Exosomes: new players in cardiovascular disease / Advanced heart failure

4.6x10^15 to 3.2x10^15 (n=5, p=0.37). In order to assess whether hnRNPU expression within EMVs might affect target cell function and phenotype, EMVs were generated from hnRNPU-kd HCAECs (EMV-hnRNPU-kd). Treatment of HCAECs using EMV-hnRNPU-kd revealed a significantly impaired target cell migration in scratch assays and Boyden chamber experiments.

Circulating exosomes from endothelial cells into EMVs is at least partially mediated by the RNA-binding protein hnRNPU. Downregulation of hnRNPU increases miR-24 level within EMVs and thereby reduces migration of endothelial cells upon treatment with EMV-hnRNPU kyd. Targeting RNA-binding proteins like hnRNPU is therefore a novel promising strategy to improve endothelial repair by control of intercellular microRNA transfer.

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Neovascularization potential of exosomes derived from blood outgrowth endothelial cells in ischemic cardiomyopathy
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Introduction: Blood outgrowth endothelial cells (BOECs) mediate therapeutic neovascularization in experimental models. We hypothesized that BOECs promote angiogenesis via secretion of exosomes.

Methods: BOECs were isolated from the peripheral blood of patients with severe ischemic heart disease and were exposed to hypoxia (1% O2) or normoxia for 12h. Exosomes were isolated from the conditioned medium using ultracentrifugation. Size and number of exosomes were determined by dynamic light scattering. In vitro angiogenesis was assessed by co-culturing BOECs with HUVECs in the presence or absence of BOEC-derived exosomes. Quantitative PCR analysis was performed to investigate angiogenic factors in BOECs from patients and controls and validated using ELISA. We measured the expression of miRNAs miR-210, miR-221, miR-126, and miR-21, with an established role in angiogenesis during normoxia and hypoxia. Finally, we compared in vivo angiogenesis of BOECs and BOEC-derived exosomes using a wound healing model in nude mice.

Results: Quantification of exosomes by NTA showed higher concentrations in the medium of BOECs incubated in hypoxia compared to normoxic conditions (14.3±0.23x10^8 vs. 12.0±0.23 x10^8 particles/ml, n=7, P<0.001). Immunoblot analysis confirmed robust expression of the exosome markers TSG101 and Flotillin-1. 2D-Tube formation assay performed to investigate the angiogenic potential of HUVECs in the presence or absence of BOEC-derived exosomes. qPCR analysis performed to investigate transcript levels of angiogenic factors both in BOECs and in BOEC-derived exosomes. Proteome analysis performed to investigate angiogenic factors in BOECs from patients and controls and validated using ELISA. We measured the expression of miRNAs miR-210, miR-221, miR-126, and miR-21, with an established role in angiogenesis during normoxia and hypoxia. Finally, we compared in vivo angiogenesis of BOECs and BOEC-derived exosomes using a wound healing model in nude mice.

Conclusion: Circulating exosomes could have an important role in the control of angiogenesis and inflammation. However, in coronary syndrome, intercellular communication via exosome might contribute with pro-inflammatory inputs by activation of TLRs/NF-kB in the acute phase and exerting a long-term maintained inhibitory effect on angiogenesis. These results could have a great impact on the progression and outcomes of coronary disease.

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ADVANCED HEART FAILURE

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Circulating microRNA profiling in serum of pediatric patients with heart failure submitted to VAD implant
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Background: There is an increasing interest on the role of circulating miRNAs (c-miRNAs) as biomarkers for diagnosis and prognosis of disease. In adult HF patients treated with VAD, changes in c-miRNAs levels have been observed. To date there are no data regarding c-miRNAs changes and role in pediatric patients with HF.

Purpose: Aims of this study were to examine: the profile of c-miRNAs in HF children; the effects of VAD on the c-miRNAs levels; and to identify potential targets of selected c-miRNAs.

Methods: Blood samples were collected from 5 pediatric patients {13±6 (means±SD) months, 17±2 LEVFR} with HF before and at 4 hrs, 1, 3, 7, 14 and 30 days after VAD implant.

Table 1

<table>
<thead>
<tr>
<th>Base Mean</th>
<th>log2 FC</th>
<th>p-value</th>
<th>p adj</th>
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<tr>
<td>miR-30a-5p</td>
<td>319.05</td>
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<td>miR-941</td>
<td>60.53</td>
<td>0.81</td>
<td>1.03E-03</td>
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</table>

Figure 1. Exosomes modulatory properties
miRNAs seem to be involved with a synergistic effect in the hemostatic process. miRNAs were confirmed by qRT-PCR (Fig. 1A). Putative targets of selected c-miRNAs were simultaneously modulated at 30 days after VAD implant compared to pre-VAD (Table 1). Among them, only 6 c-miRNAs were confirmed by qRT-PCR (Fig. 1A). Putative targets of selected c-miRNAs seem to be involved with a synergistic effect in the hemostatic process (Fig. 1B).

**Results:**
After NGS, a total of 169 c-miRNAs were detected in serum samples of HF pediatric patients. N=13 c-miRNAs were simultaneously modulated at 30 days after VAD implant compared to pre-VAD (Table 1). Among them, only 6 c-miRNAs were confirmed by qRT-PCR (Fig. 1A). Putative targets of selected c-miRNAs seem to be involved with a synergistic effect in the hemostatic process (Fig. 1B).

**Conclusions:** In HF children, levels of 6 c-miRNAs changed after 30 days of VAD treatment. These selected c-miRNAs are potentially involved in the regulation of hemostasis, a well known adverse event for this group of patients.

**Figure 1**

**Hemostasis**

**Conclusion:** In HF children, levels of 6 c-miRNAs changed after 30 days of VAD treatment. These selected c-miRNAs are potentially involved in the regulation of hemostasis, a well known adverse event for this group of patients.

**Figure 1**

**Difference in LDH between HM3 & HM2**

Analysis. Through one year, evolution of LDH was significantly higher in patients with a HM2 device compared to patients with a HM3 device, indicating lesser haemolysis and better haemocompatibility in the HM3 LVAD.

**Sex differences in pretransplantation panel reactive antibody levels and outcomes in patients undergoing heart transplantation**

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**Background:** Potential sex differences in panel reactive antibody (PRA) levels and their association with transplant-specific outcomes after heart transplantation are mostly unknown.

**Methods:** In 20,181 (24.7% women, median age 56 years (interquartile range 46–62 years)) first time heart transplant recipients included from July 2004 to March 2015 in the prospective United Network of Organ Sharing (UNOS) database with available data on PRA, we studied sex differences in most recent PRA (mrPRA) and peak PRA (pPRA) levels and outcomes and the associations of PRAs with mortality, graft failure, rejection, cardiac allograft vasculopathy (CAV) and retransplantation. Median follow-up for all-cause mortality was 5.7 years for women and 6.0 years for men. The analyses are based on Organ Procurement and Transplant Network (OPTN) data as of March 6, 2017.

**Results:** Women had significantly higher mean class I and II mrPRA and pPRA levels than men (mrPRA class I: 13.7% vs. 4.3%; mrPRA class II: 10.1% vs. 2.8%; pPRA class I: 42.7% vs. 23.9%; pPRA class II: 41.0% vs. 24.3%; P<0.001) and higher rates of graft failure (P<0.001), acute (P<0.001) and chronic (P=0.0049) rejection and retransplantation (P<0.001), while men showed a higher probability for CAV (P<0.001).

**Figure 1. Age-adjusted hazard ratios for most recent and peak PRA levels and transplant-related outcomes in patients undergoing heart transplantation by sex.**

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**Conclusion:** To the best of our knowledge, this is the first study to assess differences in LDH over time between the HM2 and HM3 using longitudinal data.

**Figure 1. Age-adjusted hazard ratios for most recent and peak PRA levels and transplant-related outcomes in patients undergoing heart transplantation by sex.**