"Survival Surgery/Skin Grafts" Presentation

2009 CCR Mouse Research Models/Mouse 101 Course

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Perspective 1: Techniques used in the Yuspa lab to investigate mechanisms of cancer development in skin

A. DMBA/PMA induced skin tumors on inbred and genetically modified mice.

B. Primary keratinocytes from these mice transduced with v-ras^{Ha} (ras-keratinocytes).

C. Orthotopic grafts of normal and ras keratinocytes onto nude mice (target cells vs host)

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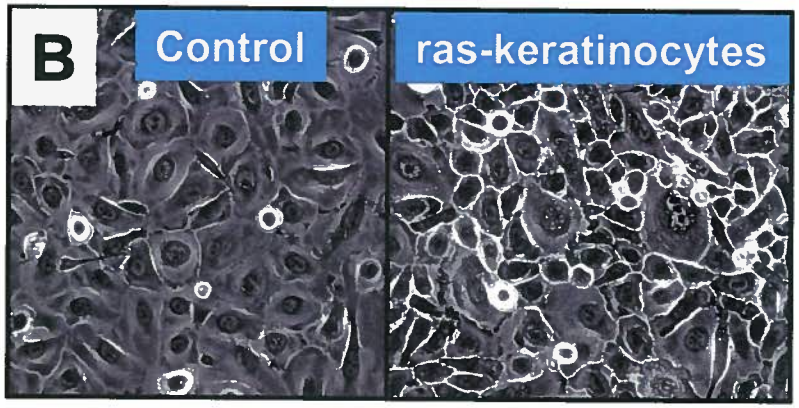
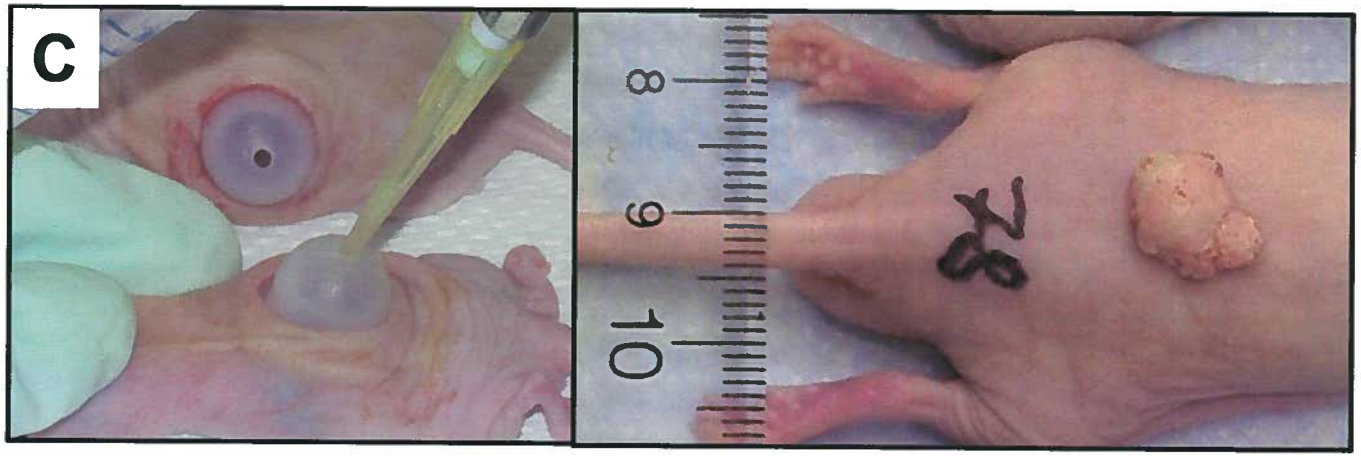
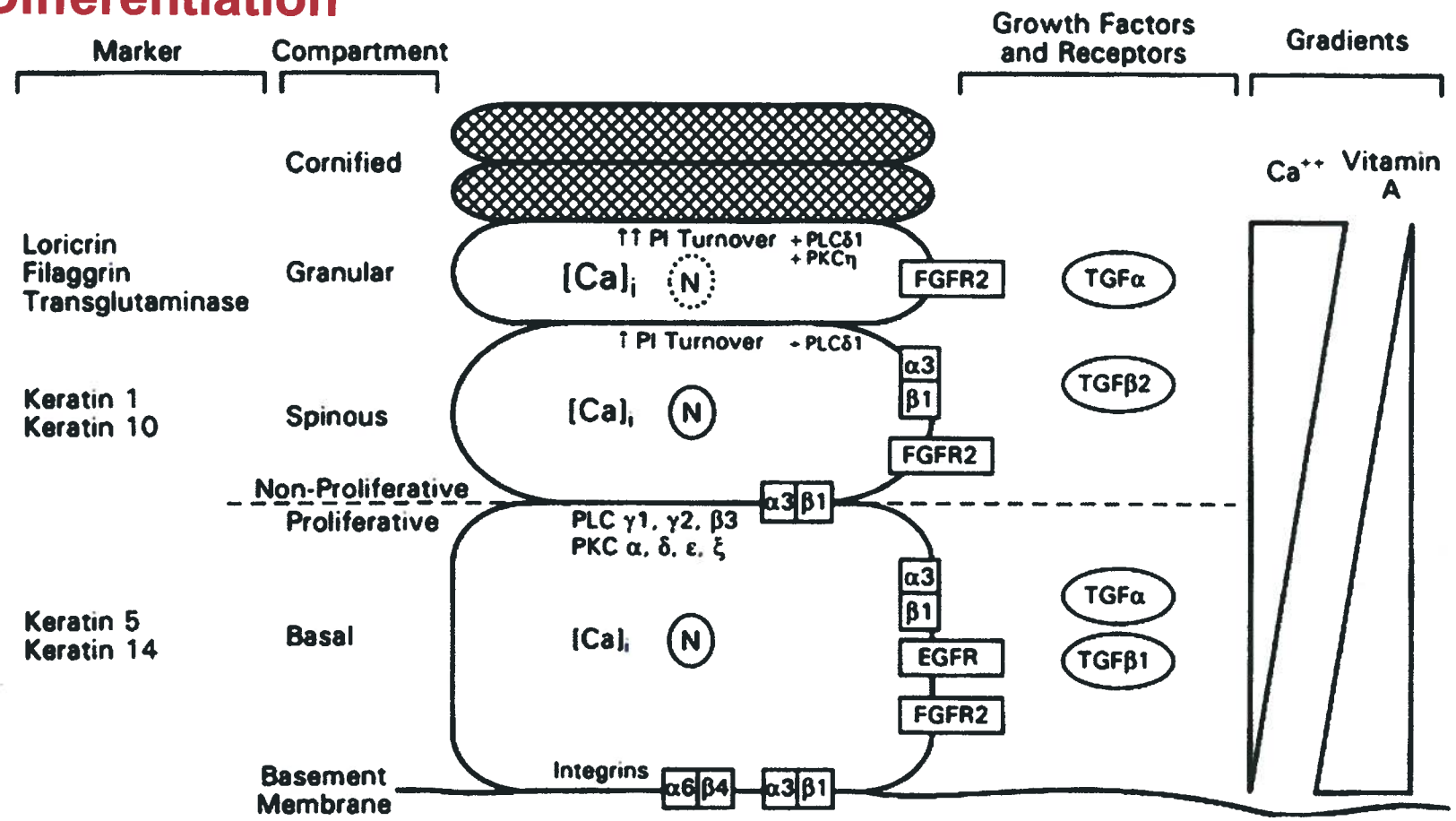




***Perspective 2*: Parallel studies have been aimed at gaining a better understanding of the controls in normal epidermal differentiation. These have revealed a highly**

Compartmentalized Control of Proliferation and Differentiation



Selected grafting bibliography:

Cell grafting:

1.Lichti, U., Anders, J, & Yuspa S. Nature Protocols 3:799-810 (2008) (Procedures for preparing primary mouse epidermal keratinocytes and dermal fibroblasts and for cell grafting)

2.Weinberg, W.C. et al., J. Invest. Dermatol. 100: 229-236 (1993) (Reconstitution of hair follicle formation in grafts)

3.Roop, D.R. et al., Nature 323: 822-824 (1986) (Benign tumor formation in grafts from keratinocytes tranduced with the Harvey *ras* oncogene)

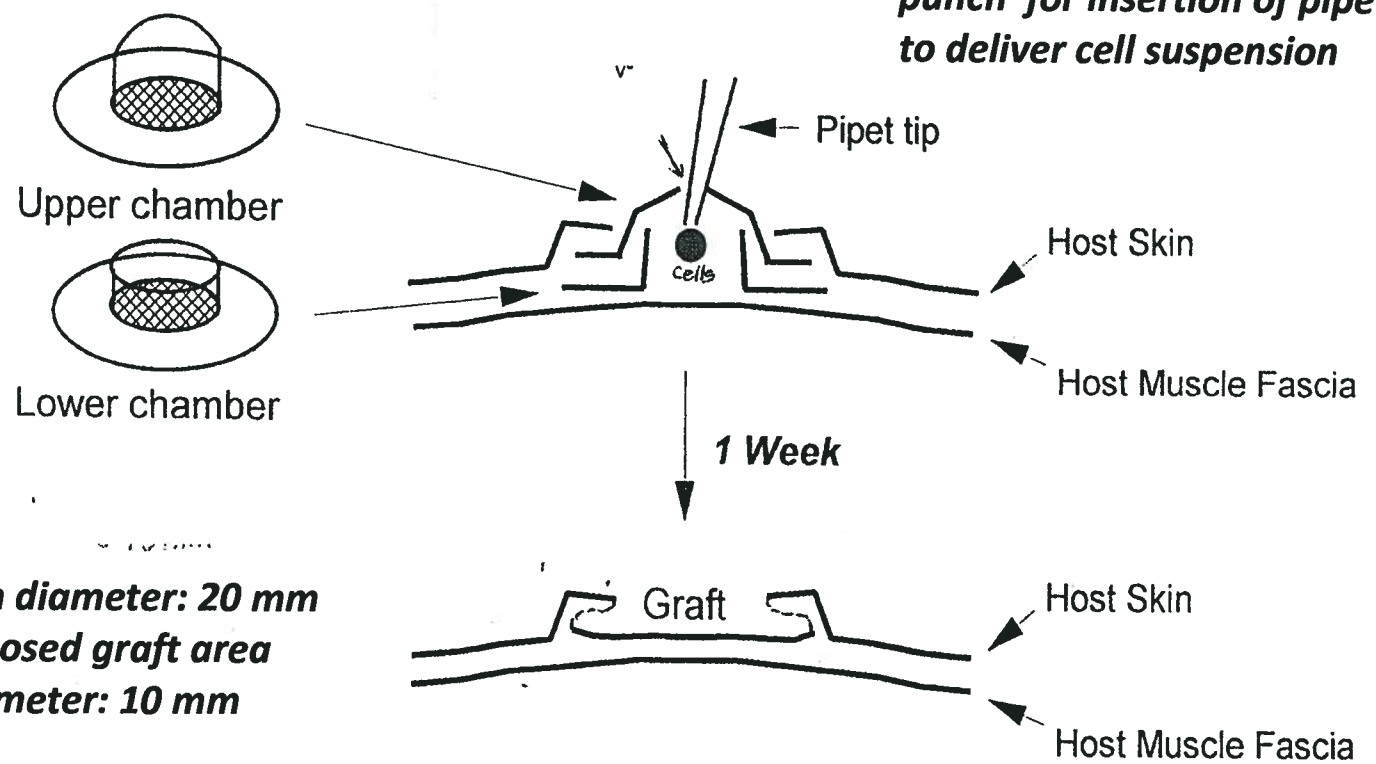
Whole skin grafting:

1.McFarland, H.I., & Rosenberg , A.S., Current Protocols in Immunology 4.4.1-4.4.13, February 2009 (detailed whole skin grafting procedure)

2.Kuhn, U., Terunuma, A., Pfutzner, W., Foster, R.A., & Vogel, J.C., J. Virol. 76:1496-1504 (2002) (delivery of GFP with lentiviral vectors to human graft skin)

Schematic of Grafting Cell Mixtures on the Backs of Nude Mice

2.5 mm diameter hole cut through dome with biopsy punch for insertion of pipet tip to deliver cell suspension

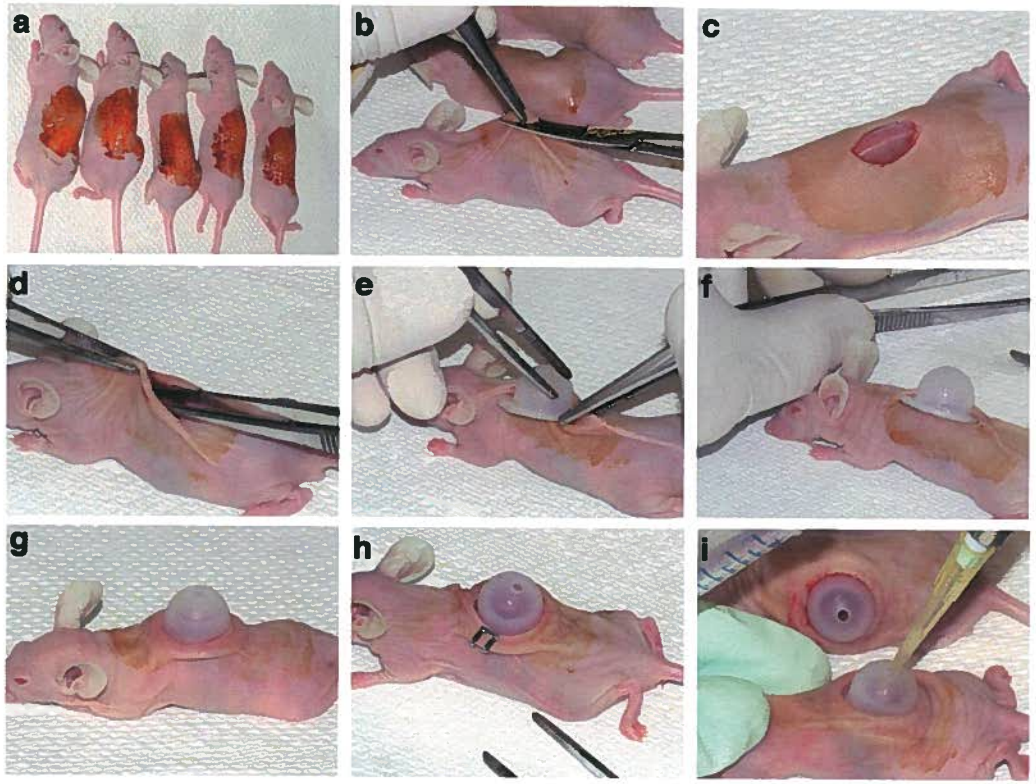


Rim diameter: 20 mm
Exposed graft area diameter: 10 mm

Cell suspension typically contains a mixture of up to 6 million each of keratinocytes and fibroblasts.

Silicone graft chambers available from:
Dave Cole, Cole Equipment Co., Phone: 301-946-4746, e-mail: coleco@erols.com

Cell Grafting Procedure



a. Decontaminate graft area, followed by alcohol wipe
b. Lift skin; cut with curved scissors
c. Exposed graft area
d. Undermine surrounding skin with PBS-moistened forceps
e. Slide moistened chamber under lifted skin on one side

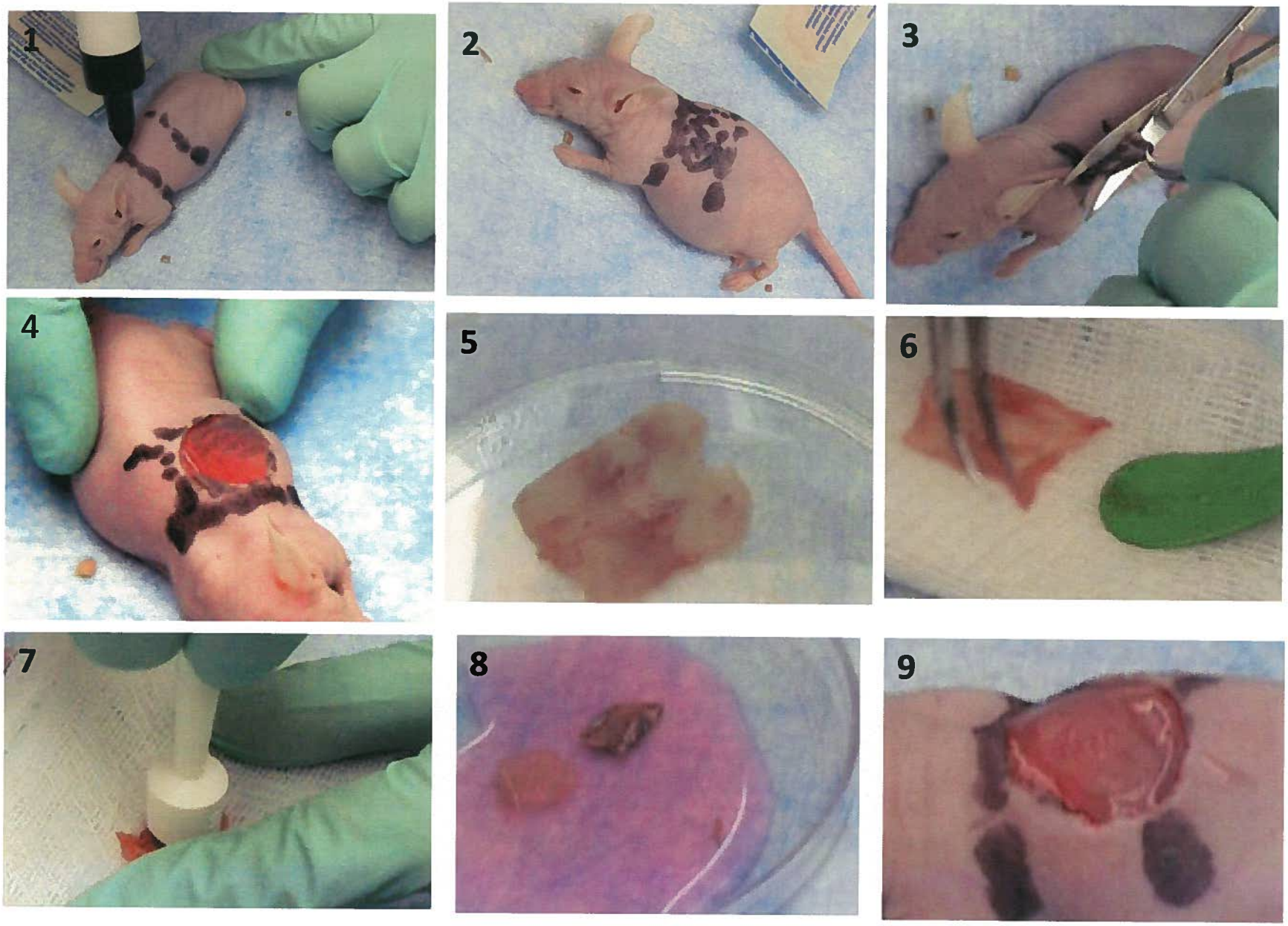
f. Ready for lifting skin on opposite side and completing chamber insertion
g. Snugly seated dome h. loosely seated dome secured with wound clip
h. Introducing suspension of graft cell mixture (100-150 ul) with micropipette
Not shown: application of "Bandguard" around hole in the silicon dome with cotton tipped applicator.

Whole Skin Grafting

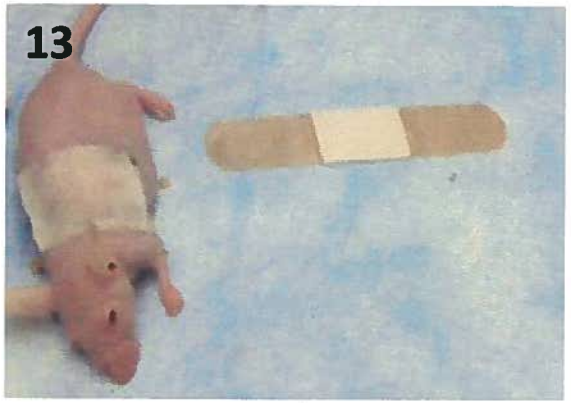
This method is essentially the only technique to determine whether the cause of severely compromised skin function in mouse mutants or in genetically altered mice is inherent in the skin itself or also has systemic components.

We have recently benefited from excellent hands-on instruction in the this technique by Dr. Atsushi Terunuma's (NCI/CCR/Dermatology Branch) and have obtained very interesting preliminary results.

STEPS IN WHOLE SKIN GRAFTING PROCEDURE #1-9



STEPS IN WHOLE SKIN GRAFTING PROCEDURE #10-18



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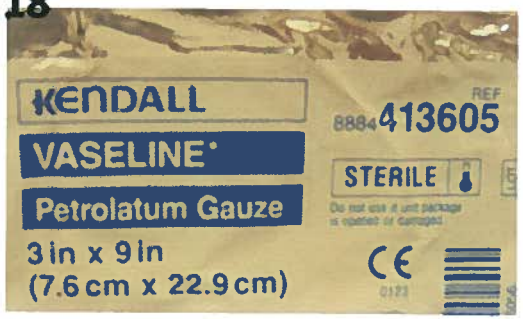
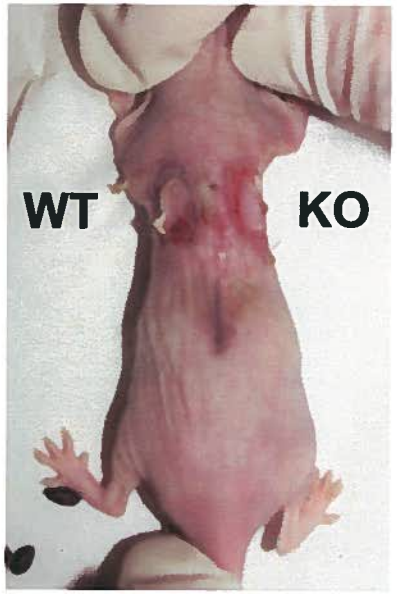
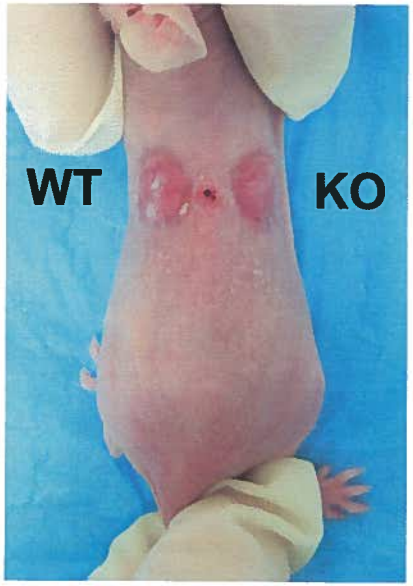


¾ in. x 3 in.

Images of graft areas at various times after grafting WT and KO skin from E18.5 mice. KO mice are not viable after birth*. Their skin is grossly abnormal in function and histology.
However, KO skin appears to develop normally in grafts!



Day 7
immediately
after removal
of bandage



Day 11

Day 15

In collaboration with Dr. Junko Okano, NIAMS