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PURPOSE: Secondary lymphedema is a debilitating disease characterized by chronic soft tissue swelling. While there is still no cure for lymphedema, we have shown that 9-cis-Retinoic acid (9-cisRA) promotes lymphangiogenesis and reduces postsurgical lymphedema in vivo. Despite the resulting efficacy, little is known about the signaling pathway of 9-cisRA in this context. 9-cisRA is thought to induce its target gene responses by binding to and activating nuclear retinoid X receptors (RXRs). The aim of this study was to determine whether RXR signaling is necessary for 9-cis-RA efficacy using lymphatic endothelial cell specific conditional deletion mutant mice.

METHODS: Tail model of lymphedema was performed on transgenic Prox1-CreER T2 RXRαfl/fl mice and an age-matched control group. Superficial lymphatic vessels were severed by a 5-mm circumferential excision 2-cm distal from the tail base. The animals were treated with 9-cisRA once daily for 40 days. The distal part of the tail was imaged weekly postoperatively and tail volumes were calculated using the truncated cone formula. Tail samples 1-cm distal to the wound edge were collected and histologically analyzed to assess soft tissue thickness.

RESULTS: Disruption of the superficial lymphatics resulted in initial surgical edema in both the control and RXRαfl/fl groups. The RXRαfl/fl group showed a significantly less decrease in swelling at post-op days 29, 36, and 40 (**p ≤ 0.01). RXRαfl/fl mice showed significantly thicker soft tissue on post-op day 45 compared to the control (****p ≤ 0.0001).

CONCLUSION: Functional RXRα in lymphatic endothelial cells is necessary for 9-cisRA to reduce postsurgical lymphedema. These detailed mechanistic studies support further translation of 9-cisRA for safe clinical use in the prevention of lymphedema in humans.

Nrf2 Dysfunction is the Basal Epidermis Coincides with Delayed Healing in Type II Diabetes

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PURPOSE: Despite optimal medical management of type II diabetes, unmitigated oxidative stress is an enduring force underlying delayed wound healing, a complication that leads to amputation, causes disability, and robs patients of their quality of life. Nrf2, the master transcriptional regulator of redox homeostasis, holds the potential to be a powerful diagnostic tool and therapeutic target against overwhelming reactive oxygen species in diabetic wounds. However, the spatial dimensions of this derangement are not well characterized. Here, we localize lost Nrf2 function to discrete locations in human wounds in tandem with dysregulated redox homeostasis.

METHODS: Primary human and diabetic epidermal keratinocytes (NHEK and DHEKs, respectively) underwent real time QT-PCR, protein quantification, and immunocytochemistry, for Nrf2 and its downstream effectors. Human surgical specimens from wounded and unwounded tissues in diabetic and nondiabetic patients underwent parallel biomolecular analysis. Keap1, a repressor of Nrf2, was silenced (siKeap1) in DHEKs, with siNonsense (siNS) as a control. siKeap1-transfected DHEKs underwent functional assays.

RESULTS: QT-PCR of DHEKs demonstrates reduced transcription of Nrf2 downstream antioxidant genes like GSR, GPX3, GSTP1, and MnSOD, and an unfavorable cytokine profile compared to NHEKs (increased TNFa and decreased TGFb). siKeap1 in DHEKs rescues the expression of GSR, GPX3, and HO-1 by 25%, 383% and 237% respectively, and restores a cytokine profile favorable to healing (5-fold increase in TGF-b, p=0.0251, and 2-fold reduction in TNF-a, p=0.019), vs that in DHEK-siNS. Compared to siNS, siKeap1 DHEKs also demonstrate decreased oxidative DNA damage as measured by 8-hydroxydeoxyguanosine (8-OHdG) assay and enhanced proliferation by 25% (54.6 vs. 32.6%, p=0.0034) in a BrdU incorporation assay. Keap1-silenced-DHEKs exhibit significant improved migration as early as 2 hours post-injury and at all following time points versus siNS. Intact diabetic skin demonstrates a 2-fold increase in 8-OHdG levels (p=0.0390), with corresponding increases in protein levels of Nrf2-P, the translocated intranuclear form of Nrf2, and downstream effector MnSOD. Compared to that of nondiabetic wounds, Keap1 expression persists at pre-wounded levels in diabetic wounds, which correlates
with low Nrf2-P. Downstream expression of antioxidant genes in diabetic wounds remains suppressed compared to normal wounded skin leading to increased 8-OHdG levels. Nrf2-P is found throughout the epidermis in unwounded tissue but becomes localized to the basal epithelium in the wounded state. However, in diabetic wounds, there is a 77% (p<0.0001) overall decrease in Nrf2-P expression with near complete absence of Nrf2-P in all but the most basal layer of the epidermis.

CONCLUSION: Dysregulation of Nrf2 leads to profound disruption of redox homeostasis in Type II diabetes. Nrf2 dysfunction in diabetic epidermal cells in vitro parallels that of the epidermal compartment in vivo. This dysfunction manifests in a loss of antioxidant gene expression, the accumulation of oxidative damage in DNA, and increased pathological wound burden among diabetic patients. Loss of active Nrf2 from the basal epithelium of the epidermis may be a sentinel event in the pathogenesis of delayed wound healing. Manipulating the Nrf2 pathway holds great promise as a novel therapeutic strategy to restore cellular redox homeostasis and the healing potential of diabetic skin.

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Association Between Repeat Alveolar Bone Grafting and Anxiety in Teenagers with Cleft Lip and Palate

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PURPOSE: In patients with cleft lip and palate (CLP), secondary alveolar bone grafting (ABG) is accompanied with a 20–30% regrafting rate due to insufficient bone stock for orthodontic rehabilitation. Due to various factors including pain and the age at which bone grafting is performed, we hypothesized that regrafting may contribute to psychosocial distress in children with CLP. In this study, we evaluate the relationship between alveolar bone regrafting and long term psychosocial functioning in patients with CLP.

METHODS: Patient Reported Outcome Measurement Information Systems (PROMIS) short-form measures (anger 5a, anxiety 8a, depression 8a, and peer relationships 8a) were administered to patients with CLP from the craniofacial clinics at the University of California, Los Angeles (UCLA) and the Orthopaedic Institute for Children (n=120). We obtained demographic information from patients’ medical records, including age at psychosocial assessment, total number of surgeries, whether or not they underwent a regraft surgery, and at what age the regraft surgery was performed. To evaluate the psychosocial functioning of patients who have completed ABG we selected children ages 14–17 (n=41). Two-tailed independent samples t-tests were conducted to compare psychosocial functioning of CLP ABG patients with a history of regraft surgery (n=11) and those without (n=30). A general linear model univariate analysis was conducted to determine if there was a significant interaction between regraft versus no regraft and total number of surgeries. A level of p<0.05 was considered statistically significant.

RESULTS: All patients had their initial ABG at the average age of 10.07±2.27 years. 26.8% of these patients required a regraft and had the regraft surgery at a mean age of 11.00±1.73 years. Patients with a history of regraft reported no significant difference in anger, depression, and peer relationship outcomes compared to those with no regraft. However, patients with CLP who underwent a regraft reported significantly higher anxiety levels (54.60±6.34 vs.45.05±9.1; p=0.003) than patients who did not. The general linear model demonstrated no significant interaction between history of regraft surgery and total number of surgeries, suggesting that regrafting was an independent surgical predictor for increased anxiety.

CONCLUSION: Increased surgical burden during childhood has been associated with decreased psychosocial functioning in teenagers. Identifying methods of decreasing psychosocial distress during childhood is necessary to improve psychosocial functioning in adolescence, especially in patients with a history of regraft. History of alveolar bone regraft surgery is independently associated with higher self-reported anxiety levels in teenagers with cleft lip and palate.