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EDITORIAL

Markers of bile duct tumors

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Abstract

Biliary tract carcinomas are relatively rare, representing less than 1% of cancers. However, their incidence has increased in Japan and in industrialized countries like the USA. Biliary tract tumors have a poor prognosis and a high mortality rate because they are usually detected late in the course of the disease; therapeutic treatment options are often limited and of minimal utility. Recent studies have shown the importance of serum and molecular markers in the diagnosis and follow up of biliary tract tumors. This review aims to introduce the main features of the most important serum and molecular markers of biliary tree tumors. Some considerable tumor markers are cancer antigen 125, carbohydrate antigen 19-9, carcinoembryonic antigen, chromogranin A, mucin 1, mucin 5, alpha-fetoprotein, claudins and cytokeratins.

Key words: Bile duct tumors; Cholangiocarcinoma; Tumor markers; Carbohydrate antigen 19-9; Chromogranin A

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INTRODUCTION

Biliary tract carcinomas are relatively rare, representing less than 1% of cancers[1]. However, an increase in their incidence and mortality in Japan and industrialized countries like the USA has been noted[2,3].

Gallbladder adenocarcinoma and cholangiocarcinoma (CCA) account for 4% and 3% of all gastrointestinal cancers respectively[4,5]. These malignancies are highly fatal with 1- and 2-year survival rates of 25% and 13% respectively[6]. CCAs can be anatomically classified into intrahepatic (including hilar) or extrahepatic tumors. 60%-70% of bile duct tumors arise in the bifurcation of the hepatic ducts (Klatskin tumors), 20%-30% in the distal common bile duct while 5%-10% of CCAs arise within the intrahepatic ducts of the liver parenchyma[7].

Intrahepatic cholangiocarcinomas (HI-CCAs) originate in the small bile ducts and tend to be grouped with hepatocellular carcinoma (HCC) as primary liver tumors[8,9].

In general, most malignant bile duct tumors present with painless obstructive symptoms which include pale stools, dark urine and jaundice. Increased symptoms in the course of the pathology such as right upper quadrant abdominal pain, fever and rigors are indicative of superimposed cholangitis[10].

The diagnosis of biliary tract disease is realized by ultrasonography (US), computed tomography (CT) or magnetic resonance imaging (MRI) of the liver and magnetic resonance cholangiopancreatography (MRCP) or endoscopic retrograde cholangiopancreatography (ERCP). Intrahepatic CCA still remains an unfortunate liver tumor with a high mortality rate as many patients diagnosed with CCA cannot be offered a curative treatment[11]. Recent studies have also shown the utility of serum and molecular markers in the diagnosis and follow up of biliary tract tumors. Since these cancers are usually detected late in the course of the disease, treatment options are often limited and of minimal utility. Thus, research efforts have concentrated on identifying potential markers of neoplasia that can be incorporated into diagnostic tests and therapeutic modalities for use in individuals at risk for these lethal malignancies. This review aims to introduce the main features of the most important serum and molecular markers of biliary tree tumors.

CANCER ANTIGEN 125

Cancer antigen 125 (CA 125) is elevated in 58% of patients with gallbladder cancer and in 40%-50% of cholangiocarcinoma patients[12] but it has a low specificity; in fact, its serum levels can increase in other gastrointestinal or gynaecological malignancies and several cholangiopathies[13]. It tends to increase in patients with ascites and is considered a possible indicator of peritoneal involvement.

CA 125 is more specific than carbohydrate antigen 19-9 (CA 19-9) in detecting CCA and in distinguishing between benign and malignant causes of bile duct obstruction. It is not easily influenced by bile duct inflammation or calculi while CA 19-9 is increased in cases of cholangitis and hepatolithiasis.

Carbohydrate antigen 19-9

CA 19-9 is a glycolipid synthesized by the pancreatic, biliary, gastric, colonic cells, endometrial and salivary epithelia. CA 19-9 is used as a screening tool for CCA in patients with primary sclerosing cholangitis (PSC). Elevated concentrations of serum CA 19-9 in bile duct cancers have been frequently reported: a rate of 97% was found in CCA patients by Torzilli *et al*[14], Hultcrantz *et al*[15] reported a rate of 76% and Caturelli *et al*[16] a rate of 68%. Nichols *et al*[17] showed that a serum CA 19-9 value greater than 100 U/mL predicted the presence of CCA in patients with a sensitivity of 89% and a specificity of 86%. CA 19-9 has an increasing role in the differential diagnosis of benign and malignant hepatobiliary and pancreatic conditions. Benign conditions of the hepatobiliary and pancreatic tract, such as Mirizzi syndrome, autoimmune pancreatitis, benign biliary stenosis secondary to PSC and pancreatic exocrine dysfunction, have been associated with abnormal elevation of CA 19-9[18-21]. If bile flow is blocked by biliary obstruction in benign conditions such as choledocolitiasis, epithelial cells will be impaired by inflammation and will proliferate at the same time[22]. As a result, more CA 19-9 may be released into the bloodstream. In such conditions, the increase of CA 19-9 is associated with hyperbilirubinemia secondary to biliary obstruction, and subsequent values of CA 19.9 tends to normalize after the restoration of biliary drainage[20,21]. The increase of CA19-9 is also useful to predict resectability of CCA[23,24]. A marked elevation of serum CA 19-9 is associated with advanced and unresectable biliary cancers. Elevated levels of CA 19.9 are correlated with advanced disease and poor prognosis and high preoperative values correlate with poor survival.

Carcinoembryonic antigen

Carcinoembryonic antigen (CEA) is a highly glycosylated cell surface glycoprotein with a molecular weight of 180 000 and is considered a colon-specific oncofetal protein[25]. CEA is often found in patients with malignant tumors of the digestive system such stomach, colon, biliary tract and pancreas cancer. Qin *et al*[26] reported a significant increase of CEA serum levels in CCA patients. In their study, the sensitivity and specificity rates of serum CEA were 68.57% and 81.52% respectively. Similar results (63.3% and 78.4%) were shown by Ramage *et al*[27]. Various studies have found a higher sensitivity of CA 19-9 in comparison with CEA values in diagnosis of CCA but the combination of these two markers increases the sensitivity and specificity. The following formula is suggested as a screening test for CCA patients with PSC: CA 19-9 + (CEA × 40). An index greater than 400 U/mL has a positive predictive value of 100%, a specificity of 100% and a sensitivity of 67%. Recently, Chalasani *et al*[28] demonstrated that an index greater than 400 U/mL was no better than a 100 U/mL one in predicting the presence of CCA.

CHROMOGRANIN A

Chromogranin A (CgA) is an acidic glycoprotein contained in the secretory granules of neuroendocrine (NE) cells[29]. It is widely used as an immunohistochemical marker of neuroendocrine tumors (NETs). It may also serve as a serum marker and is co-secreted with the amines and peptides of the neurosecretory granules[30]. It is a very sensitive, specific marker of NETs. The most common site of origin for carcinoid tumors is the gastrointestinal system, followed by the bronchopulmonary system (25%). Apart from those arising from the ampulla of Vater, intrahepatic bile duct, gallbladder and cystic duct, carcinoid tumors of the extrahepatic bile duct are extremely rare. Primary carcinoid tumors may arise from endocrine cells of the body and fundus of gallbladder or from pre-existing endocrine cells present in the

mucus membrane of the gallbladder neck.

Elevated serum levels of CgA are also reported in patients with colon, lung, breast, liver and prostate cancers[31-35] and are related to a tumor NE differentiation which has a prognostic importance in several malignancies[36]. CgA is also considered a significantly independent, negative prognostic marker for CCA patients[37].

MUCIN-1 AND MUCIN-5AC

Mucins are heavily O-glycosylated proteins, mainly expressed by ductal and glandular epithelial tissues. The human mucin family is divided into secreted and transmembrane forms. The secreted forms are mucin-2 (MUC2), mucin-5AC (MUC5AC), mucin-5AB (MUC5AB) and mucin-6 (MUC6) that are released from the apical membrane in order to form a physical barrier that protects the epithelial layer from adverse conditions such as exposure to commensally bacteria, ingested toxins and reactive oxygen species (ROS). Among the secreted mucins, MUC2 is the most correlated with inflammation and cancer. MUC2 suppresses inflammation and inhibits the development of intestinal tumors[38,39]. A recent study has shown that the loss of MUC2 expression in mice is associated with an augmented proliferation and survival of intestinal epithelial cells responsible for an increased exposure to luminal contents and induction of inflammation[40]. Transmembrane mucins include mucin-1 (MUC1), mucin-4 (MUC4), mucin-13 (MUC13) and mucin-16 (MUC16) which also contribute to forming physiological barriers and transmitting growth and survival signal to the cells. Mucin genes are expressed in several cells and tissues: in particular, specific MUC2 and MUC3 are expressed in bowel[41] while MUC5AC and MUC6 in gastric tissues[42]. Alterations of quantity and quality of mucins occur in cancer tissues, like in the case of CCA[43,44]. Various degrees of MUC1 glycosylation between normal and tumor cells have been demonstrated in several tumors.

MUC1 is a transmembrane glycoprotein frequently found in the developing intrahepatic bile ducts of fetal liver[45] but not in the normal adult intrahepatic biliary tract[46,47]. For this reason, it is an oncofetal antigen in the intrahepatic biliary tree[45]. An increased expression of MUC1 has often been demonstrated in many tumors, including CCA[48,49], and its over expression is correlated with poor prognosis and unfavourable survival. High MUC1 immunoreactivity is associated with vascular invasion and malignant progression in three different ways. Firstly, tumor cells expressing high levels of MUC1 can repel each other[50] favoring the development of metastases. Secondly, vascular invasion involves the binding between ligand (E-selectin) of endothelial cells and epitopes (sialyl Lewis) on tumor cells[51,52]. In fact sialyl Lewis, a carbohydrate epitope which is present at high levels in tumor cells and serum of CCA patients, has been identified as an epitope of MUC1[53]. Thirdly, high MUC1 levels on the membrane of tumor cells suppress the immunity of patients, inhibiting the interaction between cytotoxic lymphocytes and tumor cells[54]. The expression of MUC1 can help the development of metastases, particularly in the liver. Therefore, it is often difficult to distinguish a metastatic nodule from HCC. Xu *et al*[55] noticed that KL-6 mucin, a type of MUC1 mucin, is expressed in intrahepatic CCA but not in HCC, demonstrating its usefulness in differential diagnosis. Sasaki *et al*[56] have found that the expression of MUC1 apomucin in small bile ducts is correlated with biliary epithelial damage and the consecutive immune-mediated processes. In fact, cytotoxic T cells recognize MUC1, preferentially expressed on epithelial tumors.

While MUC1 expression reflects histological differentiation, increased proliferative activity and invasiveness, MUC2 expression is correlated with a decrease in proliferative activity.

MUC5AC is a secreted gel-forming mucin aberrantly expressed in CCA tissues; indeed, it is considered an excellent biomarker for CCA. High MUC5AC expression is correlated with tumor size and metastases which are responsible for the poor outcome of CCA patients who have positive serum MUC5AC status. Moreover, increased MUC5AC expression is related to neural invasion.

In conclusion, the expression of MUC1 and MUC5AC mucins is associated with metastasis and is considered a useful prognostic marker for a poor outcome in CCA patients.

PROMYELOCYTIC LEUKEMIA, P53 AND DPC4

Promyelocytic leukemia (PML) is a tumor suppressor gene implicated in the pathogenesis of several malignancies like leukaemia and plays a role in the regulation of apoptosis, cell growth and DNA repair[57]. It is considered a novel molecular cancer marker. PML protein expression is reduced in many tumors such as prostate, colon, breast, lung carcinomas and lymphomas. PML has a prognostic significance in human gallbladder cancer (GBC). In this case, the loss of PML expression is related to poor prognosis, metastatic lymphatic invasion and stage of GBC, suggesting that the protein may be involved in GBC progression. Patients with normal PML and p53 expression have a better prognosis than those with abnormal expression of PML and/or p53[58].

Many studies have shown a relationship between p53 mutations and GBC, reporting that the rate of p53 over expression varied from 30.6% to 72.6%[59-61]. P53 mutations are correlated to histological type, tumor grade and survival time[62]. Various studies suggest that biliary tract carcinomas have a different frequency of p53 immunoreactivity depending on their site of origin (gallbladder, intrahepatic, proximal and distal common bile duct), perhaps reflecting different pathogenic factors in their respective etiologies[62-64].

DPC4 is another tumor suppressor gene localized on the long arm of chromosome 18. It is inactivated in 55% of pancreatic adenocarcinomas and in bile duct carcinomas. Compared with their more proximal (intrahepatic

and perihilar) counterparts, distal common bile duct carcinomas show a higher frequency of loss of DPC4 gene expression and of p53 over expression.

Ki67

Ki67 antigen is exclusively expressed in proliferating cells (in the G1, S, G2 and M phases) and not in quiescent cells (G0 phase). Ki67 is considered a useful marker for clinical use because of its reliability[65]. High Ki67 serum values are associated with poor prognosis in some tumors such as breast and oesophageal cancers and lymphomas[66].

Biliary tract cancer patients with higher Ki67 index tend to have a low survival rate since such high levels lead to an increased speed in the growth of tumor cells.

Transmembrane carbonic anhydrase isoenzyme IX

Carbonic anhydrase (CA) plays a crucial role in various physiological processes. It catalyses the interconversion of carbon dioxide in bicarbonate. Eleven isoenzymes, differing in their tissue distribution and enzymatic activity, have been identified in mammals[67-70] and several isoenzymes have found in the hepatobiliary tract. Biliary epithelial cells express cytoplasmic carbonic anhydrase Ⅱ (CA Ⅱ), an apical membrane-associated carbonic anhydrase Ⅳ (CA Ⅳ) and a basolateral transmembrane carbonic anhydrase isoenzyme Ⅸ (MN/CA Ⅸ)[71,72], while in hepatocytes the most frequent isoenzyme is the mitochondrial carbonic anhydrase VA (CA VA)[73,74].

The expression of MN/CA Ⅸ is confined to the basolateral surfaces of normal biliary epithelial cells, whereas no positive reaction is found in hepatocytes.

Most of biliary epithelial tumors express MN/CA Ⅸ while only few HCCs show positive immunoreactions, suggesting that MN/CA Ⅸ can be considered a useful biomarker for the differential diagnosis of hepatobiliary neoplasms.

MATRIX METALLOPROTEINASES

Tumors invade the basement membrane through the secretion of enzymes that digest the extracellular matrix (ECM) proteins and allow angiogenesis. These enzymes are matrix metalloproteinases (MMPs). They belong to family of zinc-dependent endopeptidases, responsible for the degradation of all components of the ECM[75]. MMPs-related factors also increase the proliferation of tumor cells promoting the mitosis. MMPs activity is balanced by tissue inhibitors of metalloproteinases (TIMPs)[76,77]. In particular, matrix metalloproteinase 2 (MMP-2) and matrix metalloproteinase 9 (MMP-9) focalize their action on collagen Ⅳ, the major component of the basement membrane[76-78]. *In vitro* studies have shown that microvascular endothelial cells do not constitutively secrete MMP-9; however, when exposed to an angiogenic stimulus, like tumor necrosis factor-α (TNF-α), MMP-9 production is up-regulated[79]. Furthermore, MMP-9 plays a fundamental role in the catalytic activity of tumor cell invasion, metastasis and in the regulation of angiogenesis[80,81].

It seems that the up-regulation of MMP-9 is linked with cyclooxygenase-2 (COX-2) expression which is induced by TNF-α and increased in all inflammatory states, thus in an organism affected by cancer[82]. Recent studies have noticed that MMPs and cyclooxygenases (COXs) are over expressed in CCA cells. However, Leelawat *et al*[83] did not show significant differences in MMP-9 levels between CCA patients and the controls, as in the case of serum matrix metalloproteinase 7 (MMP-7) values. In fact, the presence of higher serum MMP-7 levels in CCA patients than those with benign biliary tract disease was demonstrated.

MMP-7 is expressed by epithelial cells[84]. Therefore, it cannot be considered as a specific marker of bile duct tumors but its expression in CCA is an unfavorable postoperative prognostic factor[85].

Another important member of this family is represented by MMP-2. Kirimlioğlu *et al*[86] analyzed the role of MMPs in every type of biliary tract cancer and reported the presence of MMP-2 expression in 75% of the distal part of the biliary ducts, and also GBC, distal CCA and ampullary carcinoma expressed MMP-2 in 30%, 37% and 40% of the cases respectively. They also showed that MMP-2 and MMP-9 levels were higher in subjects with neural invasion although they demonstrated no correlation between the expression of MMPs and tumor differentiation and the presence of metastasis.

Erb-B

The Erb-B family consists of four distinct receptors: ErbB1 (EGFR), ErbB2, ErbB3 and ErbB4. All these receptors are composed of three parts: an extracellular ligand-binding domain, a transmembrane lipophilic domain and a conserved cytoplasmic tyrosine kinase domain[87,88].

In particular, C-erb-B2 is an oncogene situated on chromosome 17 and is also known as neu or HER-2[89,90]. The protein expressed by this gene serves as receptor of the epidermal growth factor (EGF) and so it plays an important role in the angiogenesis. EGFR is involved in different human cancers such as crest, ovarian, skin, kidney, pancreas, lung, salivary glands and digestive tract tumors[91]. Zheng *et al*[92] also showed an increase of C-erb-B2 expression in extrahepatic cholangiocarcinoma (EH-CCA). Particularly, a significant difference of the expression of Erb-B in correlation with the grade and differentiation of the tumor has been found. The comparison between tumor grading Ⅲ-Ⅳ and Ⅰ and the highly and less differentiated tumors revealed a significant difference, suggesting that C-erb-B-2 could be involved in the processes of development, invasion of tumor and metastasis.

ALPHA-FETOPROTEIN

Alpha-fetoprotein (AFP) is a fetal glycoprotein with a molecular weight of about 72 kDa. It was first described in human fetus in 1956 and successfully assumed a key role in the diagnosis and follow up of HCC[93,94]. Under physiological conditions, it is synthesized by fetal hepato-

cytes, yolk sac cells and gastrointestinal cells. Some days after birth, AFP serum levels begin to decrease until they gradually get to a level lower than 10 ng/mL. The development of radioimmunoassay for AFP has increased its sensibility[95-100] and nowadays it is considered a useful marker for embryonal cell carcinoma and liver diseases. However, some studies have demonstrated the possible use of this marker in the primary neoplasm of the gastrointestinal tract. McIntire *et al*[101] showed an increase of AFP values in patients with pancreas, biliary tract and stomach carcinomas in comparison with those affected by colon, esophagus and small bowel carcinomas. AFP is not only an indicator of cell de-differentiation but also an important sign of hepatic stem cells[102]. Jalanko *et al*[103] reported a slight increase of serum AFP concentrations only in a small percent of patients.

AFP is the main serum biomarker of HCC but its variant, lectin-reactive AFP (AFP-L3), has been demonstrated to be useful in the diagnosis of intrahepatic CCA. Okuda *et al*[104] noticed that AFP-positive patients presented HCC features which were very different from those of classical intrahepatic cholangiocarcinoma (IH-CCA) whose patient were seropositive to CA 19-9. They supposed that the IH-CCA seropositive for AFP-L3, HCC and CCA might have originated from hepatic precursor cells with the characteristic of both hepatocytes and bile duct cells.

N-CADHERIN

E and N-cadherins are expressed on hepatocytes and HCC cells membrane[105,106]. N-cadherin expression is liver-specific. Hepatocytes and intrahepatic biliary epithelial cells express this marker on their plasma membrane while the extrahepatic bile ducts do not present this marker. This finding could be explained through the different embryological origins of extrahepatic and intrahepatic ducts. Mosnier *et al*[107] demonstrated that N- and E-cadherins are present on the membrane of IH-CCA tumor cells with a rate of 66% and 45% respectively. N-cadherin expression is more frequent in peripheral CCAs than in hilar ones while EH-CCAs are negative for N-cadherin expression. The N-cadherin membranous expression plays an important role in distinguishing intrahepatic and perihilar CCAs from most HCCs and digestive tract tumors such as pancreatic adenocarcinoma.

VASCULAR ENDOTHELIAL GROWTH FACTOR-C

Vascular endothelial growth factor-C (VEGF-C) is considered a lymphangiogenic factor acting *via* VEGF receptor-3 (VEGFR-3). It is expressed by lymphatic endothelial cells and stimulates the growth of tumor-associated lymphatic vessels[108,109].

One of the main features of CCA is its lymph node metastasis. The lymphatic invasion and lymph node metastasis are important prognostic factors for IH-CCA[110,111]. Aishima *et al*[112] demonstrated that the density of the lymphatic vessels is lower in the center than in the tumor periphery and the tumor invasion into the lymphatic vessels is detected in the peritumoral area. Poorly differentiated tumors present higher density of lymphatic vessels in the tumor periphery and peritumoral area than in the tumor central area. VEGF-C expression of cancer cells is correlated with lymph node metastasis in IH-CCA[113]. The lymphatic invasion in IH-CCA does not relate to the lymphangiogenesis but another mechanism is involved in the lymphatic spread. In fact, the preexisting lymphatic vessels in the tumor margin are sufficient for lymphatic metastasis. Recent studies have shown that VEGF-C is associated with lymphonodal metastasis and supports the entry of cancer cells into peritumoral lymphatic vessels[114].

CLAUDINS

Claudins are transmembrane proteins and indispensable components of tight junctions[115,116]. The tight junctions play an important role in the maintenance of epithelial cell polarity[117,118]; they function as a barrier against the paracellular diffusion of solutes and also seem to regulate the growth and differentiation of cultured cells[119-123]. Therefore, modifications of their constituents could lead to alterations of their functions.

Claudine is a family of 24 members, differentially expressed in various types of tissues. Németh *et al*[124] showed that claudins are differently expressed in various compartments of the biliary tract. They analyzed the different types of Claudine in six sample groups (normal intrahepatic and extrahepatic bile ducts, normal gallbladder and carcinomas in the correspondent locations). Significant results have been reported for claudin-2 and in claudin-4. In particular, claudin-2 expression was stronger in GBC than in intrahepatic and extrahepatic bile duct cancers but a higher presence of claudin-4 has been found in extrahepatic bile duct cancers. Previous studies had already demonstrated the importance of claudin-4 for its ability to differentiate biliary tract cancers from hepatocellular tumors[125,126]. On the contrary, claudin-2 causes leakiness to the tight junction complex which becomes less restrictive for ions[127,128]. Therefore it plays a key role in bile concentration and in the processes of reabsorption in the proximal tubule and in the thin descending limb of Henle's loop[125]. Nemeth *et al*[124] also found a stronger expression of claudin-1 and claudin-10 in intrahepatic bile duct cancer than in extrahepatic bile duct cancer and in GBC.

THROMBOSPONDIN-1

Cancer growth is made possible by angiogenesis, regulated by angiogenic and antiangiogenic factors such as thrombospondin-1 (TSP-1), interferon alpha/beta, platelet factor-4, angiostatin and endostatin[129,130]. In such a way, TSP-1 is a multifunctional matrix protein which is highly expressed, like its receptors, in the desmoplastic

stroma and the basement membrane, and is associated with tumors such as breast cancer and fibroadenoma[131]. Nevertheless, its over expression in endothelial cells has been demonstrated to inhibits tumor genesis[132]. Such results have also been shown by Kawahara *et al*[133] who compared mRNA levels of TSP-1 and VEGF in CCA and HCC tumor cells in order to find a possible correlation between their expression and the different vascularization. They demonstrated that the up-regulation of TSP-1 and the down-regulation of VEGF in tumor cells may have a role in the hypovascularity of CCA when compared with HCC which is hypervascularized by the up-regulation of VEGF[133].

CYTOKERATINS

Cytokeratins (CKs) are a complex subclass of the intermediate filaments gene family, made up of more than 20 different polypeptides. They are classified in type A or class I (CK9- CK20) and type B or class II (CK1-CK8)[134-136]. The expression of CKs is generally confined to epithelia and their neoplasms[137,138]. They are not specific tumor markers but the presence of distinct expression patterns of CKs in the various pathways of epithelial differentiation has been shown[137,139,140]. In human cells, we can find from 2 to 10 types of CKs and, especially in the human liver, there is a different distribution in hepatocytes and in bile duct cells. CK8 and 18 are present in hepatocytes whereas CK7, 8, 17, 18 and 19 characterize bile duct cells. Among them, CK7 and CK19 can be considered as markers of this tissue and they could be used to differentiate CCA from HCC[134,135]. Stroescu *et al*[141] reported that the association of CKs and CEA makes the differential diagnosis between HCC and CCA more sensible. Cytokeratin immunophenotype of an adenocarcinoma can also help in the identification of its primary site. Several studies have shown the usefulness of CK17 as antibody for the identification of pancreaticobiliary adenocarcinoma and in the distinction of pancreaticobiliary and gastric adenocarcinomas when the primary site of the tumor is occult[142].

SERUM CYTOKERATIN 19 FRAGMENT

Citokeratins are intermediate filaments which are part of the cytoskeleton of epithelium. Serum cytokeratin 19 fragment (CYFRA 21-1) is considered a useful marker for non-small-cell lung cancer[143] and a prognostic factor for many tumors such as cervical[144], esophageal[145], breast[146] and gastric[147] cancers.

Uenishi *et al*[148] showed the association between CYFRA 21-1 serum concentrations and the tumor stage of CCA. Serum CYFRA 21-1 high values are correlated to tumor progression and poor post operative outcomes in CCA patients. The treatment of these patients includes not only surgery but also adjuvant therapy with gemcitabine combined with 5-fluorouracil or platinum, considered as first-line chemotherapy for advanced biliary tract carcinomas[149,150].

Moreover, CYFRA 21-1 is considered a useful marker in the differential diagnosis between CCA and HCC. Thus, hepatocytes and bile duct cells show a different cytokeratin pattern[151] that is maintained during malignant transformation.

Other markers

It is often very difficult to distinguish biliary disorders from bile duct and pancreatic cancers. However, Alvaro[152] analyzed the possibility of discriminating CCA from benign biliary disorders and pancreatic cancers through the analysis of the biliary concentration of insulin-like growth factor 1. Furthermore, Tangkijvanich *et al*[153] recently demonstrated the usefulness of combined use of AFP and glypican-3 to differentiate HCC from other liver cancers, like IH-CCA, because of the complementary role of these two markers. Such markers could be useful to discriminate benign from malignant neoplasm or other pathologies, but the possible presence of bile duct cancers could also be indicated by immune system alteration and its specific markers.

Enjoji *et al*[154] noticed that RCAS1, a tumor associated antigen present in immune diseases, can be expressed in biliary tract carcinomas. This protein has a defensive role against the immune attack inducing apoptosis of immune cells. RCAS1 high levels lead to a rapid development of the neoplasia and its expression may be a specific event taking place in immune-mediated diseases. Its role in immune diseases is useful to distinguish malignant diseases from non-immune inflammatory biliary diseases such as drug-induced cholestasis and cholelithiasis.

Another proof that the immune system plays a fundamental role in the development of biliary tract cancers is the increase of heat shock protein expression, chaperone molecules that are present in several stress conditions[155,156]. In particular, Romani *et al*[157] noticed that the immunohistochemical expression of heat shock protein 27-kDa (HSP27) is correlated with patient survival and thus it could be considered a prognostic factor in IH-CCA. This study demonstrated that HSP27 acts to inhibit apoptotic cell death while HSP72 is correlated with the presence of necrosis and lymphoid infiltration.

CONCLUSION

Biliary tract cancers are relatively uncommon malignancies. Although the entire biliary tract is potentially at risk, the perihilar region is the most involved site, accounting for about 60% of all these tumors[158]. However, an increase in the incidence of intrahepatic cancer in the US and the UK has also been shown[159].

Biliary tract cancers are characterized by slow growth but, because of the late presentation of their symptoms, they are usually diagnosed in advanced stages when the majority of therapeutic choices are not curative[158]. This leads to integrating the use of markers in order to formulate a better diagnosis and therapeutic approach.

In general, the first approach is a complete surgical resection that is possible with acceptable morbidity and

mortality[160]. The possibility of applying this therapeutic choice is influenced by the tumor mass. Several studies have suggested a correlation between the size of the tumor and tumor markers levels. Particularly, Gerhardt *et al*[161] found that initial levels of CA 19-9 in perihilar CCA patients who underwent to resection were lower than in those affected by unresectable disease. Similar results were found by Kau *et al*[162] in relation to periampullary carcinoma. The levels of tumor markers are also important to indicate a possible prognosis. Fernández-Ruiz *et al*[163] showed a worse prognosis in patients with elevated serum levels of CA 19-9 at the time of diagnosis for values > 270 IU/L.

Chemotherapy is another possible treatment option in patients with advanced biliary tract cancer. Patients with a good performance status can benefit from a combined chemotherapy consisting of two of the following drugs: gemcitabine, 5-FU/FA or capecitabine or a platinum analog[164-166]. New discoveries about the role of markers, such as VEGF and ErbB, have led towards targeted therapy. However, trials with HER2-neu or EGFR inhibitors such as lapatinib or erlotinib have shown little or no activity in advanced biliary system adenocarcinomas[167,168].

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