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Integrated analysis of miRNA and mRNA gene expression microarrays: Influence on platelet reactivity, clopidogrel response and drug-induced toxicity

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ABSTRACT

Genetic and epigenetic variability may influence the efficacy and safety of antiplatelet therapies, including clopidogrel. Therefore, the miRNA-mRNA interactions and drug toxicity were investigated *in silico* using available microarray data. Expressions profiles of platelet miRNA (GSE59488) from acute coronary syndrome and mRNA in peripheral blood cells (GSE32226) from coronary artery disease patients were used to miRNA-target mRNA integrated analysis by Ingenuity Pathways Analysis 6 software (IPA). Results showed that *ST13* mRNA is regulated by hsa-miR-107 (miR-103-3p); *BTNL3* and *CFD* mRNAs are regulated by hsa-miR-4701-3p (miR-1262); *SLC7A8* is regulated by hsa-miR-145-5p (miR-145-5p); and *SENP5* mRNA is regulated by hsa-miR-15b-5p (miR-16-5p) and hsa-miR-26a-5p (miR-26a-5p). Drug toxicity IPA tool showed that these miRNAs/mRNAs are associated with clopidogrel-related liver and renal injury. In conclusion, these results demonstrate that differential expression of miRNAs in platelets and interactions with their target mRNAs are associated with variability in platelet reactivity, clopidogrel response and drug-induced toxicity.

1. Introduction

Platelets play essential roles in homeostasis, inflammation and thrombus formation, especially following to atherosclerotic lesions (Badimon and Vilahur, 2014; Katz et al., 2011; Paniccia et al., 2015). After atherosclerotic plaque rupture, the sub-endothelial matrix is exposed, mattering circulating blood components, and followed by platelets activation and aggregation (thrombus formation), that lead to vascular occlusion (Camici et al., 2012; Ueno et al., 2011; Wildgruber et al., 2013). A better understanding of the thrombosis mechanisms is crucial for treatment and prevention of thrombotic and hemorrhagic disorders, increasing the possibility of novels preventives and therapeutics interventions (Wang et al., 2014). Therefore the antiplatelet drugs have become essential for prevention and treatment of the atherothrombotic disease (Fintel, 2012).

Antiplatelet therapy is essential in the primary and secondary prevention of several cardiovascular disorders characterized by pathological prothrombotic states (O'Connor et al., 2015). Coronary artery disease (CAD) accounts for approximately 30% of all deaths from cardiovascular causes, and atherosclerotic plaque disruption with subsequent thrombus formation, which is the main cause of acute coronary syndrome (ACS) event (Husted, 2015). Thus, dual antiplatelet therapy with aspirin and clopidogrel is currently the standard treatment to prevent thrombotic events in patients with coronary artery disease (CAD) or acute coronary syndrome (ACS) (O'Connor et al., 2015). The prasugrel and ticagrelor are alternative antiplatelet agents in patients with altered response to clopidogrel (Lewis et al., 2015; Shuldiner et al., 2009).

Genetic and non-genetic factors, such as sex, age, smoking, and drugs interaction, may contribute to poor antiplatelet response, which has an important impact on clinical outcomes, including increased risk for thrombosis, ischemic events, and bleeding (Fintel, 2012; Karaźniewicz-ŁAda et al., 2014; Nguyen et al., 2005; Topçuoglu et al., 2011). Moreover, differences in gene expression maybe related to variability of clopidogrel response. We have shown that low *ABCC3* mRNA expression in peripheral blood cells (PBC) of CAD patients was associated with increased clopidogrel response (Luchessi et al., 2012). It is likely that dysregulation of genes involved in drug transport, such as *ABCC3*, may provide valuable information on clopidogrel response, contributing with the management of also ACS patients and reducing the risk of adverse effects (Luchessi et al., 2012, 2013).

Abbreviations: ACS, acute coronary syndrome; ASA, acetylsalicylic acid; CAD, coronary artery disease; CVD, cardiovascular disease; GEO, Gene Expression Omnibus; HPR, high platelet reactivity; IPA, Ingenuity Pathways Analysis; LogFC, Log2-fold change; LPR, low platelet reactivity; PBC, peripheral blood cells; PRU, platelet reactivity units.

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MicroRNAs (miRNAs) are single stranded, short and non-coding RNAs that play important gene-regulatory roles by pairing to target mRNAs to direct their repression (Guo et al., 2010; Sondermeijer et al., 2011). It has been suggested that miRNAs are potential novel biomarkers to monitor progression and therapy response of a broad range of diseases, including cardiovascular disease (CVD) susceptibility, prognosis, or treatment (Guo et al., 2010; McManus and Freedman, 2015; Sondermeijer et al., 2011). miRNAs have been shown to participate in platelet function, vascular homeostasis, and inflammation. In addition, levels of platelet miRNAs in the circulation are associated with the risk for CVD, suggesting that platelet-derived miRNAs might have important roles as biomarkers of CVD susceptibility, prognosis or treatment (McManus and Freedman, 2015).

miRNAs regulate simultaneously multiple target mRNAs, as well as multiple miRNAs may regulate a single target gene (Nunez et al., 2013). The study of miRNA-mRNA interactions can be laborious and time consuming because experimental models are difficulty to set up (Li et al., 2014). *In silico* analysis of miRNAs can be used to predict mRNA targets based on information on complementary sequence, evolutionary conservation, free energy and/or target site accessibility. In addition, *in silico* analyses may help to develop a new strategy to study miRNAs-mRNA interactions in patients with CVD using antiplatelet therapy and identify potential biomarkers of drug response (Liu et al., 2014).

Platelets contain a diverse array of biologically important RNA species, including mRNAs and mRNA-regulatory microRNAs. Inherited from their megakaryocytic precursors (Osman et al., 2015). In addition, previous studies have been showed that platelet-derived miRNAs play a role in several regulatory functions, including mRNA expression in others peripheral blood cells, such as leucocytes circulating (Pan et al., 2014). Therefore, the present results can help to clarify the influence of platelet-derived miRNAs in the regulation of mRNA expression in PBC, as well as the association of them with clopidogrel toxicity.

Thus, in this study the interactions between miRNAs and mRNAs and drug-related toxicity in response to clopidogrel treatment were investigated by bioinformatics tools using available microarray data from platelets and PBC of ACS and CAD patients, respectively. To the best of our knowledge, this is the first study attempting to identify possible interaction between miRNAs and mRNAs effects in ACS and CAD patients through *in silico* analyze.

2. Material and methods

2.1. Data sampling

Expression profile data set GSE59488 and GSE32226 were down loaded from NCBI (National Center for Biotechnology Information) through of Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/). GSE59488 explored the global miRNAs expression in platelets from ACS patients between high platelet reactivity (HPR) and low platelet reactivity (LPR), performing miRNA expression profiling of platelets from four HPR and four LPR patients using human miRNA microarray system. Platelets were isolated from venous blood and the VerifyNow P2Y12 assay was used to measure platelet reactivity. HPR was considered as ≥300 platelet reactivity units (PRU) while LPR was <170 PRU. miRNA expression was analyzed by microarray system using the platform GPL18402 Agilent-046064 Unrestricted_Human_miRNA_V19.0_Microarray (miRNA ID version).

GSE32226 data set is a global mRNA expression analysis in PBC from CAD patients with differential response to antiplatelet drugs using the platform GPL5175 by Affymetrix Human Exon 1.0 ST Array (Luchessi et al., 2013). CAD patients were treated continuously with acetylsalicylic acid (ASA) (100 mg/day) and clopidogrel (75 mg/day). Platelet reactivity was evaluated by VerifyNow® ASA and P2Y12 assays. The study design is shown in Fig. 1.

2.2. Analysis of miRNAs differentially expressed in platelets

Platelet miRNA expression profiles (GSE59488) were analyzed using GEO2R bioinformatics tool (available at http://www.ncbi.nlm.nih.gov/geo/geo2r/) accessed from GEO web server (Barrett et al., 2013a). GEO2R allows the identification and visualization of the top 250 miRNAs, which results are reported as graphic profiles. miRNA expression of ACS patients with HRP platelets ($n = 4$) were compared with whose with LRP platelets ($n = 4$). Among the top 250 miRNAs, we selected those with differential expression higher than Log2-fold change (LogFC) and p-value < 0.05.

2.3. Selection of target genes from differentially expressed miRNAs in platelets

Target mRNAs of the miRNAs differentially expressed in platelets (GSE59488) were selected using the microRNA (http://microrna.org), miRBase (http://www.mirbase.org), miRDB (http://mirdb.org/miRDB/), Target Scan Human (http://www.targetscan.org/), and miRTarBase (mirtarbase.mbc.nctu.edu.tw) databases. mRNAs ($n = 75$) with high score for strong miRNA-mRNA interactions (score ≤ 1.0) were selected.

2.4. Analysis of mRNAs differentially expressed in peripheral blood cells

Global mRNA expression in PBC (GSE32226) from CAD patients treated chronically with clopidogrel (75 mg/day) was evaluated using a microarray platform. CAD patients were grouped as responders and non-responders to clopidogrel based on platelet reactivity (Luchessi et al., 2013). GEO2R bioinformatics tool was also used to select the top 250 differentially expressed mRNAs in PBC. Significant differences were considered LogFC (FC > 2.0) and p-value < 0.05.

2.5. Analysis of miRNAs and mRNAs interactions

The miRNAs and target mRNAs interactions were further analyzed using the software Ingenuity Pathways Analysis v.6 (IPA) (Ingenuity® Systems, Ca., Redwood City, EUA), which generated a miRNA regulatory network and the related mRNA targets.

IPA is a web-based software application that enables researchers to analyze data derived from expression and SNP microarrays, RNA-sequencing, proteomics and metabolomics experiments, and small-scale experiments (such as PCR) that generate gene or protein lists. It also allows search for targeted information on genes, proteins, chemicals, diseases, and drugs, as well as building your own biological models. IPA's data analysis and experimental modeling enables understanding the significance of data or target(s) of interest in relation to larger biological or chemical systems (Krämer et al., 2014).

The mRNA differentially expressed in PBC (20 mRNAs) and the target mRNA predicted by miRNA databases (75 mRNAs) were screened to identify the mRNA targets of the 20 miRNAs download from GSE59488 dataset, using IPA. The IPA analysis between all mRNAs indicated above and the regulatory miRNAs in platelets was performed for evaluated the miRNA-RNA interaction using many database available by the software. Significant miRNA-RNA interactions were considered when LogFC (FC > 2.0) and mirSVR score between 0 and −1.3.

2.6. Analysis of miRNAs and mRNAs associated with toxicity of the antiplatelet therapy

IPA-Tox® is a IPA tool useful to screen molecules that have been associated with toxicity of drug therapy (Toxicity function). This tool analyses toxicogenomics data generated from multiple platforms to better understand the functions, pathways, and tox response gene sets that are likely perturbed by the compound/drug under investigation. The differentially expressed mRNAs and miRNAs from experimental and *in silico* data were screened using the IPA-Tox tool.