Abstract 107: Requirement Of ALX1 Homeobox Transcription Factor In Craniofacial Development

Citation
PINI, Jonathan, and Eric C. Liao. 2018. “Abstract 107: Requirement Of ALX1 Homeobox Transcription Factor In Craniofacial Development.” Plastic and Reconstructive Surgery Global Open 6 (4 Suppl): 85-86. doi:10.1097/GOX.0000533972.55205.57. http://dx.doi.org/10.1097/GOX.0000533972.55205.57.

Published Version
doi:10.1097/GOX.0000533972.55205.57

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Accessibility
PURPOSE: Diabetes affects a significant proportion of the population in the United States. Microsurgical procedures are common in this patient population, including free flap reconstruction. However, studies evaluating the direct association between diabetes and outcomes following microsurgical free flap reconstruction are limited. The aim of our study is to evaluate the impact of diabetes on outcomes following microsurgical free tissue transfer.

METHODS: We reviewed the American College of Surgeons National Surgical Quality Improvement Program (ACS-NSQIP) database to identify patients undergoing free flap microsurgical reconstruction between 2010 and 2015. Preoperative variables and outcomes were compared between diabetic and non-diabetic patients. Chi-square analysis and Fisher’s exact test were used for categorical variables and t tests for continuous variables. Multivariate regression was performed to control for potential confounders.

RESULTS: Database review identified 6,030 eligible patients. Diabetic patients presented significantly higher rates of wound complications, including deep incisional surgical site infection (SSI) (OR = 1.35; 95% CI: 1.23–1.47; \( p=0.01 \)) and wound dehiscence (OR = 1.17; 95% CI: 1.02–1.24; \( p=0.03 \)). No significant difference in flap failure was observed. Diabetic patients also presented a significantly longer hospital length of stay (LOS) (B = 0.62; 95% CI: 0.26–0.97; \( p<0.001 \)).

CONCLUSION: Our study evaluated the largest national cohort of free flap procedures. These results suggest that diabetic patients are at significantly higher risk of postoperative deep incisional (SSI), wound dehiscence and longer LOS. However, importantly diabetes was not associated with increased rates of flap failure. Our findings highlight the critical need for heightened clinical vigilance and wound care in the diabetic patient population following free flap reconstruction for optimal outcomes.

R.S. Kantar: None. W.J. Rifkin: None. J.A. David: None. M.J. Cammarata: None. J. Diaz-Siso: None. A.R. Golas: None. J.P. Levine: None. D.J. Ceradini: None.

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Requirement of ALX1 homeobox transcription factor in craniofacial development

PURPOSE: Frontonasal dysplasia (FND), or median facial cleft syndrome, is a class of developmental abnormalities caused by incomplete growth and fusion of the central frontonasal protrusion with the paired maxillary facial prominences. Genetic aspects of FND are not well defined. Recently, mutations in the ALX gene family have been associated with FND. ALX1 is expressed in the facial mesenchyme of vertebrate embryos, in cranial neural crest cells and its cartilage derivatives. However, little is known about the function of this transcription factor during craniofacial development.

METHODS: Using a human pedigree of 3 subjects in a family with heritable FND, we defined a loss-of-function ALX1 gene variant (A). Control and patient blood samples were used to generate induced Pluripotent Stem Cells (iPSCs), using Sendai virus infection. iPSCs were characterized based on their embryonic stem cells (ESCs) properties i.e. self-renewal and pluripotence. Moreover, karyotype, Sendai virus gene expression and ALX1 gene sequencing were performed to finalize the iPSCs characterization. To go further, iPSCs for both genotypes were subjected to neural crest cells differentiation to study the role of ALX1 transcription factor in craniofacial formation.

RESULTS: Control and ALX1-/- iPSCs displayed typical embryonic stem cells properties, such as self-renewal and ESC markers expression (B). Moreover, iPSCs from both genotype display normal karyotype and don’t express Sendai virus genes anymore. Finally, ALX1 coding region sequencing reveals the presence of pathogenic L165F mutation whereas Control iPSCs have a normal ALX1 gene. The iPSC are differentiated into neural crest cells, and demonstrate phenotypes that reveal their mechanism of developmental anomaly, such as loss of function in chondrocytes differentiation.

CONCLUSIONS: Our results show that ALX1 is dispensable for iPSC reprogramming process, and initiation to form embryoid body. The use of ALX1 null iPSCs will provide a better comprehension of ALX1 biology and its essential role in craniofacial regulatory network. Importantly, we show how patient derived iPSC can be used to model craniofacial

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anomalies. Analysis of ALX1 function and identification of its target genes will help to better understand and innovate future treatments, through pharmacologic or CRISPR editing approaches.

J. Pini: None. E.C. Liao: None.

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Maintenance of Mammalian Stem Cell States and Enhanced Wound Healing by Honey Bee Royal Jelly

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PURPOSE: Royal jelly is the well-known queen-maker for the honey bee Apis mellifera, and has documented cross-species effects on longevity, fertility, and regeneration in mammals. Given larger body size and shorter developmental times in honey bee queens despite genetic similarity to workers, we reasoned that royal jelly may impact stem cell function. Here we show, for the first time, stemness maintenance activities in mouse embryonic stem cells and enhanced wound healing in dorsal mouse wounds.

METHODS: J1 mESCs were grown in medium containing KnockOut D-MEM and 1,000U/ml mouse LIF. For 2i conditions, mESCs were cultured in serum-free 2i media containing 1,000U/ml mouse LIF, 1uM PD0325901, and 3uM CHIR99021. To assess teratoma formation capacity, -LIF+Royal Jelly mESCs were injected subcutaneously into flanks of 8-week-old SCID/Beige mice. RNA-seq libraries were generated and deep sequencing was performed. Royal jelly mass spectrometry was performed, and data-dependent collision induced fragmentation was evaluated on the top 10 most intense +2 and +3 ions. Individual royal jelly components were also injected into full-thickness dorsal wounds in mice and wound healing rates were determined.

RESULTS: mESCs readily differentiated upon LIF withdrawal, however addition of royal jelly, in the absence of LIF, resulted in formation of undifferentiated colonies and upregulation of pluripotent genes. Royal jelly maintained a high pStat3 state, suggesting activation of a Stat3-driven LIF-independent pathway for mESC self-renewal. mESCs grown in -LIF+Royal Jelly were also capable of teratoma formation in mice. RNA-seq analysis revealed 1445 upregulated and 1885 downregulated genes in response to royal jelly. GO term analysis revealed enrichment of genes involved with proliferation and stemness among upregulated gene sets. Furthermore, GO term analysis of ‘royal jelly-responsive’ genes demonstrated enrichment for metabolic and biosynthetic processes, similar to ground state conditions. To obtain an unbiased view of royal jelly and what components may be responsible for its stemness effects, we performed liquid chromatography-mass spectrometry. Six of 10 predominant proteins belonged to a highly homologous group known as major royal jelly proteins, one of which was capable of maintaining mESCs in a pluripotent ground state and enhancing wound healing in mice.

CONCLUSION: Royal jelly can functionally replace LIF and maintain self-renewal and pluripotency in mESCs, effecting a phenotype similar to ground state pluripotent stem cells. Our findings indicate that royal jelly can regulate the dynamic state of stem cells in non-insect species, uncovering an important heretofore unappreciated innate program for stem cell self-renewal with broad implications in understanding the molecular regulation of stem cell fate and wound healing.

D.C. Wan: None. K.C. Wang: None.