(-35); TLR pathway= HSPA1A (-58), HRAS (-36), MAP2K3 (-23), TOLLIP (-23), RELA (-18), and FOS (-16).

CONCLUSIONS: These results provide a new insight into the potential role played by keratinocytes to drive inflammatory responses in severe burned patients. These epithelial cells play a key role in triggering the formation of several innate and adaptative proinflammatory cytokines and in activating members of the toll-like receptor pathway that might be disrupted by extensive lesions of the skin. Therefore, this study aims to contribute to understanding the molecular mechanisms underlying wound infection in severe burned patients and to provide new strategies that would restore the normal expression of these genes to enhance the inflammatory process and drive these patients to a better outcome.

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MicroRNA Regulates Hemangioendothelioma Growth by Targeting the Nox-4/MCP-1 Pathway

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PURPOSE: MicroRNA (miR) are emerging as biomarkers to identify aberrant signaling pathways and potential therapeutic targets in tumors. Endothelial cell tumors are the most common soft tissue tumors in infants yet little is known about the role of miR in promoting their growth. A validated mouse endothelial cell (EOMA) tumor model was used to demonstrate that post-transcriptional gene silencing of dicer, the enzyme that converts pre-miR to mature miR, can prevent tumor formation in vivo. We also sought to determine how dicer activity regulates the nox-4/monocyte chemoattractant protein-1 (MCP-1) pathway, which we have previously shown is required for hemangioendothelioma formation.

METHODS: EOMA cells were transfected with either control or dicer lentiviral shRNA particles and injected (5×10^6 cells/100 ul PBS) subcutaneously into 6 week old 129 P3 mice. For in vitro experiments EOMA cells were transfected with control or dicer siRNA and samples collected 72 hours after transfection for measurement of miR, mRNA, or protein. Sybr green real-time PCR was used to measure miR and mRNA. Western blots and ELISA were used to measure protein. Plasmid transfections were done with reporter vectors containing firefly luciferase and co-transfected with a renilla luciferase vector as a transfection efficiency control. Luciferase levels were measured using a dual luciferase reporter assay. At least three independent replicates were conducted for all experiments. Two-sided 2 sample t-test was used to compare the difference between two groups, and ANOVA for comparison among more than 3 groups with Tukey's adjustment for the multiple pairwise comparisons among groups. Non-parametric procedures were used when normality assumption of the data was violated even after proper data transformation. A p-value of ≤ 0.05 was considered statistically significant.

RESULTS: Tumors formed in 4/4 mice injected with EOMA cells transfected with control short hairpin RNA (shRNA), but only formed in 1/5 mice injected with EOMA cells transfected with dicer shRNA and the single tumor in the dicer knockdown group was 91% smaller than the average size of tumors in the control group. This response to dicer knockdown was mediated by enhanced miR 21a-3p targeting of the nox-4 3'UTR. EOMA cells were transfected with miR 21a-3p mimics and luciferase reporter plasmids containing either intact nox-4 3'UTR or with mutation of the proposed 3'UTR miR21a-3p binding sites. Mean luciferase activity was decreased by 85% in the intact versus the site mutated vectors ($p<.01$). Loss of

nox-4 activity resulted in decreased hydrogen peroxide production and decreased production of oxidant inducible monocyte chemoattractant protein-1.

CONCLUSIONS: These are the first reported results to demonstrate the significant contribution of dicer activity and miR production in promoting hemangioendothelioma growth in vivo. These are also the first reported results of miR21a-3p targeting nox-4 mRNA and inhibiting reactive oxygen species production in endothelial cells. Collectively, these results indicate that targeting microRNA and specifically, miR-21a-3p, represent potential therapeutic strategies for the treatment of endothelial cell tumors including hemangioma and hemangioendothelioma.

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Characterization of The Koliber Mutant: A New Model for Craniosynostosis Research

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PURPOSE: Zebrafish (Danio rerio) is an established model for craniofacial development studies. Here, we investigated cranium development in a recently identified kolibernu7 mutant. The kolibernu7 is characterized by a misshapen body, reduced length, and malformed skull as a result of hyperossified endochondral bones, bone fusions, and loss of cartilage (Anderson, in preparation). In this project, we investigated development of intramembranous calvaria bones and cranial sutures in kolibernu7 mutant and wildtype siblings. Our lab has established baseline biostatistical data for cranial vault development through progressive developmental stages (Shoela, in preparation). Here we expand on this method by using landmark-based morphometric analysis, with an aim to quantify the growth of individual bones of the cranial vault, the cranium as a whole, and the asymmetrical positioning of bilateral structures in kolibernu7 mutants.

METHODS: Wildtype (WT) (n=25) and kolibernu7 (n=23) zebrafish were collected at different developmental stages and double-stained with Alizarin Red and Alcian Blue to visualize bone and cartilaginous structures, respectively. Images of whole fish and isolated cranial vaults were taken using standard light microscopy. Following initial measurements of cranial width and length, total area, and overlapping areas of the anterior frontal, posterior frontal, parietal, and supraoccipital bones, the calvaria were enzymatically cleared using a trypsin solution with sodium borate. Individual bones were separated and photographed and similar measurements to those described above were taken. Morphometric analysis was conducted using landmarks and allowed for the creation of deformation grids of wildtype versus kolibernu calvaria. Similar analysis was conducted for individual cranial bones.

RESULTS: The ossification pattern and developmental schedule of cranial sutures are noticeably different between wildtype and kolibernu7 mutant. In contrast to the wildtype, where we observed a strong linear correlation between size of individual calvaria bones, total calvaria size, and standard length, in the