Biochimica et Biophysica Acta 1844 (2014) 988–1002

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

Review

Proximal fluid proteomics for the discovery of digestive cancer biomarkers ☆

Annarita Farina *

Biomedical Proteomics Research Group, Department of Human Protein Sciences, Geneva University, Geneva CH-1211, Switzerland

ARTICLE INFO

Article history:
Received 11 July 2013
Received in revised form 15 September 2013
Accepted 22 October 2013
Available online 1 November 2013

Keywords:
Bile
Pancreatic juice
Cyst fluid
Gastric juice
Saliva
Ascites

ABSTRACT

Most digestive malignancies have asymptomatic course, often progressing to poor outcome stages. Surgical resection usually represents the only potentially curative option but a prior assumption of the malignant nature of the lesion is mandatory to avoid exposing patients to unnecessary risks. Unfortunately, currently available diagnostic tools lack accuracy in many cases, consequently more reliable markers are needed to improve detection of malignant lesions. In this challenging context, fluids surrounding digestive malignancies represent a valuable source for the search of new potential biomarkers and proteomic tools offer the opportunity to achieve this goal. The new field of proximal fluid proteomics is thus emerging in the arena of digestive cancer biomarker discovery.
In the present review, the state-of-the-art of proteomic investigations aimed at identifying new cancer biomarkers in fluids surrounding gastrointestinal malignancies is summarized. A comprehensive catalog of proteomic studies in which potential cancer biomarkers from gastrointestinal fluids have been identified and assessed for their diagnostic performances is also provided. This article is part of a Special Issue entitled: Biomarkers: A Proteomic Challenge.

1. Introduction: Unmet clinical needs in the diagnosis of digestive malignancies

Digestive malignancies refer to a heterogeneous group of cancers affecting he gastrointestinal tract and associated organs. Among them, liver, stomach, colon-rectus, esophagus and pancreas cancers represent five of the ten leading causes of cancer death, accounting for nearly 3 million of all estimated deaths per year in the world [1]. The reason for such a high mortality is mainly related with the common asymptomatic course of these malignancies, which often results in late detection, at an advanced stage of the disease [2]. Surgical resection usually represents the only potentially curative option but it requires a reliable assumption of the malignant nature of the lesion because oncological digestive surgery is associated with an important morbidity and mortality [3]. Unfortunately, gastroenterologists and digestive surgeons are frequently challenged by clinical situations where the lesion is difficult to detect or to diagnose. Currently available diagnostic tools (e.g., imaging, serum biomarkers) lack accuracy in many cases to differentiate between nonmalignant and malignant lesions. Resulting diagnostic uncertainties expose patients to potential harmful risks. In the next sections, I report on the clinical details of digestive cancers for which proteomic studies of proximal fluids have been reported. The limitations of differential diagnosis between malignant and nonmalignant diseases are also discussed.

1.1. Liver cancer

Liver cancer represents the fifth most frequently diagnosed cancer and the second most frequent cause of cancer death in men. The highest number of cases is diagnosed in Asia, with China accounting for 50% of all estimated cases [1]. A significant increase in liver cancer incidence and death rates was observed in USA from 2005 to 2009 and an overall 5-year survival rate of 15% has been reported. Early stage diagnosis occurs in only 40% of cases and is associated with a 28% 5-year survival rate [2]. Hepatocellular carcinoma (HCC) mostly arises from liver cirrhosis [4] and accounts for 70% to 85% of the total primary liver cancers [1]. HCC is clinically difficult to distinguish from other hepatic benign masses (e.g., focal nodular hyperplasia, hepatocellular adenoma, regenerative nodule and hemangioma) with which it shares common histologic features [5]. Pre-neoplastic hepatocellular lesions in cirrhotic patients (e.g., dysplastic nodules mimicking small HCC) may also complicate the differential diagnosis [6]. In a recent study involving 638 liver transplanted patients with cirrhosis, ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI) disclosed a sensitivity of 46%, 65%, and 72%, respectively, for the detection of HCCs ranging between 2 cm and 4 cm, and 21%, 40%, and 47%, respectively, for smaller lesions. The current gold standard serum biomarker for HCC, alpha-fetoprotein (AFP), also showed an inadequate sensitivity in detecting HCC (53% at 10 ng/dL cutoff level) [7]. Other existing serum

☆ This article is part of a Special Issue entitled: Biomarkers: A Proteomic Challenge.
* Tel.: +41 22 379 5451; fax: +41 22 379 5502.
E-mail address: Annarita.Farina@unige.ch.

1570-9639/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbapap.2013.10.011

biomarkers (e.g. lens culinaris agglutinin-reactive AFP (AFP-L3), des-γ-carboxy prothrombin (DCP)) display suboptimal sensitivity when assessed alone [8]. Their contribution to the current standard AFP is currently being evaluated and their clinical use is not yet recommended [9,10].

1.2. Pancreas cancer

In the USA, pancreas cancer accounts for 44,000 new cases each year and has become the fourth leading cause of cancer mortality with 37,000 new estimated deaths in 2013 [2]. From 2004 to 2008, the incidence and death rates of pancreatic cancer have been increasing by 1.5% and 0.4% per year, respectively [11]. The 5-year survival rate is 6% for all diagnosed patients and 2% for patients diagnosed at an advanced stage [11]. Infiltrating ductal adenocarcinoma (or pancreatic adenocarcinoma (PAC)) represents about 90% of all pancreatic tumors [12]. Among imaging techniques, multi-detector computed tomography (MDCT) and endoscopic ultrasonography (EUS) are widely accepted as the methods of choice for diagnosing and staging pancreatic cancer [13]. A recent retrospective study, conducted on 117 patients, revealed MDCT to have limited performances for detecting pancreatic tumors (93% sensitivity, 72% specificity, 95% PPV, 65% NPV and 90% accuracy). The sensitivity, specificity and diagnostic accuracy of EUS–FNA for differentiating nonmalignant vs. malignant pancreatic solid masses is 83%, 100% and 88%. In patients with indeterminate or negative findings at initial EUS–FNA and a high clinical suspicion for pancreatic cancer, repetition of EUS–FNA is strongly advised [14]. However, these figures have been reported by dedicated endosonographers and may be significantly lower in the community [15].

No specific biomarkers exist for pancreatic cancer and the gold standard serum carbohydrate antigen 19-9 (CA 19-9) has a low specificity and limited clinical utility to differentiate nonmalignant from malignant masses [16,17].

1.2.1. Pancreatic cystic neoplasms

Pancreatic cysts represent approximately 10%–15% of the primary masses of the pancreas and consist in a heterogeneous group of malignant and nonmalignant lesions sharing many common clinical features [18,19]. Although cystic tumors of the pancreas are generally uncommon (2% of all pancreatic tumors), the prevalence of pancreatic cystic lesions is rising due to improved detection related to the increased use of cross-sectional imaging. Recent studies estimated the prevalence of cystic lesions between 2.6% and 44.7% [20]. The differential diagnosis between malignant, premalignant and nonmalignant pancreatic cysts, as well as the identification of the histological type, is a complex and highly relevant clinical problem. Actually, the accuracy of CT and MRI in making a correct diagnosis ranges between 40% and 60%. EUS also shows limited performances to distinguish between cystic tumors (56% sensitivity, 48% specificity) [20]. Finally, fluid cytology shows a specificity of about 100% but a much lower sensitivity for identifying malignancies [20].

1.3. Malignant biliary stenosis

Malignant biliary stenosis may arise due to several of the above-mentioned malignancies, as a consequence of the extrinsic compression of intra- or extra-hepatic bile ducts by a tumor affecting an adjacent organ (e.g., pancreas, liver, gallbladder), or it may be caused by an intrinsic tumor of the bile duct (cholangiocarcinoma (CC)) or of the ampulla (ampullary carcinoma (AC)). Pancreatic head adenocarcinoma represents the most common cause of malignant biliary stenosis, followed by biliary tract tumors: gallbladder cancer (GBC) and CC. GBC usually arises in the fundus or neck of the gallbladder [21]. It is a rare malignancy but represents, by itself, 80–95% of all biliary cancers [22]. CC, instead, arises from the biliary epithelium mainly at the bifurcation of the hepatic ducts (60–70%). Neoplasms in the distal common bile duct or peripheral intrahepatic ducts are also possible although less frequent (20%–30% and 5%–10%, respectively) [23]. Both CC and GBC have a very poor prognosis [21,24]. Adenocarcinoma of the ampulla of Vater is the third cause of malignant biliary stenosis. For this malignancy, obstructive jaundice occurs relatively early during the course of the disease and its evolution is slower. Consequently, AC presents a relatively better prognosis, with a 5-year survival rate ranging from 33% to 68%, compared to 6% for PAC and 5% for CC [11,21,24]. Finally, bile duct compression by liver cancer is also possible [25].

Besides malignant causes of bile duct stenosis, nonmalignant obstructions can also develop following inflammation associated with bile ducts injuries (e.g., surgery, trauma, pancreatitis, bile duct stones, primary sclerosing cholangitis (PSC)) [26] and represent about 25% of all biliary stenoses [27]. Differentiating malignant vs. nonmalignant biliary stenoses is clinical challenging. All currently available techniques, including cross-sectional imaging (e.g., EUS, CT, MRCP), endoscopic retrograde cholangiopancreatography (ERCP) and biliary brush or grasp cytology, show suboptimal accuracy. As a consequence, uncertainty about the nature of the stenosis persists in up to 50% examinations [14,28–31]. Furthermore, standard serum biomarkers are, on the one hand, generally unreliable to detect bilio-pancreatic malignancies and, on the other hand, significantly altered by the presence of a biliary obstruction (e.g., CA 19-9) [32].

1.4. Stomach cancer

Stomach cancer accounts for 8% of new cancer cases and 10% of cancer deaths in the world; it represents the third most frequent cause of cancer death in men. Over 70% of new cases occur in developing countries [1]. The incidence of stomach cancer has declined in most parts of the world due to a decreasing prevalence of *Helicobacter pylori* infection as well as to improved diet, hygiene and food storage practices [1,11]. Nevertheless, this malignancy carries a poor prognosis with an overall 5-year survival rate of 26% (62% in the case of early diagnosis). Accuracy of many common screening tools (including barium-meal X-ray, US, CT and MRI) has shown to be inadequate for detecting both, advanced and early-stage gastric cancers [33]. The differential diagnosis between malignant and nonmalignant gastric ulcers may also be difficult due to common morphological characteristics. Virtual gastroscopy (VG) and ES actually offer the best performances in distinguishing malignant from nonmalignant gastric ulcers, with an almost similar accuracy (92.0% and 88.6%, respectively) [34,35]. Finally, a recent screening conducted on a total of 13,118 participants in Portugal revealed that serum pepsinogen (PG) shows an insufficient performance to detect gastric cancer, with an estimated sensitivity, specificity, positive predictive value, and negative predictive value of 67%, 47%, 2% and 99%, respectively [36].

1.5. Peritoneal cancer

The inner membrane lining the abdominal cavity (peritoneum) can be affected by primary or secondary neoplasms. Primary peritoneal neoplasms are rare and usually arise from the mesothelial cell layer. They include malignant peritoneal mesothelioma, well-differentiated papillary mesothelioma, multicystic mesothelioma, desmoplastic small round cell tumor and peritoneal serous carcinoma [37]. Secondary peritoneal neoplasia is much more common and can occur by direct invasion from contiguous organs or through the seeding of cancer cells via the intraperitoneal fluid [37]. Metastases of the peritoneum are frequent in the presence of advanced ovarian and gastrointestinal (e.g., colorectal, pancreatic, gastric) tumors and represent the leading cause of death in most cases. Peritoneal carcinomatosis has long been considered virtually incurable, with an average life expectancy of 6 months, and chemotherapy protocols have proven to increase median survival by only 4 to 14 months [38,39]. The combination of cytoreductive surgery with hyperthermic intraperitoneal chemotherapy