ELSEVIER

Experimental Hematology

Experimental Hematology 2010;38:341–350

Proteome of platelets in patients with coronary artery disease

Cristina Banfi[a,b,*], Maura Brioschi[a,b,*], Giancarlo Marenzi[a,c], Monica De Metrio[a,c], Marina Camera[a,b], Luciana Mussoni[b], and Elena Tremoli[a,b]

[a]*Centro Cardiologico Monzino, I.R.C.C.S, Milan, Italy;* [b]*Department of Pharmacological Sciences, University of Milan, Milan, Italy;* [c]*Institute of Cardiology, University of Milan, Italy*

(Received 6 October 2009; revised 26 February 2010; accepted 2 March 2010)

Objective. **This study aimed at investigating the protein patterns of platelets from patients with stable or acute coronary atherosclerosis (CAD), in which platelets play a key role.**

Materials and Methods. **A proteomic approach was adopted to investigate specific protein patterns in platelets of patients with non-ST elevation acute coronary syndrome, stable angina, or of subjects with no history of CAD.**

Results. **Six differentially expressed proteins were identified: two involved in energy metabolism (2-oxoglutarate dehydrogenase [OGDH], and lactate dehydrogenase [LDH]); three were associated with cytoskeleton-based processes (γ-actin, coronin 1B, and pleckstrin); and one involved in protein degradation (proteasome subunit type 8). Expression levels of OGDH and a cleaved form of γ-actin were significantly higher in the platelets of patients than in controls, whereas that of LDH was higher only in the platelets of patients with acute coronary disease. The increases in protein expression of OGDH and LDH are paralleled by changes in their functional activities. Coronin and proteasome subunit type 8 were less expressed in the platelets of patients, as were the basic isoforms of pleckstrin.**

Conclusion. **The platelet proteome is altered in CAD patients with stable or acute coronary syndrome possibly because of the ongoing atherosclerotic process. The identified protein changes not previously connected with CAD were an increase in the energy metabolism enzymes and alterations in the proteins associated with cytoskeleton-based processes, both of which indicate platelet activation.** © **2010 ISEH - Society for Hematology and Stem Cells. Published by Elsevier Inc.**

Coronary atherosclerosis (CAD) is a chronic inflammatory disorder mainly a result of interactions between major risk factors (e.g., dyslipidemia, hypertension, diabetes) and genetic susceptibility. CAD can remain stable for many years, or can induce the formation of an occlusive coronary thrombus at the site of atherosclerotic plaque rupture and lead to the development of cardiovascular events, such as unstable angina or myocardial infarction (acute coronary syndromes [ACS]) [1,2].

Platelets play a role in the initiation, development, and progression of atherosclerotic plaque. An expanding body of evidence suggests that they can adhere to vascular lesions without forming a clinically significant intracoronary thrombus. Platelet aggregates stimulate vascular reactions that can lead to vascular healing or disease progression. Furthermore, platelets bind to leukocytes and endothelial cells and initiate the transformation of monocytes into macrophages; internalize oxidized phospholipids and promote foam cell formation; and recruit progenitor cells capable of differentiating into foam or endothelial cells [3].

Moreover, platelet activation is elicited by plaque rupture or fissuring that leads to formation of an occlusive or subocclusive thrombus [4]. It has been reported that increased platelet activation in the presence of ACS and stable CAD is a result of coordinated interactions between evolving atherosclerotic plaque and various cardiovascular risk factors, which can also increase the susceptibility of platelets to thrombus formation. However, it is still not known whether CAD induces specific changes in the expression, distribution, or amount of proteins in platelets.

Proteomics has the potential to transform the way we analyze platelet biology by determining the composition of platelet proteins and their changes upon stimulation and/or disease [5,6]. Platelets make an attractive and simple model

*Drs. Banfi and Brioschi contributed equally to this work.

Offprint requests to: Cristina Banfi, Ph.D., Centro Cardiologico Monzino, I.R.C.C.S., Via Parea 4, 20138 Milano, Italy; E-mail: cristina.banfi@unimi.it

0301-472X/10 $–see front matter.
doi: 10.1016/j.exphem.2010.03.001

for proteomic studies because they lack nuclear DNA, their genome consists of a small set of megakaryocyte-derived messenger RNA transcripts, and the complete pool of platelet RNAs is significantly smaller than the transcriptome of a nucleated cell [7]. It is particularly relevant that changes in various proteins might reflect a pathological profile, and thus allow the pathological condition to be identified.

The application of proteomics to normal stimulated platelets has so far identified multiple signaling responses activated by specific agonists, such as thrombin and its peptides, collagen, and adenosine diphosphate [8]. Among the few published proteomic studies of human diseases [9,10], a phosphoproteomic study of unstimulated platelets has found significantly greater protein phosphorylation in the unstimulated platelets of stroke patients, but no specific protein was identified [11].

The aim of this study was to use a proteomic approach to assess protein patterns and potential changes in protein levels in platelets isolated from patients with stable CAD, stable or non−ST-segment elevation ACS, and subjects with no history of CAD. In order to obtain information concerning in vivo platelet activation, the proteome was assessed in resting platelets.

Materials and methods

Patients and controls

The study population consisted of 26 patients with CAD: 14 with non−ST-segment elevation ACS and a final diagnosis of non−ST-segment elevation myocardial infarction (NSTEMI), and 12 with stable angina (Table 1). NSTEMI was defined as chest pain at rest with documented transient ST-segment depression or T-wave inversion in at least two contiguous electrocardiographic leads, without pathological Q-waves, but with enzymatic evidence of myocardial necrosis. The last spontaneous episode of chest pain had to have occurred within the 24 hours preceding study entry. The patients with stable angina had typical exertional chest pain associated with diagnostic ST segment depression of >1.0 mm upon exercise testing. All of the patients underwent coronary angiography in order to confirm the presence of significant coronary artery stenosis, which was defined as the presence of at least one $>75\%$ stenosis in any major epicardial coronary vessel. The exclusion criteria were an age older than 80 years, angina precipitated by correctable factors, valvular heart disease, atrial fibrillation, recent acute myocardial infarction (<6 months), thyrotoxicosis, a history of hemorrhagic diathesis, platelet disorder or thrombocytopenia, malignancies, inflammatory diseases, major surgery or trauma within the preceding month, or severe liver disease or renal insufficiency.

The study also included a control group of 10 subjects without CAD (5 male and 5 female patients aged 56 ± 5 years) who were comparable with the patients in terms of age and gender, free of major cardiovascular risk factors, and not taking drugs affecting atherosclerotic or thrombotic processes (Table 1).

All of the study subjects gave their written informed consent, and the study protocol was approved by our local Ethics Committee.

Platelet preparation

Fresh blood (40 mL) was drawn from an antecubital vein using a 19-gauge needle without venous stasis and collected into Vacutainer tubes containing acid-citrate-dextrose 15% v/v (trisodium citrate 22.0 g/L; citric acid 8.0 g/L; dextrose 24.5 g/L) as anticoagulant. The blood of the patients with NSTEMI was collected immediately after they were admitted to our emergency department, before the start of antithrombotic therapy; the blood of the patients with stable angina was collected before angiography.

The blood was centrifuged for 20 minutes at 160*g* at room temperature in order to obtain platelet-rich plasma. The upper third of the platelet-rich plasma was centrifuged at 1,000*g* in

Table 1. Clinical characteristics

	Controls (n = 10)	SA (n = 12)	NSTEMI (n = 14)
Age, y, mean ± SE	56 ± 5	60 ± 11	61 ± 9.9
Males/females, n	5/5	9/3	10/4
Hypertension, n (%)	0	5 (42)	8 (57)
Diabetes, n (%)	0	4 (33)	2 (14)
Current smokers, n (%)	0	2 (17)	6 (43)
Dyslipidemia, n (%)	0	8 (67)	8 (57)
Previous MI, n (%)	0	1 (8)	3 (21)
Previous PCI, n (%)	0	2 (17)	5 (36)
Previous ACBPG, n (%)	0	3 (25)	0
Single-vessel disease, n (%)	0	8 (67)	9 (64)
Multivessel disease, n (%)	0	4 (33)	5 (36)
Statins, n (%)	0	7 (58)	9 (64)
Aspirin, n (%)	0	6 (50)	12 (86)
Platelets ($\times 10^3/\mu L$), mean ± SE	205 ± 68.9	223 ± 19	220 ± 17
Mean platelet volume (fL), mean ± SE	6.9 ± 0.66	6.7 ± 0.2	6.8 ± 0.2
Leukocytes ($\times 10^3/\mu L$), mean ± SE	5.44 ± 0.90	6.4 ± 0.6	6.5 ± 0.4
Monocytes ($\times 10^3/\mu L$), mean ± SE	0.21 ± 0.12	0.32 ± 0.03	0.26 ± 0.03

ACBPG = aortocoronary bypass graft; MI = myocardial infarction; NSTEMI = non−ST-segment elevation myocardial infarction; PCI = percutaneous coronary intervention; SA = stable angina; SE = standard error.