Biochimica et Biophysica Acta 1844 (2014) 1018–1025

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Bile carcinoembryonic cell adhesion molecule 6 (CEAM6) as a biomarker of malignant biliary stenoses ☆

Annarita Farina [a,*], Jean-Marc Dumonceau [b], Paola Antinori [a], Isabelle Annessi-Ramseyer [a,c], Jean-Louis Frossard [b], Denis F. Hochstrasser [a,c], Myriam Delhaye [d], Pierre Lescuyer [a,c]

[a] Biomedical Proteomics Research Group, Department of Human Protein Sciences, Geneva University, Geneva CH-1211, Switzerland
[b] Division of Gastroenterology and Hepatology, Geneva University Hospitals, Geneva CH-1211, Switzerland
[c] Clinical Proteomics Laboratory, Department of Genetic and Laboratory Medicine, Geneva University Hospitals, Geneva CH-1211, Switzerland
[d] Department of Gastroenterology, Erasme Hospital, Free University of Brussels, Brussels BE-1070, Belgium

ARTICLE INFO

Article history:
Received 14 March 2013
Received in revised form 13 June 2013
Accepted 14 June 2013
Available online 24 June 2013

Keywords:
Pancreatic cancer
Cholangiocarcinoma
Proteomics
iTRAQ
CEACAM6
NCA-90

ABSTRACT

Differentiating malignant from nonmalignant biliary stenoses is challenging. This could be facilitated by the measurement of cancer biomarkers in bile. We aimed at (i) identifying new cancer biomarkers by comparative proteomic analysis of bile collected from patients with a malignant or benign biliary stenosis (exploratory phase) and (ii) verifying the accuracy of the newly identified potential biomarkers for discriminating malignant versus nonmalignant biliary stenoses in a larger group of patients (confirmation phase). Overall, 66 proteins were found overexpressed (ratio > 1.5) in at least one cancer condition using proteomic analysis and 7 proteins were increased in all malignant/nonmalignant disease comparisons. Preliminary screening by immunoblot highlighted carcinoembryonic cell adhesion molecule 6 (CEAM6), a cell surface protein overexpressed in many human cancers, as an interesting candidate biomarker. ELISA subsequently confirmed CEAM6 as a potential bile biomarker for distinguishing malignant from benign biliary stenoses with a receiver operating characteristic (ROC) area under the curve (AUC) of 0.92 (specificity 83%, sensitivity 93%, positive predictive value 93%, and negative predictive value 83%). No significant difference in serum CEAM6 level was found between malignant and nonmalignant samples. Combining bile CEAM6 and serum CA19-9 in a panel further improved diagnostic accuracy for malignant stenoses (AUC 0.96, specificity 83%, sensitivity 97%, positive predictive value 93%, and negative predictive value 91%). CEAM6 measurement in bile could be clinically useful to discriminate between malignant and nonmalignant causes of biliary stenosis. This article is part of a Special Issue entitled: Biomarkers: A Proteomic Challenge.

1. Introduction

Common bile duct stenoses are most often caused by pancreatic adenocarcinoma (PAC), the fourth leading cause of cancer death in the United States [1]. Other etiologies include primary bile duct carcinoma (i.e., cholangiocarcinoma, CC), adenocarcinoma of ampulla of Vater, and several benign diseases, such as chronic pancreatitis (CP) and primary sclerosing cholangitis [2,3]. Not rarely, biliary stenoses are also caused by bile stones (BS) or surgical injuries [4,5].

Etiological diagnosis of biliary stenosis is a critical problem for clinicians since early identification of malignant stenoses would enable the rapid resort to surgical resection, which currently represents the only potentially curative option [6,7]. A number of methods have been proposed for this purpose, including imaging techniques and pathological examination of endoscopic biliary samples, but all are plagued by relatively poor accuracy and negative predictive value [8,9]. In particular, the negative predictive value of the most recognized technique, i.e. endoscopic ultrasound–guided fine needle aspiration (EUS–FNA), for the diagnosis of pancreatic malignant masses was found to be of only 72% in a large review [10]. Of note, these results were almost exclusively reported by large, dedicated, centers and a recent survey suggests that the diagnostic performance of EUS–FNA is much poorer in the community [11]. Furthermore, standard serum biomarkers of pancreatic cancer and of cholangiocarcinoma, such as the carbohydrate antigen 19-9 (CA19-9), have not proved to be of any benefit because of an insufficient positive predictive value [12–14]. Resulting diagnostic uncertainties regularly lead to inadequate and potentially harmful disease management. New biomarkers of malignant

Abbreviations: PAC, pancreatic adenocarcinoma; CC, cholangiocarcinoma; CP, chronic pancreatitis; BS, bile stones; EUS–FNA, endoscopic ultrasound–guided fine needle aspiration; ERCP, endoscopic retrograde cholangiopancreatography

☆ This article is part of a Special Issue entitled: Biomarkers: A Proteomic Challenge.

* Corresponding author at: Biomedical Proteomics Research Group, Department of Human Protein Sciences, Faculty of Medicine, Geneva University, Rue Michel Servet, 1, Geneva CH-1211, Switzerland. Tel.: +41 22 3795451; fax: +41 22 3795502.
E-mail address: Annarita.Farina@unige.ch (A. Farina).

1570-9639/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbapap.2013.06.010

biliary stenoses are therefore needed to complement current diagnostic tools.

We and others have shown that bile represents a valuable source of potential biomarkers for malignant biliary stenoses [15–20]. If a tumor obstructs the duct, bile accumulates upstream from the stenosis and proteins released from the tumor tissues gather in there, becoming more easily detectable and quantifiable than in any other circulating body fluid (e.g., serum). In this context, proteomic analysis of bile allowed the discovery of proteins whose level is directly related to the presence of the tumor. Proteomics relies on powerful analytical methods that are able to identify hundreds of proteins in a complex biological sample and to provide quantitative information about changes in their expression in response to pathological conditions. It is based on rapidly evolving technologies (e.g. mass spectrometry) allowing one to gain increasing knowledge of the human proteome in health and disease [21].

In the present study, we first conducted a comparative proteomic analysis on bile samples collected from four patients with malignant and nonmalignant biliary stenoses, with the goal of identifying proteins overexpressed in malignant conditions. Immunoblots were then performed to highlight the best potential biomarker candidate. Finally, results obtained with the selected protein, CEAM6, were verified on crude bile samples from a larger cohort of patients using a commercial enzyme-linked immunosorbent assay (ELISA).

2. Materials and methods

2.1. Sample collection

A total of 41 bile samples were collected during endoscopic retrograde cholangiopancreatography (ERCP) from patients presenting with biliary stenoses of various etiologies, including PAC (n = 23), CC (n = 4), ampullary adenocarcinoma (n = 2), CP (n = 8), biliary stones (BS, n = 2), and other benign stenoses (n = 2) (Table 1). Bile was collected upstream to the bile duct stenosis before contrast medium injection. Clinical diagnosis was determined by pathological examination. All of the patients were previously uninstrumented. The study protocol was approved by the Ethics Committees of the Geneva University Hospitals (Geneva, Switzerland) and the Erasme University Hospital (Brussels, Belgium). Written informed consent to the study protocol was obtained from all the patients.

Table 1
Demographic data, serum concentrations of total bilirubin, direct bilirubin, CA19-9, bile concentrations of CEAM6 in 41 patients enrolled in the study. Corresponding iTRAQ labels, Western Blot lane numbers and densitometric values are also reported for the sub-cohort of 4 and 37 patients subjected to quantitative analysis and immunoblot verification.

Gender	Age (years)	Diagnosis	TBIL (μmol/L)	DBIL (μmol/L)	CA19-9 (kU/L)	CEAM6 (pg/mL)	iTRAQ label	WB[a] lane no.	WB[a] CA19-9 (O.D.)	WB[a] CEAM6 (O.D.)
F	83	Pancreatic cancer	252	134	2484	155,768		1	2452	3574
M	65	Pancreatic cancer	289	163	602	86,764		2	3471	7332
M	76	Pancreatic cancer	396	234	675	111,663		3	5828	13,181
M	60	Pancreatic cancer	351	180	615	360,478		4	13,050	17,346
M	77	Pancreatic cancer	80	40	883	262,360		5	11,000	10,125
M	72	Pancreatic cancer	127	63	98,8	297,285		6	2286	14,488
F	73	Pancreatic cancer	210	114	3599	168,748		7	5201	11,205
M	52	Pancreatic cancer	129	73	4972	81,126	115	8	6609	7343
F	90	Pancreatic cancer	19	9	>5000	17,104		9	2113	943
F	61	Pancreatic cancer	245	141	4002	311,304		10	9764	19,295
F	78	Pancreatic cancer	164	87	778	90,001		11	3539	11,186
F	94	Pancreatic cancer	319	173	>5000	315,704		12	15,643	16,870
F	81	Pancreatic cancer	22	12	384	713,524		27	5286	23,351
M	66	Pancreatic cancer	206	108	53,7	141,208		28	5858	15043
F	94	Pancreatic cancer	171	88	2,78	76,458		29	0	6841
M	95	Pancreatic cancer	576	312	<2,5	88,676		30	0	5819
F	75	Pancreatic cancer	250	153	615	461,335		31	50	13,928
M	61	Pancreatic cancer	212	122	>5000	764,742		32	18,662	19,884
M	63	Pancreatic cancer	256	157	494	208,794		33	639	12472
M	57	Pancreatic cancer	66	38	458	98,195		–	–	–
F	68	Pancreatic cancer	63	26	52,1	18,285		–	–	–
M	99	Pancreatic cancer	389	216	>5000	908,490		–	–	–
M	69	Pancreatic cancer	106	51	10,6	482,601		–	–	–
M	73	Cholangiocarcinoma	245	120	934	290,653		17	4754	15,435
M	69	Cholangiocarcinoma	192	93	1235	139,728		18	297	7136
M	77	Cholangiocarcinoma	93	52	274	79,917	114	19	1886	8790
F	80	Cholangiocarcinoma	293	167	226	182,825		20	1039	13,016
F	75	Ampullary adenocarcinoma	147	82	<2,5	207,532		23	0	9180
M	62	Ampullary adenocarcinoma	202	107	196	109,225		24	5682	8689
M	70	Chronic pancreatitis	29	3	7,88	89,710	117	13	713	239
M	50	Chronic pancreatitis	10	2	16,2	8816		14	0	0
M	49	Chronic pancreatitis	10	2	3,42	14,285		15	0	413
M	50	Chronic pancreatitis	11	3	<2,5	29,694		16	0	2957
M	45	Chronic pancreatitis	14	5	27,7	100,246		34	526	1865
M	43	Chronic pancreatitis	24	14	24	59,276		35	0	0
F	61	Chronic pancreatitis	10	3	22,5	29,573		36	0	646
M	54	Chronic pancreatitis	124	67	<2,5	40,059		37	0	0
F	66	Biliary stones	75	29	9,08	3938	116	21	0	0
F	54	Biliary stones	12	5	4,29	36,550		22	130	0
F	83	Pancreatic necrosis	21	10	118	1618		25	543	0
F	88	Benign stenosis of Oddi's sphincter	4	1	23	34,148		26	0	0

TBIL, total bilirubin (normal levels 7–25 μmol/L); DBIL, direct bilirubin (normal levels 2–9 μmol/L); CA19-9, carbohydrate antigen 19-9 (normal levels, <37 kU/L); CEAM6, carcinoembryonic cell adhesion molecule 6; O.D., optical density.
[a] Fig. 1.