

CYTOKERATIN 7/20 EXPRESSION PATTERNS AND ASSOCIATION WITH PROGNOSTIC VARIABLES IN GASTRIC ADENOCARCINOMAS

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ABSTRACT

Aim: The histologic features, potential behavior, genetic pathways, epidemiological characteristics and response to treatment of gastric carcinomas, is showing heterogeneity. Cytokeratin (CK), an intermediate-sized filament detected primarily in epithelial cells. Most commonly used CKs in immunohistochemical studies are CK7, CK20 and these molecules are used as a markers to help identify the site of origin of metastatic carcinomas. The aim of this study to evaluate the expression pattern of CK7 and CK20 molecules in primary adenocarcinoma of the stomach and their relation between clinicopathologic prognostic factors.

Material and method: Gastric adenocarcinoma specimens were obtained from 103 patients who underwent total / subtotal gastrectomy with extended lymphadenectomy between December 2011-December 2013.

Results: The 103 gastric adenocarcinomas included 80 (77.60%) cases with CK7 expression and 56 (54.30%) cases with CK20 expression, and were classified into 4 (3.80%) cases with CK7-/CK20 - pattern, 43 (41.70%) cases with CK7+/CK20 - pattern, 19 (18.40%) cases with CK7-/CK20+ pattern and 37 (36.10%) cases with CK7+/CK20+ pattern. CK expression patterns was not associated with additional variables which were age, gender, tumor location, size, depth of invasion, lymphatic and perineural invasion, lymph node and distal organ involvement, existence of *H. pylori* and intestinal metaplasia, histological type according to Lauren classification system and p53 expression.

Conclusion: The CK expression patterns cannot helpful in determining prognostic factors for gastric adenocarcinoma.

Key words: Gastric adenocarcinoma, cytokeratin 7, cytokeratin 20, clinicopathologic factors.

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Introduction

Cytokeratin (CK), an intermediate-sized filament detected primarily in epithelial cells. At least, approximately 20 subtypes of CKs have been categorized. These have various isoelectric pH, molecular weight and showed differential expression in distinct tumors depend on the degree of cellular differentiation^(1,2). Most commonly used CKs in immunohistochemical studies are CK7, CK20 and these molecules are used as a markers to help identify the site of origin of metastatic carcinomas^(3,4). CK7 is expressed in many glandular and ductal epithelia, including the lung, breast, ovary, and endometrium^(5,6) CK20 is expressed in the gastroin-

testinal epithelium, urotelium, and Merkel cells⁽²⁾. Gürbüz et al. approved that CK7 as an aberrant cytokeratin expressed by chronic inflammatory process and CK20 as an indicator of gastric mucosa maturation⁽⁷⁾.

The histologic features, potential behavior, genetic pathways, epidemiological characteristics and response to treatment of gastric carcinomas, is showing heterogeneity. Therefore, new prognostic markers in this tumor group is needed. Several studies in the gastrointestinal tract including colorectal carcinomas cytokeratin expression patterns and significance was investigated^(8,9). These studies have also been conducted in gastric carcinomas with a few studies⁽¹⁰⁻¹²⁾.

In this study, we examined the expression pattern of CK7 and CK20 molecules in primary adenocarcinoma of the stomach and their relation between clinicopathologic prognostic factors.

Methods

Clinical Materials

Primary gastric adenocarcinoma specimens were obtained from 103 patients who underwent total / subtotal gastrectomy with extended lymphadenectomy between December 2011-December 2013. The resected and examined lymph nodes were at least 15 for each case. No patients received chemotherapy or radiotherapy before surgery. The group of patients consisted of 70 males and 33 females with median age 63 (18-89) years. According to the seventh edition of the classification TNM-UICC/AJCC, depth of tumor invasion was recorded using T classification. Mucosal or submucosal tumor invasion was defined as T1, muscularis propria or subserosal tumor invasion was defined as T2, serosal tumor invasion was defined as T3, and invasion of adjacent structures was defined as T4⁽¹³⁾ But for statistically significant results in the calculation T2/T3/T4 were evaluated as a group. The existence of intestinal metaplasia and *Helicobacter pylori*, extent of lymphatic and perineural invasion, lymph node and distant organ involvement were also recorded and marked as present or absent. Also in cases of tumor size, tumor localization, and classification of the histological type was performed by Lauren system.

In the Lauren system, the gastric carcinomas classified as intestinal, diffuse, and mixed⁽¹⁴⁾. p53 immunoreactivity was assessed as being positive only nuclear staining, and expression was categorized into 2 groups: negative expression (less than 10% positive tumor cells) and positive expression (at least 10% positive tumor cells).

Immunohistochemistry

All specimens were fixed in formalin and embedded in paraffin. Serial sections (4- μ m) were prepared and one of them was stained with hematoxylin and eosin, while the others were used for immunohistochemistry. CK 7 - CK20 immunohistochemistry were carried out on the automated slide stainer (Ventana Benchmark XT). The primary antibodies we used OV-TL-12/30 (Novocastra, Newcastle Upon Tyne, UK, 1:75) for CK7 and Ks 20.8 (Novocastra, Newcastle Upon Tyne, UK,

1:250) for CK20.

The sections were counter-stained with hematoxylin.

CK Expression Profiles: CK7 and CK20 was evaluated assessing the cytoplasmic staining respectively and evaluated according to the CK7 and CK20 staining pattern and classified into four main groups:

- 1 no expression of CK7 and CK20 (CK7-/CK20-),
- 2 only CK7 expression (CK7+/CK20-)
- 3 only CK20 expression (CK7-/CK20+)
- 4) coexpression of CK7 and CK20 (CK7+/CK20+).

Statistical Analysis

Variables were expressed as median (minimum-maximum) or frequency and related percentage values. Between groups comparisons were performed by using Kruskal Wallis or Fisher-Freeman-Halton test. The level of significance was set at $\alpha=0.05$ and SPSS v.21 software was used for statistical analysis.

Results

We studied 103 patients with primary gastric adenocarcinoma. 11 (10.60%) had tumors with invasion of mucosa or submucosa (T1), 92 (89.40%) had tumors with invasion of muscularis propria (T2), subserosa (T3) and adjacent structures (T4). When evaluated according to tumor location, in 63 (%61.10) cases the proximal stomach and in 40 (%38.90) cases distal stomach were affected. In 60 of the cases (58.20%) while the tumor is larger than 5 cm in diameter, distant organ involvement, 70 of the patients (67.90%) viewed. According to Lauren classification system; the series comprised 59 (57,3%) intestinal types, 28 (27,2%) diffuse types, and 16 (15,5%) mixed types.

Lymph node metastases were detected histologically in 72 (69.90%) patients. Lymphatic invasion was seen in 51 (49.50%) patients and perineural invasion was present in 52 (50.40 %) patients. *H. pylori* was detected in 11 (10.60%) patients and patients with intestinal metaplasia were 56 (54.30%). In total, 53 cases (51,4%) were positive for p53.

CK Expression Profiles in GAs and Their Relation with Clinicopathologic Parameters

The 103 GAs included 80 (77.60%) cases with

CK7 expression and 56 (54.30%) cases with CK20 expression, and were classified into 4 (3.80%) cases with CK7-/CK20-pattern, 43 (41.70%) cases with CK7+/CK20-pattern, 19 (18.40%) cases with CK7-/CK20+ pattern and 37 (36.10%) cases with CK7+/CK20+ pattern.

Although not statistically significant, we observed that CK20 expression was more frequent in males and gastric proximal location. Additionally, lymph node involvement and distant organ metastases are seen more commonly in this expression pattern.

		CK7-/CK20- (n=4)	CK7+/CK20- (n=43)	CK7/CK20+ (n=19)	CK+/CK20 +(n=37)	p-value
Age		58.50(53-80)	65(18-89)	59(38-82)	61(32-88)	0.41
Gender	Male	2(50)	27(62.80)	13(68.40)	28(75.70)	0.495
	Female	2(50)	16(37.20)	6(31.60)	9(24.30)	
H. pylori	Absence	4(100)	38(88.40)	18(94.70)	32(86.50)	0.859
	Present	0(0.0)	5(11.60)	1(5.30)	5(13.50)	
Lymphovascular invasion	Absent	1(25)	24(55.80)	7(36.80)	20(54.10)	0.388
	Present	3(75)	19(42.20)	12(63.20)	17(45.90)	
Perineural invasion	Absent	1(25)	22(51.20)	10(52.60)	18(48.60)	0.858
	Present	3(75)	21(48.80)	9(47.40)	19(51.40)	
intestinal Metaplasia	Absent	2(50)	20(46.50)	8(42.10)	17(45.90)	0.987
	Present	2(50)	23(53.50)	11(57.90)	20(54.10)	
pT Stage	pT1	1(25)	2(4.70)	2(10.50)	6(16.20)	0.189
	pT2/3/4	3(75)	41(95.30)	17(89.50)	31(83.80)	
Lymph node metastasis	Absent	1(25)	16(37.20)	3(15.80)	11(29.70)	0.403
	Present	3(75)	27(62.80)	16(84.20)	26(70.30)	
Stomach location	Proximal	3(75)	25(58.10)	12(63.20)	23(62.20)	0.937
	Distal part	1(25)	18(41.90)	7(36.80)	14(37.80)	
Distant metastasis	Absent	4(100)	24(55.80)	14(73.70)	28(75.70)	0.127
	Present	0(0.0)	19(44.20)	5(26.30)	9(24.30)	
Tumor diameter	≤5 cm	1(25)	21(48.80)	5(26.30)	16(43.20)	0.367
	>5 cm	3(75)	22(51.20)	14(73.70)	21(56.80)	
Histologic type	Intestinal	2(50)	27(62.80)	9(47.40)	21(56.80)	0.575
	Diffuse	2(50)	12(27.90)	5(26.30)	9(24.30)	
	Mixed	0(0.0)	4(9.30)	5(26.30)	7(18.90)	
P53 expression	Negative	3(75)	23(53.50)	9(47.40)	15(40.50)	0.483
	Positive	1(25)	20(46.50)	10(52.60)	22(59.50)	

Table 1: Association between CK expression patterns and clinicopathologic characteristics.

Next, we analyzed the association between CK expression patterns and the clinicopathologic parameters including as age, gender, tumor location, size, depth of invasion, lymphatic and perineural invasion, lymph node and distal organ involvement, existence of H. pylori and intestinal metaplasia, histological type according to Lauren classification system and p53 expression. However, CK expression patterns was not associated with additional variables which mentioned above ($p>0.05$) (Table-1).

Discussion

One of the common causes of death due to cancer is gastric carcinoma. These tumors have a poor prognosis and in patients with advanced gastric carcinoma the median survival is 7-10 months⁽¹⁵⁾. Nowadays, for this tumor group many therapeutic strategies are developed. But, there are significant differences between patients with the same stage, for responses to treatment or prognosis.

Therefore more researches are essential to discover new distinct prognostic factors for effective therapies.

CK is the main intermediate filament protein of normal epithelium and epithelial neoplasm⁽¹⁾. Especially, in the gastrointestinal tract, while colorectal neoplasms show a CK7-/CK20+ expression pattern, gastric neoplasms show CK7+/CK20-expression pattern⁽²⁾. Some researches in the gastrointestinal tract including colorectal carcinomas cytokeratin expression patterns and significance was investigated^(8,9). Whereas, in literature there are few studies have data for CK expression patterns and prognostic significance in gastric carcinomas.

In previous studies, different CK expression patterns of gastric carcinoma was determined. The ratio of CK20+ gastric carcinomas has been declared to range from 30% to 70%, that of CK7+ tumors, from 30% to 80%^(16,17). The results achieved in this study are consistent with the findings of previous reports.

Takami et al. examined CK expression patterns in a large GAs series (n:870) and they demonstrated that were no significant differences between CK expression patterns and clinicopathologic prognostic parameters⁽¹⁰⁾. Our results are likewise consistent with outcome of this study. Also, similar findings were also obtained in other studies^(11,12).

In conclusion, our study fails to confirm that CK7/20 expression patterns have role in association between gastric adenocarcinomas and clinicopathologic prognostic factors. Gastric carcinomas show heterogeneous clinical behaviour, therefore no single factor has diagnostic value for prognosis. Additional studies are needed for its molecular process of development and progression.

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