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Cytokeratin 7 and 20 expression in gallbladder carcinoma

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Cytokeratin expression is being frequently used for the differential diagnosis of carcinomas originating from different sites. Among the various cytokeratins, the combination of cytokeratins (CKs) 7 and 20 is considered to be the most useful for this purpose. However, there are very few reports in the literature regarding CK7 and CK20 expression in gallbladder carcinoma (GBC). In this paper we studied the immunohistochemical expression of CK7 and CK20 in 42 GBC cases. In addition, we studied 25 randomly selected cases of lithiasis associated chronic cholecystitis (LaCC) as controls. CK7/CK20 immunoprofile was assessed in relation to tumour differentiation, depth of invasion, and p53 protein expression. Twenty-nine GBC cases (69.05%) were CK7-positive, and 12 cases (28.57%) were CK20-positive. Tumour differentiation was not correlated with CK7 or CK20 immunoreaction. Regarding tumour depth of invasion, the CK7+/CK20– group presented a significant correlation with early stage (T1) disease ($p = 0.04$). p53 expression was correlated with both CK7 ($p = 0.05$) and CK20 ($p = 0.023$) expression. All the cases of LaCC demonstrated diffuse intense CK7 positivity of the mucosal epithelium, while CK20 was only focally positive in 13/25 cases. Our results along with the data from the literature indicate that CK7/CK20 expression may be of clinical significance, and further investigation in this direction is needed.

Key words: gallbladder carcinoma, cytokeratin 7, cytokeratin 20, p53 protein.

Introduction

The cytoplasm of eukaryotic cells contains a cytoskeletal system of intermediate filaments, which include the keratin family of proteins. The various keratins are encoded by 54 distinct functional genes [1, 2]. Intermediate filaments are expressed in a highly specific cell type manner. In this regard, keratins are characteristic of epithelial cells. Traditionally, these proteins are divided into type I (acidic) and type II (basic to neutral) keratins. Keratins can constitute their filamentous stage by heteropolymeric pair formation of type I and type II (1 : 1) molecules. Beyond the biological importance of keratins in cell functions, keratin expression patterns do not only characterize cells as epithelial, they are also characteristic of different epithelial cell types [3]. In addition, epithelial tumours (carcinomas) usually retain the keratin expression profile of their normal epithelial origin. Thus, the determination of keratin expression by various carcinomas has been extensively used as a tool of tumour typing.

Cytokeratin 7 (CK7) is a type II cytoskeletal keratin that in humans is encoded by the *KRT7* gene [4], located on chromosome 12q13.13 [5]. Cytokeratin 20 (CK20) is a type I cytoskeletal protein that in humans is encoded by the *KRT20* gene [6], located on chromosome 17q21.2 [5]. Studies on various carcinomas suggest that the combined use of CK7 and CK20 may provide helpful information for the discrimination of the origin of metastatic tumours of unknown primary location [7-10]. Therefore, the CK7/CK20 expression pattern in primary gallbladder carcinoma (GBC) may facilitate the evaluation of

metastatic deposits in the region of the hepatic hilum and the abdomen and prevent potential pitfalls. On the other hand, comparison of the expression pattern of CK7 and CK20 in cases of chronic cholecystitis with lithiasis and in GBC may highlight possible alterations linking inflammatory and metaplastic changes and carcinoma. The CK7/CK20 expression investigation of a small number of primary gallbladder carcinomas has been included in a few published studies, usually in conjunction with intrahepatic and extrahepatic biliary tree tumours [11-14]. These studies provided discrepant results. In addition, only one study was performed on more than 11 cases of GBC and a substantial number of cases of adenoma, dysplasia and normal mucosa [11].

The aim of the present study was to investigate the coordinated expression of CK7 and CK20 in GBC, to compare the expression pattern in GBC with the expression pattern in the gallbladder mucosa in cases of lithiasis associated chronic cholecystitis (LaCC), to explore possible relationships of different expression patterns with histological parameters such as tumour differentiation, depth of tumour invasion and p53 protein expression, and to present previous experience on the subject through a systematic review of the literature.

Material and methods

The material of this study consisted of a total of 67 cholecystectomy specimens accessioned in the pathology departments of Agios Pavlos First General Hospital of Thessaloniki, and Euromedica General Clinic of Thessaloniki (Thessaloniki, Greece). It comprised 42 cases of gallbladder adenocarcinoma and 25 randomly selected cases of lithiasis associated chronic cholecystitis. Tissue sections fixed in 10% buffered formalin and embedded in paraffin were retrieved from our archives, re-cut, stained and reviewed by two pathologists using a double-headed light microscope. In GBC cases, tumour differentiation and depth of invasion were assessed. Tumours were divided into two groups regarding differentiation (well vs. moderate/poor), while the tumours were also divided into two groups regarding depth of invasion (T1 vs. T2/T3/T4). In 15 GBC cases where non-neoplastic mucosa was also available, we examined the immunoprofile of the normal tissue as well. Cases of LaCC were evaluated for the presence of intestinal and pseudopyloric metaplasia. Intestinal metaplasia was identified by the presence of goblet cells and/or columnar intestinal-type epithelial cells, while pseudopyloric metaplasia was identified as glands outside the neck region of the gallbladder resembling gastric antral glands in their general architecture and in the appearance of individual cells [15].

Immunohistochemistry

The immunohistochemical staining was performed on 4 μm thick sections using an avidin-biotin-peroxidase (ABC) staining kit (Novocastra, Newcastle, UK). The primary antibodies used and the corresponding antigen retrieval methods were as follows: anti-CK7 (Biogenex, San Ramon, CA, USA, clone OV-TL 12/30, dilution 1:80, trypsinization), anti-CK20 (Novocastra, clone Ks20.8, dilution 1 : 25, microwave pretreatment in Tris-EDTA buffer pH 9), and anti-p53 protein (YLEM, Rome, Italy, clone DO-7, dilution 1 : 70, microwave pretreatment in citrate buffer). Diaminobenzidine (DAB) was used as chromogen, and haematoxylin as counterstain. Sections in which the primary antibody was replaced by non-immune rabbit serum served as negative controls. Sections of colon carcinoma which was known to be positive for CK20 and p53, and sections of breast carcinoma which was known to be positive for CK7 were used as positive controls. Both cytoplasmic and cell membrane staining were considered as positive immunoexpression of CK7 and CK20, whereas only nuclear staining was accepted as positive for p53 protein expression. Immunoreaction was graded as follows: 0 = negative, 1 = focal staining (<25% of cells), 2 = moderate staining (25-75% of cells), and 3 = diffuse staining (>75% of cells).

Statistical analysis

The Fisher's exact test was used to analyse the frequency of each group in relation to the parameters studied.

Results

Gallbladder carcinoma

The mean age of the patients with GBC was 74.15 (±8.29, range 55-89). Twenty-nine cases (69.05%) were CK7-positive (Fig. 1), and 12 cases (28.57%) were CK20-positive (Fig. 2). CK7 positivity was strong and diffuse (3+) in 7 cases, moderate (2+) in 20 cases, and focal (1+) in 2 cases. CK20 positivity was strong and diffuse (3+) only in one case, moderate (2+) in 6 cases, and focal (1+) in 5 cases. The cytokeratin 7 and 20 immunoprofile in GBC was grouped in 4 distinct types of coordinate expression:

- CK7+/CK20+ (9 cases, 21.43%),
- CK7+/CK20– (20 cases, 47.62%),
- CK7–/CK20+ (3 cases, 7.14%),
- CK7–/CK20– (10 cases, 23.81%).

Tumour differentiation was correlated with neither CK7 nor CK20 immunoreaction considered alone, nor with any of the four distinct types of coor-

dinate CK7/CK20 expression (Table I). Regarding tumour depth of invasion, the CK7+/CK20- group presented a significant correlation with early stage (T1) disease ($p = 0.04$), while none of the rest immunoprofile groups showed any association with this parameter (Table II). p53 expression was correlated with both CK7 ($p = 0.05$) and CK20 ($p = 0.023$) expression considered alone, and even more with the CK7+/CK20+ group ($p = 0.0045$) where all nine tumours were p53-positive (Table III).

Epithelium adjacent to carcinoma

The neighbouring non-carcinomatous mucosa was CK7+ in 14 of the 15 cases in which it was available for study, whereas it was CK20– in all cases.

Lithiasis associated chronic cholecystitis

All the cases of LaCC demonstrated diffuse intense CK7 positivity of the mucosal epithelium. CK20 staining was generally negative with the exception of one or more small isolated foci of CK20 positivity in 13 out of 25 cases. In 8/13 cases the isolated foci of CK20 positivity were associated with histological evidence of intestinal metaplasia. Four of these cases also showed pseudopyloric metaplasia.

Discussion

Carcinomas constitute the vast majority of malignant tumours that arise in humans, and have a propensity to metastasize to regional lymph nodes and then to other organs, such as the lungs, the brain, the liver, and the bones. Many times a malignant tumour presents itself to the patient, and the clinician by its manifestations at the metastatic site. The determination of the primary site of the metastasis is a challenge to both oncologists and pathologists, having potentially important clinical and therapeutic consequences. For this purpose, many

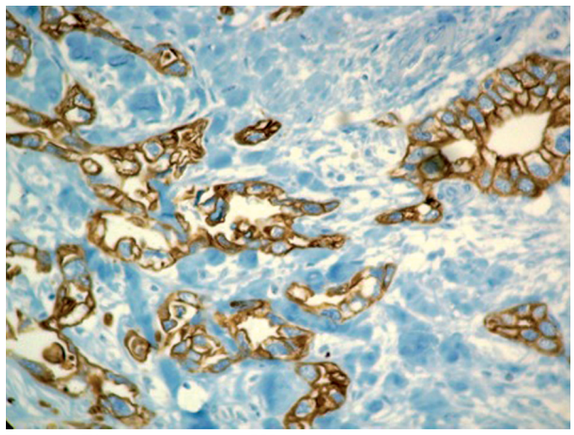

Fig. 1. Fine membrane CK7 staining of gallbladder adenocarcinoma (DAB/haematoxylin, magnification 400×)

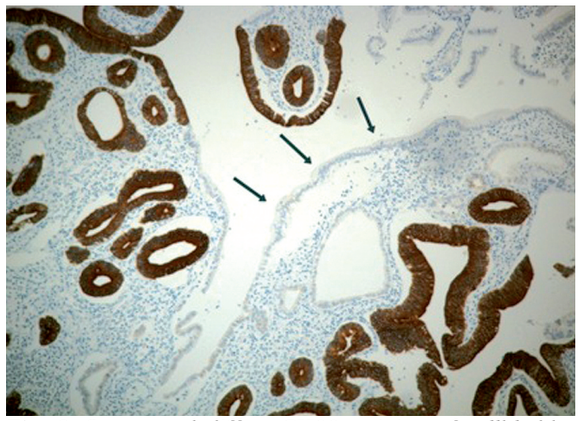

Fig. 2. Strong and diffuse CK20 staining of gallbladder carcinoma. Note that the adjacent normal mucosa (arrows) is negative (DAB/haematoxylin, magnification 100×)

Table I. Carcinoma immureaction profile in relation to tumor differentiation

IMMUNOREACTION	WELL DIFFERENTIATED	MODERATELY/POORLY DIFFERENTIATED	STATISTICS
CK7+	12 (41.37%)	17 (58.62%)	NS*
CK7–	3 (23.07%)	10 (76.92%)	
CK20+	4 (33.33%)	8 (66.66%)	NS
CK20–	11 (36.66%)	19 (63.33%)	
CK7+/CK20+	3 (33.33%)	6 (66.66%)	NS
Remainder	12 (36.36%)	21 (63.63%)	
CK7+/CK20–	9 (45%)	11 (55%)	NS
Remainder	6 (27.27%)	16 (72.72%)	
CK7–/CK20+	1 (33.33%)	2 (66.66%)	–
Remainder	14 (35.90%)	25 (64.10%)	
CK7–/CK20–	2 (20%)	8 (80%)	NS
Remainder	13 (40.62%)	19 (59.37%)	

**NS – not significant*

Table II. Carcinoma immureaction profile in relation to the tumour depth of invasion

IMMUNOREACTION	EARLY	ADVANCED	STATISTICS
CK7+ CK7–	8 (27.58%) 1 (7.69%)	21 (72.41%) 12 (92.31%)	NS*
CK20+ CK20–	1 (8.33%) 8 (26.66%)	11 (91.66%) 22 (73.33)	NS
CK7+/CK20+ Remainder	1 (11.11%) 7 (21.87%)	8 (88.88%) 25 (78.12%)	NS
CK7+/CK20– Remainder	7 (35%) 2 (9.09%)	13 (65%) 20 (90.90%)	p = 0.04
CK7–/CK20+ Remainder	0 9 (23.07%)	3 (100%) 30 (76.92%)	–
CK7–/CK20– Remainder	1 (10%) 9 (27.27%)	9 (90%) 24 (72.72)	NS

**NS – not significant*

Table III. Carcinoma immunoreaction profile in relation to p53 protein expression

IMMUNOREACTION	P53 (+)	P53 (–)	STATISTICS
CK7+ CK7–	20 (68.96%) 5 (38.46%)	9 (31.03%) 8 (61.53%)	p = 0.05
CK20+ CK20–	11 (91.66%) 14 (46.66%)	1 (8.33%) 16 (53.33%)	p = 0.023
CK7+/CK20+ Remainder	9 (100%) 16 (48.48%)	0 17 (51.51%)	p = 0.0045
CK7+/CK20– Remainder	11 (55%) 6 (27.27%)	9 (45%) 16 (72.72%)	NS*
CK7–/CK20+ Remainder	2 (66.66%) 23 (58.97%)	1 (33.33%) 16 (41.02%)	–
CK7–/CK20– Remainder	3 (30%) 22 (68.75%)	7 (70%) 10 (31.25%)	p = 0.030

**NS – not significant*

immunohistochemical markers have been used in the past two decades in order to aid the diagnosis of the origin of a carcinoma. Some of them lack organ specificity at all, such as carcinoembryonic antigen (CEA) and epithelial membrane antigen (EMA), but still are widely used in order to define a tumour as a carcinoma instead of another large category of tumours like lymphoma or sarcoma. Some other markers, such as prostate specific antigen (PSA) or thyroglobulin, are very organ-specific.

Even though CKs are not considered specific tumour markers, their highly diverse expression patterns enable the classification of epithelial cells and their neoplasms into different subtypes [3, 16]. The combined expression of CK7 and CK20 in carcinomas has been found to be useful in the differential diagnosis of some carcinomas of epithelial origin, and especially in the differential diagnosis between metastatic colon and ovarian adenocarcinomas [8, 9, 17-20] and the differential diagnosis between lung, endometrial, and breast adenocarcinomas and colon adenocarcinoma [10, 21-23]. In addition, CK7 is almost always positive in cholangiocarcinoma, while hepatocellular carcinoma is consistently CK7 and CK20 negative [7, 24].

CK7 expression was found in more than two thirds of our GBC cases, while CK20 expression was confined to less than one third of our cases. CK7 expression in the vast majority of GBC cases was also found in the previously published studies [11-14]. In the largest published study, which included 131 GBC cases, Chang *et al*. reported that 87% of tumours were CK7-positive, and 18% were CK-20 positive, proportions of cases that are similar to ours [11]. Likewise, Duval *et al*. reported that most of the extrahepatic bile duct tumours (including 11 from

the gallbladder) were CK7-positive and CK20-negative [12]. On the contrary, Cabibi *et al*. found that all extrahepatic bile duct carcinomas (including 10 from the gallbladder) were CK7+/CK20+ [13]. In addition, on the basis of the latter findings, these authors suggested that CK20 expression by extrahepatic bile duct carcinomas is the hallmark of the sequence metaplasia-dysplasia-carcinoma.

Chang *et al*. on the other hand, who studied the expression of CK7/CK20 as well as the expression of mucins, stated that normal gallbladder mucosa has a gastric phenotype in terms of mucin expression, which is gradually lost during malignant transformation [11]. The intestinal phenotype (including CK20 expression), they continue, is uncommon in the gallbladder, but it may play a role in the malignant transformation of premalignant lesions of the organ. In accordance with this view, we found a CK7+/CK20– profile in the normal mucosa adjacent to carcinoma, and frequent CK20-positive foci in cholecystitis associated intestinal metaplasia. Moreover, we found that early stage (T1) carcinomas had a CK7+/CK20– immunoprofile, i.e., the same as that of normal gallbladder mucosa. Chang *et al.* did not find any association between CK7/CK20 immunoprofile and depth of invasion, but they observed a correlation between loss of CK7 expression and survival of the patients, which was worse in this event [11]. In this regard, we found an association between CK7-positive and CK20-positive tumours and p53 protein expression, which was stronger in double positive tumours. *P53* is known to be the gene most frequently mutated in human malignant tumours, and very often associated with an aggressive phenotype [24]. So, it could be that CK20-positive tumours may present a more aggressive behaviour, even though there is no evidence to support this view at present.

In conclusion, similarly to cholangiocarcinoma, and the majority of extrahepatic bile duct carcinomas, most GBCs are CK7-positive. The CK7+/CK20– immunoprofile of the normal gallbladder mucosa seems to be retained in early stage GBCs, while loss of this phenotype may be related to tumour progression. A minority of GBCs is CK20-positive, representing perhaps an alternative pathway of carcinogenesis in the gallbladder through an intestinal phenotype. The limited data of the literature combined with our results indicate that CK7/CK20 expression may be of clinical significance, so further investigation in this direction is warranted.

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