequency of Occurrence of Associated Proteins in Human Gastric Tumor Cells, %

Feature, p	Accessory tumor proteins	Disappearing proteins	Tumor content	
			increase	decrease
71	-	-	4	-
56	16	-	-	-
51	24	12	-	-
50	8	40	-	-
48	-	4	-	-
46	12	8	-	-
45	4	-	-	4
42	-	32	-	-
37	-	-	-	4
30	-	-	-	8
28	4	-	-	-
24	-	4	-	-
20	8	28	-	-
19	10	8	-	8
18	16	10	4	-
16.6	-	4	-	-
15-15.5-16.6	-	-	12	-
15-15.5	-	-	-	8

peptides, p18, p20, and p22, displayed clear qualitative differences and the presence of p18 or p20 was characteristic of the majority of extracts studied. As a rule, synthesis of one of these peptides excludes the other two and in 10% of all cases none of these polypeptides were found in cell extracts of control MCS. In 8% of cases the level of the p50 content in normal MCS cells substantially exceeded the usual level, often correlating with the presence of p20; sometimes p50 was not found at all in normal MCS cell extracts. The specific characteristics found in the spectra of different parts of intact MCS confirmed the wisdom of comparing the polypeptides in tumors of different patients as related to those of normal MCS.

Proteins of tumor extracts exhibited resistance to autoproteolytic degradation and in the majority of cases differed distinctly from normal MCS protein composition (Fig. 1). Proteins whose content in tumors was lower than normal were found more often than proteins whose content was higher. In particular, the increased content of the p15-p15.5-p16.6 polypeptide cluster in more than 10% of patients is of significant interest due to the possible coordinated regulation of the expression of the corresponding genes (Table 1). There are two groups of very pronounced changes in tumors, namely accessory proteins and proteins disappearing from the spectrum (Table 1). The first group include p56, p51, p50, p46, p45, p28, p20, p19, and p18, p51 being found most often (in more than 20% of patients). Some proteins are not found in the spectra of tumor cell extracts, although they are detected in normal MCS, for example, p18, p20 (more than 25%), p42 (more than 30%), and p50 (in about 40% of patients).

In several cases the changes in the tumor spectra of different patients are identical or very like each other. In general the noted phenotypic diversity is restricted to 7 polypeptides associated with gastric tumor cells (p56, p51, p50, p46, p20, p19, and p18), poly- and monoclonal antibodies against which are of particular interest for the screening of corresponding cDNA-expressing libraries for the identification of the genes involved in the tumor process.

It was shown previously [12] that the activity of cAMP-dependent protein kinase type I rises in gastric tumors. The discovery of endogenous substrate for tyrosine protein kinase, pp36, along with the detection of pp60 *c-src* protein kinase activity in intestinal cells [2], lay the basis for a detailed investigation of Mg-dependent phosphorylation in normal MCS cells and gastric tumor cells.

It was found that some 20 gastroproteins are phosphorylated depending on the Mg content in the presence of gamma-[^{32}P]ATP as phosphate donor with the use of detergent-processed extracts in the studied pair of "intact MCS cells — tumor cells" (Fig. 2, Table 2). Intact MCS cells and tumor cells possessed different time kinetics of posttranslation

modification of this type, which was taken into account when choosing the optimal incorporation of radioactive label in proteosubstrates of Mg-dependent protein kinases.

A comparative study of gastroprotein phosphorylation in studied pairs in different patients revealed additional phosphorylation or increased content of pp94, pp80, pp54, pp49, pp34, and pp30 phosphoproteins, in particular, of pp18 (Table 2). Here the content of pp62 and pp42 phosphoproteins drops markedly and pp6 often disappears. Phosphoproteins whose content is high in tumors are found more often than phosphoproteins synthesized additionally as related to normal MCS. Although the phosphoprotein spectrum is less diverse, it reacts more dynamically to the switch to the transformed state than the spectrum of unmodified polypeptides. This phenomenon may be of interest in the study of regulatory mechanisms of gastric tumor cell proliferation and the role of Mg-dependent phosphorylation of gastroproteins in this process.

The comparative analysis of extracts from intact MCS cells revealed 4 main types, containing p18, p20, p22, or none of these components. About 80% of the patients possessed the "p18 type" or the "p20 type." The comparison of characteristics in pairs of "MCS cells — tumor cells" samples allow us to distinguish three classes of gastric tumors. Class I consists of tumors without differences of polypeptide and phosphoprotein patterns as related to the homologous mucous coat, class II possesses a few differences in patterns, and class III is characterized by numerous qualitative and quantitative differences in gastropolypeptides and their phosphoforms.

Tumors of classes II and III were found with approximately the same frequency (about 40% of cases each), while tumors of class I were revealed much less often (20% of cases). It cannot be ruled out that the marked pp42 variations in compared extracts are due to modulation of the functioning of the channel for transfer of the proliferation signals coupled with mitogen-activated protein kinase [9].

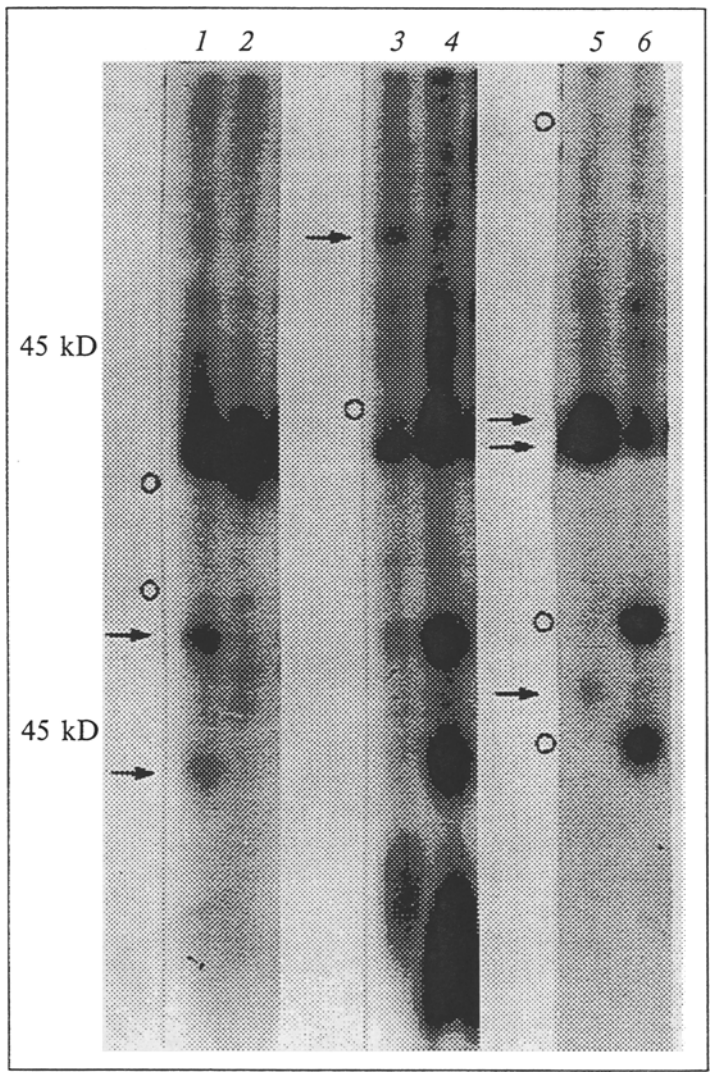

Fig. 2. Characteristics of Mg – dependent phosphorylation of gastropeptides in cells of mucous coat of the stomach and in tumor cells. *1*) transformed cells and *2*) nontransformed cells of patient P.: additional pp11 and pp18 phosphoproteins (arrows) and disappearance of pp20 and pp34 phosphoproteins in tumor cells in *1* (circles); *3*) transformed cells and *4*) nontransformed cells of patient Ts.: increased content of pp 94 phosphoprotein in the tumor in *3* (arrow) and disappearance of pp42 phosphoprotein in tumor cells (circle); *5*) transformed cells and *6*) nontransformed cells of patient Pi.: marked increase in content of pp12, pp38, and pp42 phosphoproteins tumor cells in *5* (arrows), and disappearance of pp11, pp18, and pp129 phosphoproteins in tumor cells (circles). At left is the molecular weight scale.

TABLE 2. Frequency of Occurrence of Associated Phosphoproteins in Human Gastric Tumor Cells, %

Feature, pp	Accessory tumor proteins	Disappearing proteins	Tumor content	
			increase	decrease
141	9	-	-	-
129	-	9	9	-
111	-	-	-	9
94	-	-	18	-
92	-	9	-	-
80	18	-	9	-
62	-	-	-	27
54	9	-	18	9
49	-	9	18	9
42	-	27	18	18
34	-	9	27	18
30	18	-	-	-
24	-	-	9	-
20	-	9	-	-
18	9	9	45	18
13	9	-	-	-
12	-	-	9	9
11	-	9	18	9
6	18	36	-	9

The discovery of discrete phenotypic classes in gastric tumors points to the existence of various, possibly alternative mechanisms of human MCS cell transformation.

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