Abstract

Extracellular vesicles in systemic sclerosis as potential mediator for pulmonary vascular disease

Authors:
A Albini, R Casella, A Santaniello, L Beretta, V Bollati, F Rota, L Cantone, L Dioni, F Lombardi, M Vicenzi

1Fondazione IRCCS Cà Granda, Cardiovascular Unit, Department of Clinical Sciences and Community Health, University of Milan - Milan - Italy,
2Fondazione IRCCS Cà Granda, Immunological Unit, Department of Clinical Sciences and Community Health, University of Milan - Milan - Italy,
3University of Milan, EPIGET Lab, Department of Clinical Sciences and Community, Fondazione IRCCS Ca' Granda - Milan - Italy,

On behalf: Cardio-Epigenetic Team

Topic(s):
Hypertension, Pulmonary Hypertension

Citation:
Cardiovascular Research (2018 ) 114 (Supplement 1 ), S7

Background: Pulmonary vascular disease (PVD) is characterized by media muscular hypertrophy and/or hyperplasia. Molecular mechanisms underlying this condition are not yet fully understood, but novel circulating mediators as the extracellular vesicles (EVs) are getting attention. Recently, the deregulation of EVs in some forms of pulmonary hypertension studies has been reported, but data on pulmonary vascular disease are still lacking. Focusing on a high-risk population for PVD like systemic sclerosis (SSc) patients, this condition can be approached in a translational way through EVs-miRNAs characterization.

Purpose: To investigate whether EVs from SSc patients with or without established PVD can induce hypertrophy in in vitro smooth muscle cells and to study vesicular miRNAs expression.

Method: The population included 12 female subjects (mean age: 72 + 6): 3 SSc-PAH patients with established PVD under target therapy [PH+], 3 SSc patients with high clinical risk without hemodynamic features/PVD [PH-], 3 early SSc patients with low clinical risk [Ea] and 3 healthy control subjects. EVs from each subject were isolated from fresh plasma. Smooth muscle cells were cultured PBS (phosphate buffered saline) culture medium enriched with EV purified from each study subject. Real-time cell growth was analysed with xCELLigence RTCA (real time cell analysis). miRNAs from vesicles were isolated, characterized and target prediction was performed via Diana Tools mirPath 2.0.

Results: Real-time analysis of cellular growth showed a brisker growth in every aliquot exposed to EVs with respect to the one exposed only to the culture medium. The intergroup comparison showed that EVs from controls induced an inferior growth in terms of cell index and doubling time. Between SSc subjects, PH- showed the greatest effect on cell growth with respect to Ea and PH+ treated subjects. Considering miRNA content, the most deregulated miRNA was miR-324-3p which was strongly downregulated in PH-, weakly downregulated in PH+ and upregulated in Ea. Bioinformatics prediction for 324-3p and other differential miRNAs showed them to target intracellular metabolic pathways, especially lipids synthesis and metabolism.

Conclusions: EVs can influence in vitro smooth muscle cells growth suggesting a potential in vivo role in the pathogenesis of PVD in SSc. These results provide a first evidence that bio-molecular mediated mechanisms may predispose to PVD even before hemodynamic features of vascular remodelling. The observed miRNA expression pattern is potentially linked with the effect on cellular growth, suggestive of a protective or damaging role in subjects with high risk to develop PVD. The potential implication of deregulated miRNAs, especially 324-3p, on lipids metabolism indicates that this pathway could be involved in the pathogenesis of SSc-PVD. Further data are needed to deeply investigate these preliminary evidences.
Abstract: Extracellular vesicles in systemic sclerosis as potential mediator for pulmonary vascular disease

Authors: A Albini, R Casella, A Santaniello, L Beretta, V Bollati, F Rota, L Cantone, L Dioni, F Lombardi, M Vicenzi

1 Fondazione IRCCS Cà Granda, Cardiovascular Unit, Department of Clinical Sciences and Community Health, University of Milan - Milan - Italy,
2 Fondazione IRCCS Cà Granda, Immunological Unit, Department of Clinical Sciences and Community Health, University of Milan - Milan - Italy,
3 University of Milan, EPIGET Lab, Department of Clinical Sciences and Community, Fondazione IRCCS Ca' Granda - Milan - Italy,

On behalf: Cardio-Epigenetic Team

Background: Pulmonary vascular disease (PVD) is characterized by media muscular hypertrophy and/or hyperplasia. Molecular mechanisms underlying this condition are not yet fully understood, but novel circulating mediators as the extracellular vesicles (EVs) are getting attention. Recently, the deregulation of EVs in some forms of pulmonary hypertension studies has been reported, but data on pulmonary vascular disease are still lacking. Focusing on a high-risk population for PVD like systemic sclerosis (SSc) patients, this condition can be approached in a translational way through EVs-miRNAs characterization.

Purpose: To investigate whether EVs from SSc patients with or without established PVD can induce hypertrophy in in vitro smooth muscle cells and to study vesicular miRNAs expression.

Method: The population included 12 female subjects (mean age: 72 ± 6): 3 SSc-PAH patients with established PVD under target therapy [PH+], 3 SSc patients with high clinical risk without hemodynamic features/PVD [PH-], 3 early SSc patients with low clinical risk [Ea] and 3 healthy control subjects. EVs from each subject were isolated from fresh plasma. Smooth muscle cells were cultured PBS (phosphate buffered saline) culture medium enriched with EV purified from each study subject. Real-time cell growth was analysed with xCELLigence RTCA (real time cell analysis). miRNAs from vesicles were isolated, characterized and target prediction was performed via Diana Tools mirPath 2.0.

Results: Real-time analysis of cellular growth showed a brisker growth in every aliquot exposed to EVs with respect to the one exposed only to the culture medium. The intergroup comparison showed that EVs from controls induced an inferior growth in terms of cell index and doubling time. Between SSc subjects, PH- showed the greatest effect on cell growth with respect to Ea and PH+ treated subjects. Considering miRNA content, the most deregulated miRNA was miR-324-3p which was strongly downregulated in PH-, weakly downregulated in PH+ and upregulated in Ea. Bioinformatics prediction for 324-3p and other differential miRNAs showed them to target intracellular metabolic pathways, especially lipids synthesis and metabolism.

Conclusions: EVs can influence in vitro smooth muscle cells growth suggesting a potential in vivo role in the pathogenesis of PVD in SSc. These results provide a first evidence that biomolecular mediated mechanisms may predispose to PVD even before hemodynamic features of vascular remodelling. The observed miRNA expression pattern is potentially linked with the effect on cellular growth, suggestive of a protective or damaging role in subjects with high risk to develop PVD. The potential implication of deregulated miRNAs, especially 324-3p, on lipids metabolism indicates that this pathway could be involved in the pathogenesis of SSc-PVD. Further data are needed to deeply investigate these preliminary evidences.