Esrp1 Loss Disrupts MicroRNAs Which Target Wnt Signaling Required For Palatogenesis

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Purpose Orofacial clefting is one of the most prevalent congenital defects, affecting 1/800 live births. Surgical repair of palate deformities constitutes a costly challenge to pediatric care which the reconstructive surgical community encounters commonly. In previous studies, reduced expression of an epithelium-specific alternative splicing regulator, Esrp1, caused cleft palate in a mouse model. Upon noting that Esrp1 physically interacts with a battery of microRNAs, small non-coding transcripts which negatively regulate mRNA expression, we investigated a possible role for Esrp1 in microRNA expression to explore the central hypothesis that Esrp1 loss results in disruption of microRNAs required for palate formation.

Methods Due to the epithelium-specific expression and function of Esrp1, we employed an in vitro model of mouse epithelium, the Py2T mammary epithelial cell line. Our collaborator had previously employed the CRISPR-Cas9 gene editing system to disrupt Esrp1 expression in this cell line. We employed a microarray-based approach for all known mouse microRNAs, between unmodified Py2T cells and Esrp1: CRISPR Py2T cells.

Results Here we report that upon CRISPR-induced disruption of Esrp1 in a mouse epithelial cell line there is a distinct upregulation of three micro-RNAs, miR-342-5p, miR-374c-5p and miR-181c-5p, each of which have been shown to target the Wnt pathway, whose necessity in palatogenesis has already been extensively documented. Specifically, miR-342-5p downregulates the expression of Wnt3 and Wnt7b, as well as a variety of downstream effectors of Wnt signaling which drive the progression of the cell cycle required for the outgrowth of the palatal shelves.

Conclusions This work establishes that Esrp1 negatively regulates the expression of key microRNAs which must be suppressed in order for orofacial primordia to undergo successful proliferation and migration to form the mature secondary palate. Ongoing work is focused upon identifying the mechanism by which Esrp1 loss disrupts specific microRNA expression levels and how such disruptions interfere with the process of palatogenesis.

S-nitrosylation Therapy To Improve Ex-vivo Parameters Of Vascular Composite (vca): Study On Brain Dead Porcine

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S-Nitrosylation Improves ex-vivo parameters of vascular composite (VCA): Study on brain dead swine

Background: Brain death (BD) results in disruption of S-Nitrosohemoglobin (SNO-Hb) leading to decrease tissue micro perfusion. In a pre-clinical study on bd swine, we found that repletion of SNO-Hb by S-nitrosylation agent as ethyl nitrite (ENO) improves organ function. Vascularized composite allograft (VCA) is a viable therapeutic option for correction of composite tissue loss as limb amputation, abdominal trauma and severe facial damage. Although significant progress has been made with VCA surgical techniques and anti-rejection drug regimens, less attention has been directed to care of the tissue composite preservation. It is the physiologic status of the donor that often drives tissue availability and short-term VCA outcome. We hypothesized that additional of ENO during composite storage could restore composite microcirculation and thus improves outcome.

Methods: VCA (n=8) and hind limb (n=6) were harvested from induced bd swine. Each composite was placed on a Life-port storage machine and cold pulsatile perfusion was started at a targeted pressure 22 ± 5 mmHg for VCA and 13±2 for limb. Flow and resistance measurements were recorded during the experiment. ENO was aerated to the preservative solution for 2 hours (50ppm) for the treated composites. Data points were extracted from machine-generated values. Rate of
change (slope) in flow rate and resistance were calculated. We compared values from control group to ENO treated group using Wilcoxon rank sum tests. GraphPad Prism software was used and considered a p < 0.05 to be statistically significant.

Results: Flow and resistance measurements were expressed as percent change from baseline. For the duration of the pumping period. VCA flow rate in the ENO-treated group (n=2) was significantly higher (p<0.0001; 95% CI -17.22 to -9.743) than the control group (n=6), As well as, resistance was significantly decreased in ENO-treated group (p=0.0004; 95% CI 11.86 to 33.96). Similarly, limbs flow rate in the ENO-treated group (n=3) was significantly higher (p<0.0001; 95% CI -11.52 to -2.36) than control limb (n=3) as well as resistance significantly decreased in ENO-treated group (p<0.0001; 95% CI 11.51 to 24.13).

Conclusions: S-nitrosylation therapy (ENO) during ex vivo storage of VCA holds promise to increase the number and quality of VCA available for transplant.

Venous Malformations Have Collagen IV Deposition Defects Associated With Increased Endothelial Cell Death

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Purpose: Collagen IV (COLIV) is an essential component of the blood vessel basement membrane (BM). COLIV functions as a scaffold to stabilize vessels, that can also be proteolytically cleaved to release angiogenic peptides that regulate endothelial cell (EC) fate. In murine ECs, constitutive RAS/ERK signaling leads to defects in COLIV BM deposition, which in turn promotes EC death. We previously demonstrated ERK hyperactivation in the endothelium of venous malformations (VMs). Thus, we hypothesized that COLIV deposition is altered in VMs leading to EC death.

Methods: Clinically confirmed VM lesions (n=11) were resected and specimens divided in two and either paraffin or fixed-frozen embedded. Neonatal dermis (n=5) served as controls. VM and control sections were stained for the EC marker, VECADHERIN, and either phospho-ERK (pERK) or COLIV. Endothelial cell apoptosis was determined by TUNEL assay and co-staining for VECADHERIN. COLIV localization was assessed and scored as normal continuous BM expression, abnormal nuclear localization, or discontinuous BM deposition. Mean endothelial pERK expression and percent TUNEL+ ECs was quantified using ImageJ. Student’s T-Test was used for statistical analysis by Graphpad, and a p value <0.05 was considered statistically significant.

Results: Activated pERK expression was significantly increased in VM ECs (n=8), compared to control vessel ECs. COLIV defects were noted in a majority of VM tissues (10/11, 82%) which was not observed in control vessels. COLIV nuclear localization (arrowheads) was observed in 27% of VM ECs, discontinuous BM deposition in 9% of VM vessels, and both abnormal COLIV deposition phenotypes were observed in 55% of VMs. VMs (n=9) had a significant increase in TUNEL+ ECs (arrowheads), when compared to control neonatal skin. Control vessels had 0.12% TUNEL+ ECs (0-0.6%), while VMs had an average of 31.17% (11.77%-66.43%) TUNEL+ ECs (p=0.002).0.12% TUNEL+ ECs (0-0.6%), while VMs had an average of 31.17% (11.77%-66.43%) TUNEL+ ECs (p=0.002).

Conclusion: We observed that VM vessels had increased ERK activation associated with COLIV mislocalization and BM deposition defects. Consistent with murine studies, the loss of COLIV in the BM correlated with a significant increase in EC apoptosis in VM tissues. These results suggest that pathological ERK hyperactivation, leads to a loss of COLIV deposition in the BM leading to endothelial cell death in VMs.

Loss Of Arnt Limits Skeletal Muscle Performance In Response To Aerobic Exercise

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Purpose: The hypoxia signaling pathway is essential for angiogenesis and metabolic regulation during muscular adaptation to exercise. We have previously demonstrated that hypoxia signaling and levels of aryl hydrocarbon receptor nuclear translocator (ARNT), in particular, decline...