

## **Wound Healing Society Annual Meeting May 2002. Baltimore, Maryland**

### **Second Messenger Eps8 Is A Gene Therapy Candidate In Wound Healing**

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Eps8 (EGFR pathway substrate 8) is a substrate for certain receptor tyrosine kinases and a second messenger in the signal pathway from Ras to Rac. Eps8-null fibroblasts lack PDGF-induced actin reorganization and membrane ruffling, and eps8 enhances the mitogenic response to EGF in fibroblasts. Using a cDNA subtraction strategy, it was found that eps8 mRNA was strongly induced in skin excisional wounds in both normal and diabetic mice. In situ hybridization confirmed local expression. To establish the role of eps8 in wound healing, we transferred murine eps8 cDNA by particle-mediated (gene gun) or adenoviral-mediated methods into various animal wound models. In a rat polyvinyl alcohol sponge implantation model, we injected  $10^7$ - $10^9$  PFU of either Ad-eps8 or Ad-LacZ into sponges 3 days post-implantation. At d7 Ad-eps8 infected sponges displayed much better organization of granulation tissue than controls. The contents of collagen, DNA and protein in Ad-eps8 infected sponges increased 11.2% ( $p < 0.05$ ), 61.2% ( $p < 0.001$ ), and 28.0% ( $p < 0.05$ ). By d10, only protein content was elevated (13.5%,  $p < 0.05$ ). In a rat incisional wound model, gene gun DNA delivery at d0 produced a 52.1% increase of wound strength in normal rats and an 86.8% increase in diabetic rats at d10, although the increase did not reach significance. On d7 after Ad-eps8 injection, the breaking pressure of Ad-eps8 infected wounds in normal rats increased by 23% ( $p < 0.05$ ). In the rabbit ear ulcer model, particle-mediated transduction of eps8 cDNA increased the rate of wound closure by 44% (d5) and 24% (d10) and collagen content 70%. These findings show that eps8 can augment experimental granulation tissue and improve wound strength. Because of its position in the signal transduction pathway, eps8 gene therapy of wounds has the intriguing possibility of sensitizing target cells to multiple growth factors.

Supported by SWITCH Biotech AG