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AAO Foundation Final Report Form
(01/31/2018)

Please prepare a report that addresses the following:

Type of Award, e.g., Orthodontic Faculty Development Fellowship Award, Postdoctoral Fellowship Award, Biomedical Research Award, Center Award, Educational Innovation Award, Program Award, Research Aid Award

Name(s) of Principal Investigator(s)
Melih Motro

Title of Project
TGF-ß3: Does it Induce Epithelial-Mesenchymal Transition of Median Edge Epithelial Cells of Fetal Palatal Shelves?

Period of AAOF Support :
07-01-16 to 12-31-2017

Amount of Funding
$18,998

Summary/Abstract of Completed Project Results
This project involved multiple objectives for faculty development in research, educational, teaching and clinical skills. Within the project period as a part of the proposal, I have completed several courses focuses both in clinical and research development, attended professional development meetings and worked on the research project. The details of the courses I attended can be found below.
Results of the Research Project:
Mouse embryos that were collected from time-pregnant mice were euthanized at embryonic stages (ES) 12.5, 13.5, 14.5 and 15.5. Maxillae were dissected and embedded for immunohistochemistry/immunoflorescence on frozen sections.
The vertical position of the palatal shelves was shown at ES12.5 and ES13.5, followed by the elevation at ES14.5, along with the palatal seam formation. The palatal seam was shown to disappear at ES15.5. At ES14.5, expression of s100a4 which is one of the main epithelial-mesenchymal transition markers was elevated at the palatal seam, where the median edge epithelial cells are located along with the e-Cadherin and fibronectin levels at the same stage which may present that there is an EMT process in that region at ES14.5 stage where fusion of the palatal shelves takes place. (Figure1) At ES15.5 when the palatal seam was completely

disappeared, the s100a4 levels were all similar throughout the palatal mesenchymal region. Also, at the same stage e-cadherin expression was totally lost in the fusion area meaning that there were no more epithelial cells in the region.
For primary cell culture, palatal tissue was scraped off the dissected maxillae of the embryos at ES15.5 and cultured on 60mm dishes. Once the cells reached 80% confluency, e-cadherin positive cells were sorted by flow-cytometry to differentiate the epithelial cells. Epithelial cells were transfected by TGFb3 siRNA to knockdown the TGFb3 expression. qPCR results showed that there was 30% reduction in expression of TGFb3, 24 hr after gene silencing.
Control and TGFb3 groups were constituted to assess the effects of TGFb3 on palatal tissue originated epithelial cells. In the TGFb3 group, expression of e-cadherin declined, however, the s100a4 and fibronectin expression levels were elevated.
The results present that TGFb3, like other TGFb isotypes, has a direct role in EMT process which may underlie the mechanism of EMT during palatal fusion.

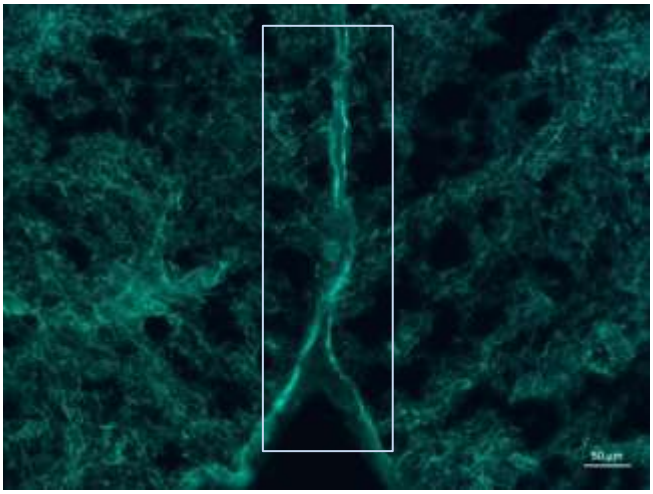

Figure 1A: Elevated expression level of S100A4 in the palatal seam/median edge epithelial cells

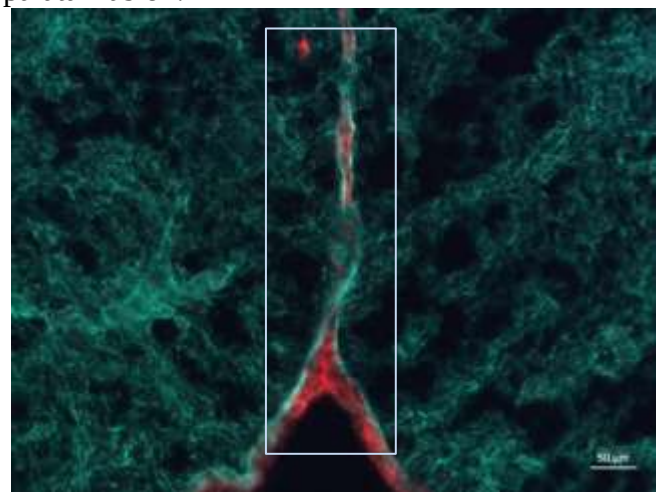

Figure 1B: E-cadherin (red) and S100A4 (green) stained palatal seam.

Response to the following questions:

1. Were the original, specific aims of the proposal realized?

   The aims of this project were realized. It was shown by this project that during palatal fusion median edge epithelial cells are going through an EMT process. Also, it was shown that TGF-b3 has a role in inducing the EMT process. Further analysis is needed to realize our overall aim of illuminating the mechanisms behind the palatal fusion and the EMT processes occurring during the fusion.

2. Were the results published?

We are in the process of writing the manuscript to submit it for publication soon.

3. Have the results of this proposal been presented?

The results have recently been compiled and planned to present at the 2019 AAO annual session.

4. To what extent have you used, or how do you intend to use, AAOF funding to further your

career?

I used most of the AAOF funding for the research project and to fund the courses I took to further my career.

I completed “Biochemistry and Cell Biology” and “Techniques in Biochemistry, Cell and Molecular Biology” courses which were both one semester courses given by biochemistry department of Boston University, School of Medicine. Both of these courses covered extensively the topics that benefited my research goals and this particular research project funded by AAOF.

I am planning to use the remaining funds to run further analyses that may shed more light to the mechanisms of palatal fusion on both the primary cell cultures, histological slides and cDNA, I prepared and stored for this project.

Please return to AAOF via email attachment to aaofevp@aaortho.org