

# IL-24 AND ITS ROLE IN WOUND HEALING

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*Making Cancer History*

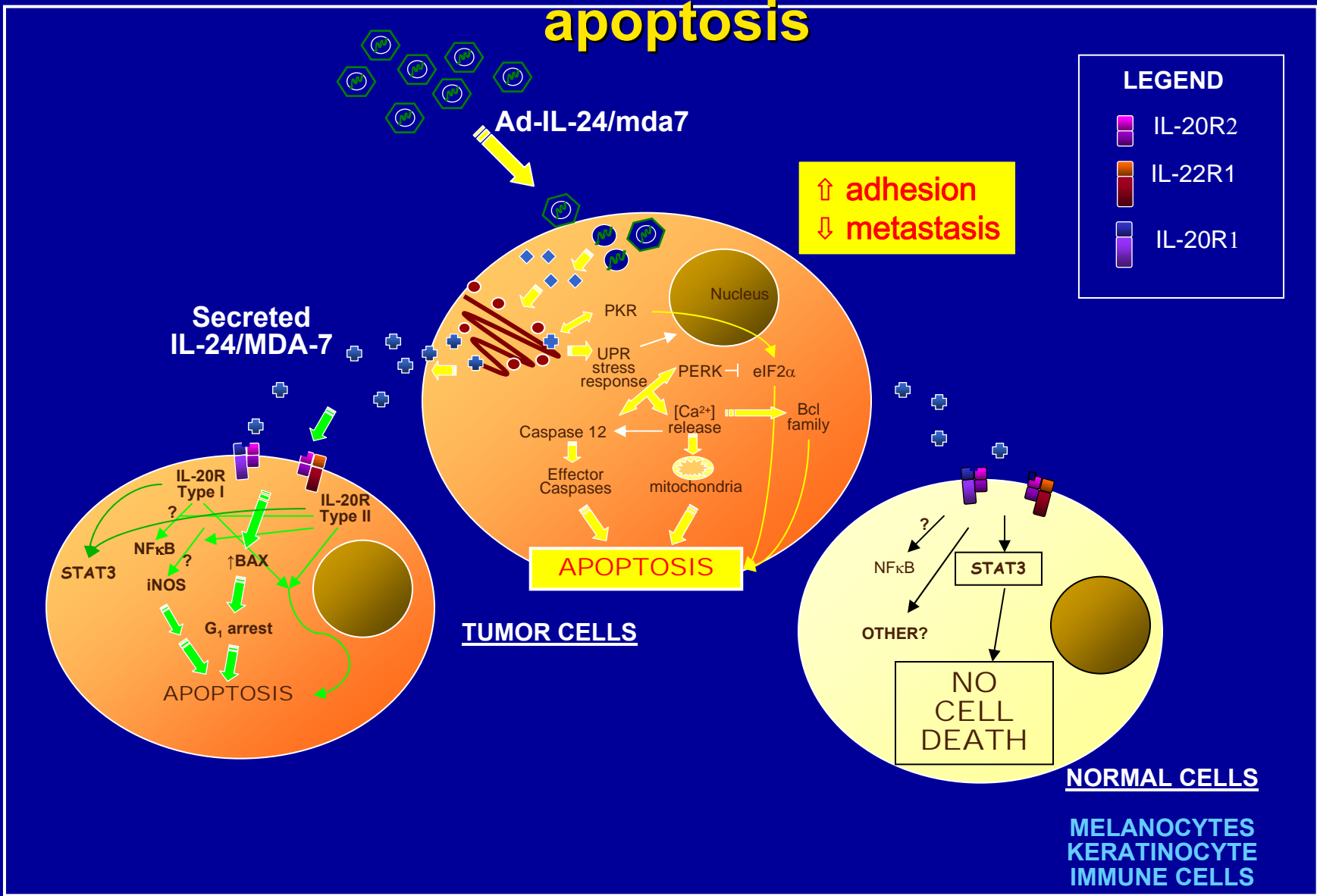
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Introgen Therapeutics, Inc.,  
Houston, TX

# IL-24/MDA-7 is a Tumor Suppressor

- mda-7 gene transfer inhibits growth of a broad spectrum of tumor cells while not affecting normal cells
- Ad-mda7 induces apoptosis in tumor cells and the mechanism of apoptosis is dependent on the cell type
- Ad-mda7 blocks tumor cell growth *in vitro* and *in vivo* through G2/M cell cycle blockade
- Ad-mda7 is currently being tested in Phase II clinical trial.
- Tumor suppressor functions have been duplicated *in vitro* and in mouse models with the pure protein.

# Signaling pathways of IL-24/MDA-7 induced apoptosis



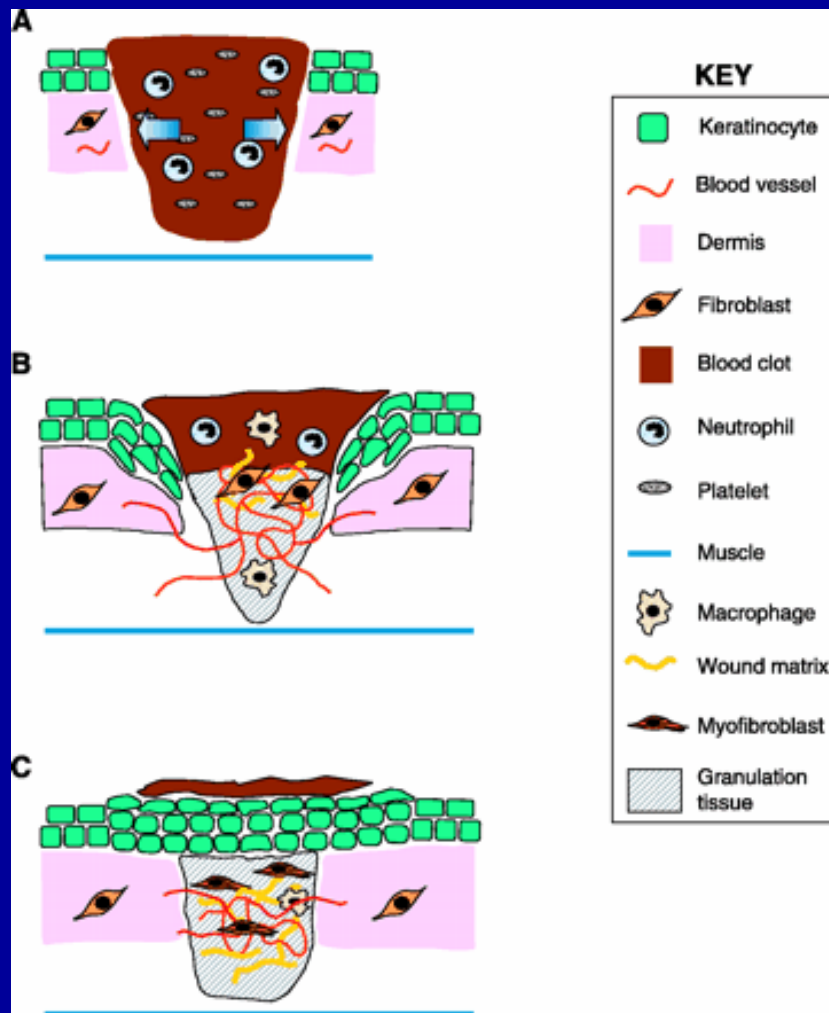
# IL-24 Expression and Function in Normal Cells

- IL-24 gene maps to chromosome 1q32 in the IL-10 family cytokine cluster. It shares 19% amino acid identity with IL-10 as well as similar homology with other IL-10 family cytokines, IL-19, IL-20, IL-22, and IL-26.
- IL-24 is expressed and secreted by in cytokine activated monocytes. IL-24 is constitutively expressed by melanocytes in the skin.
- IL-24 protein stimulates the secretion of secondary cytokines from peripheral blood mononuclear cells.
- Regulation of expression in monocytes is at the post-transcriptional level with cytokine stimulation stabilizing IL-24 mRNA

# IL-24 in the Skin

- Is IL-24 involved in inflammatory responses in the skin?
- What cells express IL-24?
- What is the function of IL-24 in skin inflammation?
- How does this relate to melanoma development

# Wound Healing



**12-24 hour:** wound area filled with a blood clot with neutrophils

**2-7 days:** Blood vessel formation in the clot, lots of macrophages with keratinocytes proliferating at the wound edge

**1-2 weeks:** wound contraction and collagen deposition

# Wounding Experiment n=4

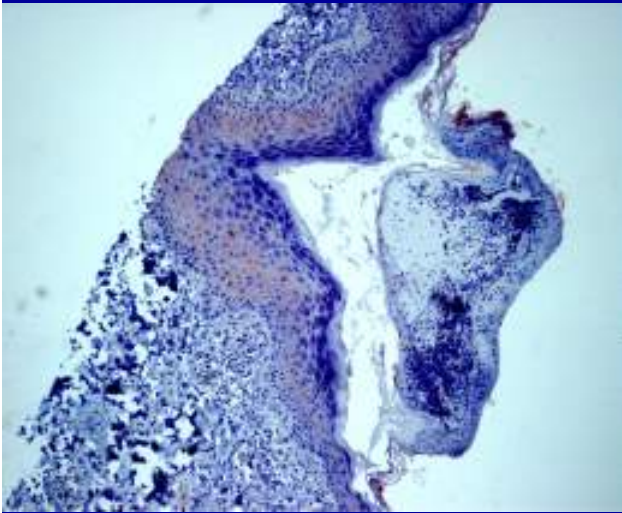


Biopsy #1 Day 2

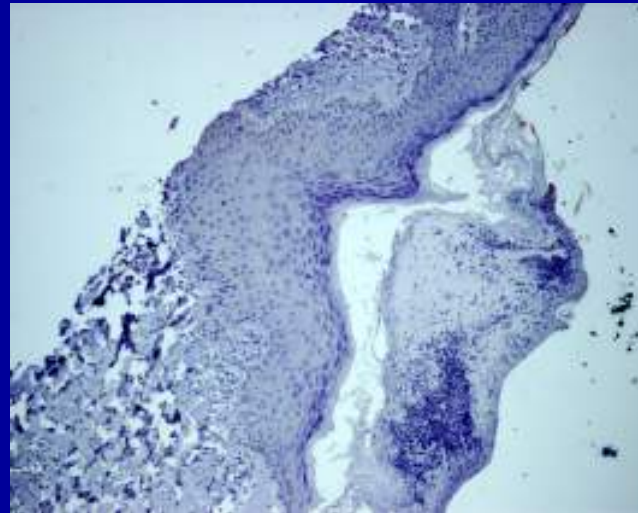
Biopsy #2 Day 6

Biopsy #3 Day 10

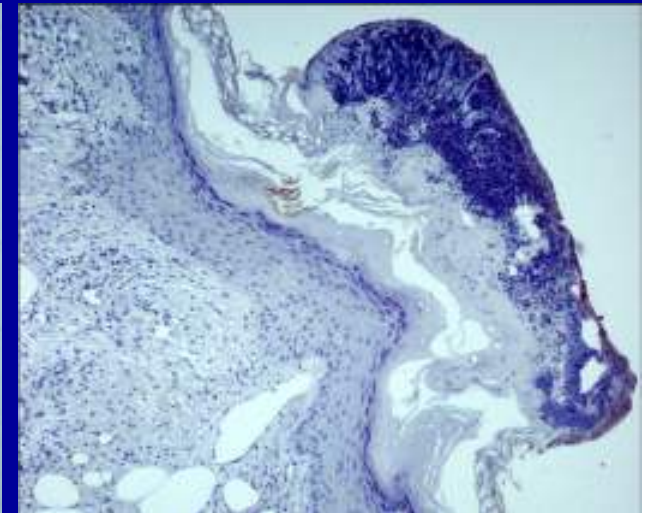
# IL-24 Expression During Wound Repair



**Day 2**

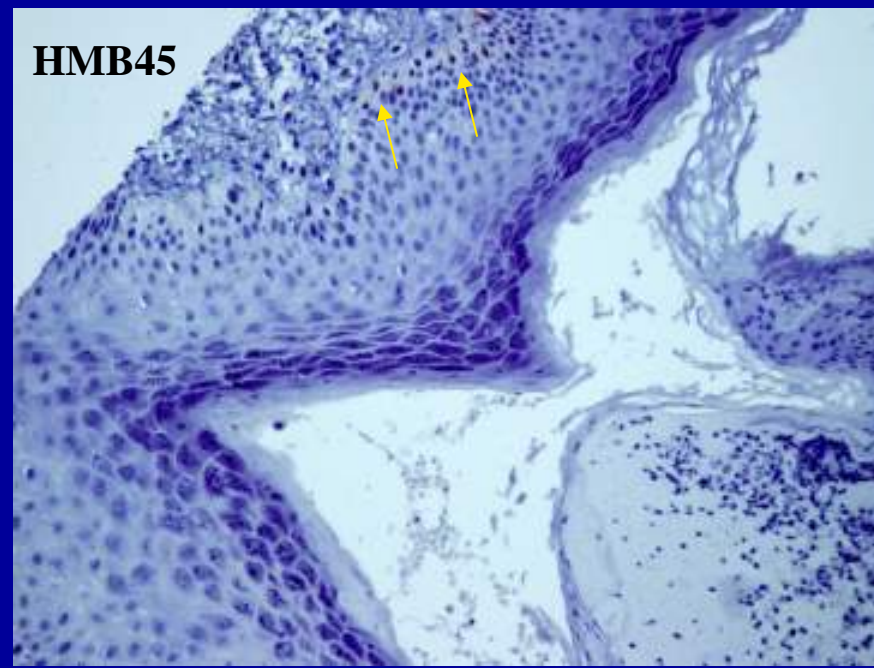
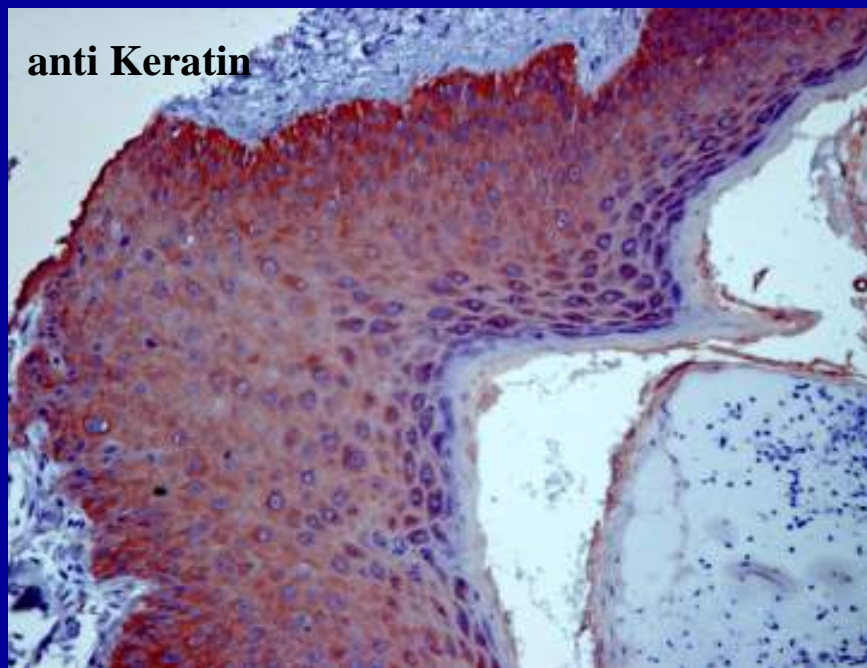
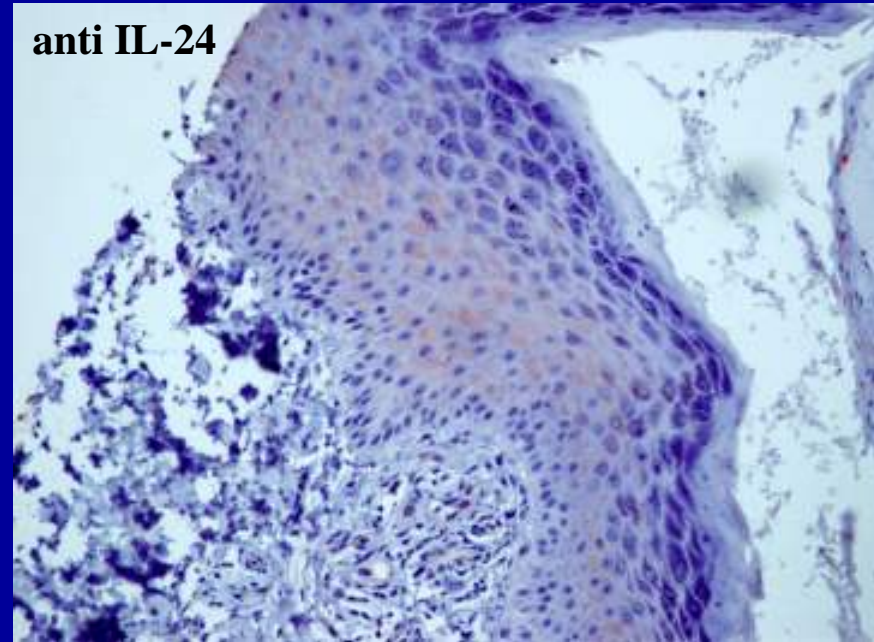
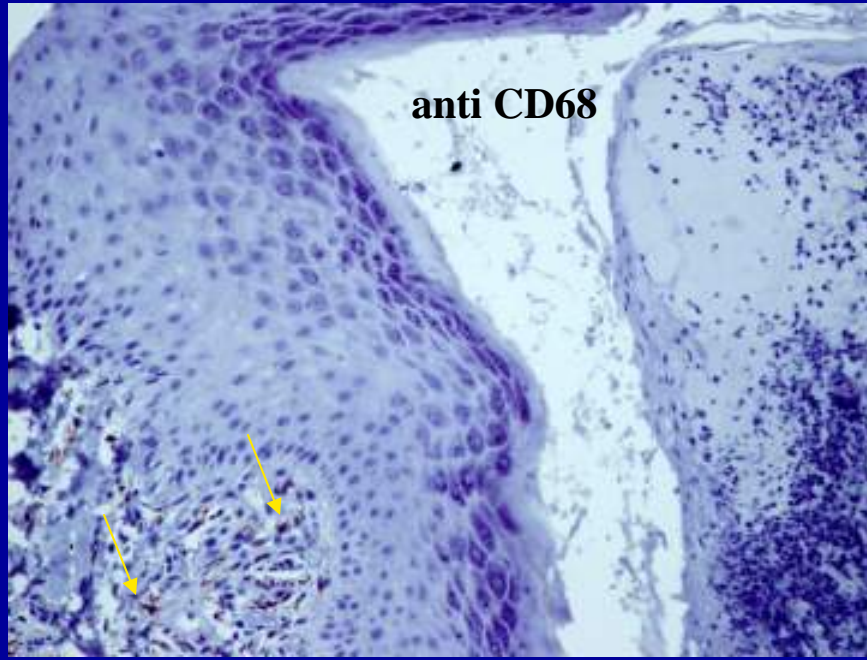


**Day 6**



**Day 10**





# IL-24 Expression during Wound Healing

<i>Patient</i>	<i>Day 2</i>	<i>Day 6</i>	<i>Day 10</i>
1	+++	++	-
2	++	+	-
3	++	-	-
4	+	++	-

**+** 5-25% cells staining positive

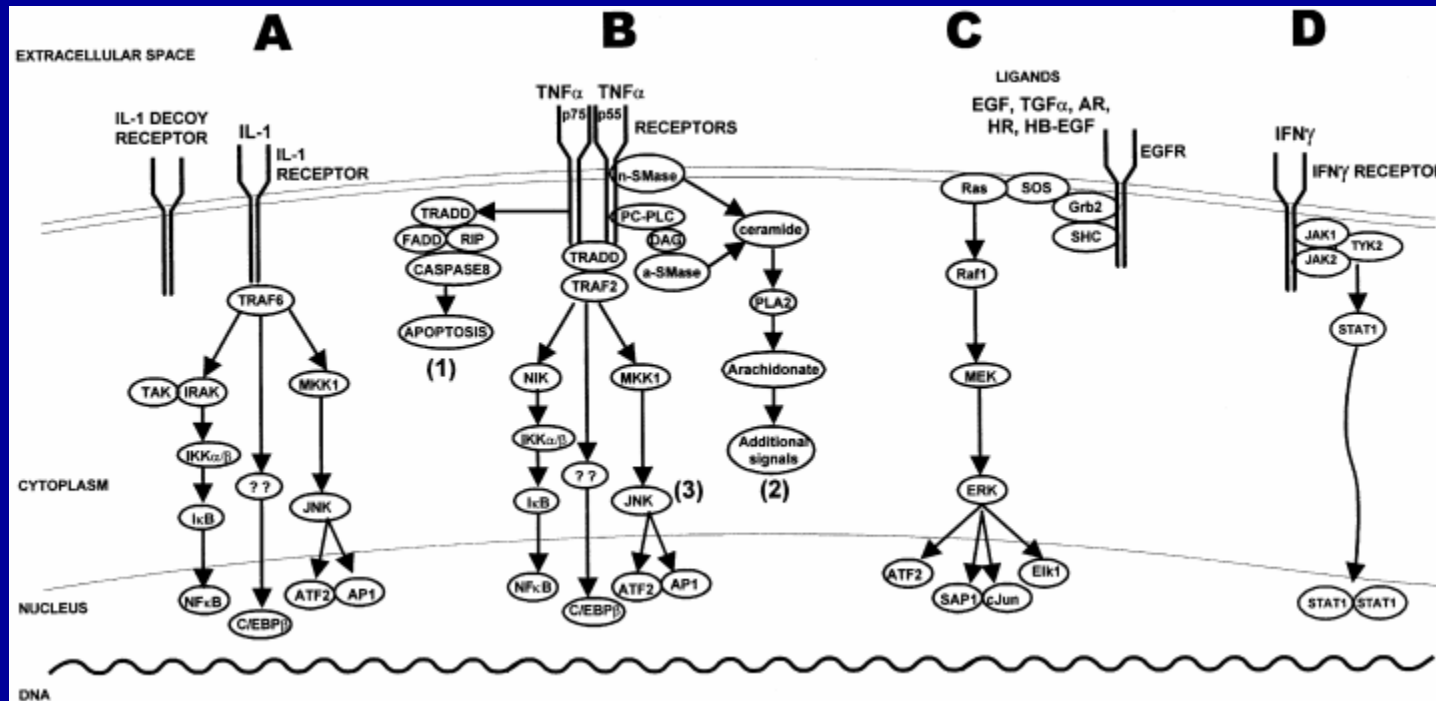
**++** 25-75%

**+++** >75%

# IL-24 in the Skin

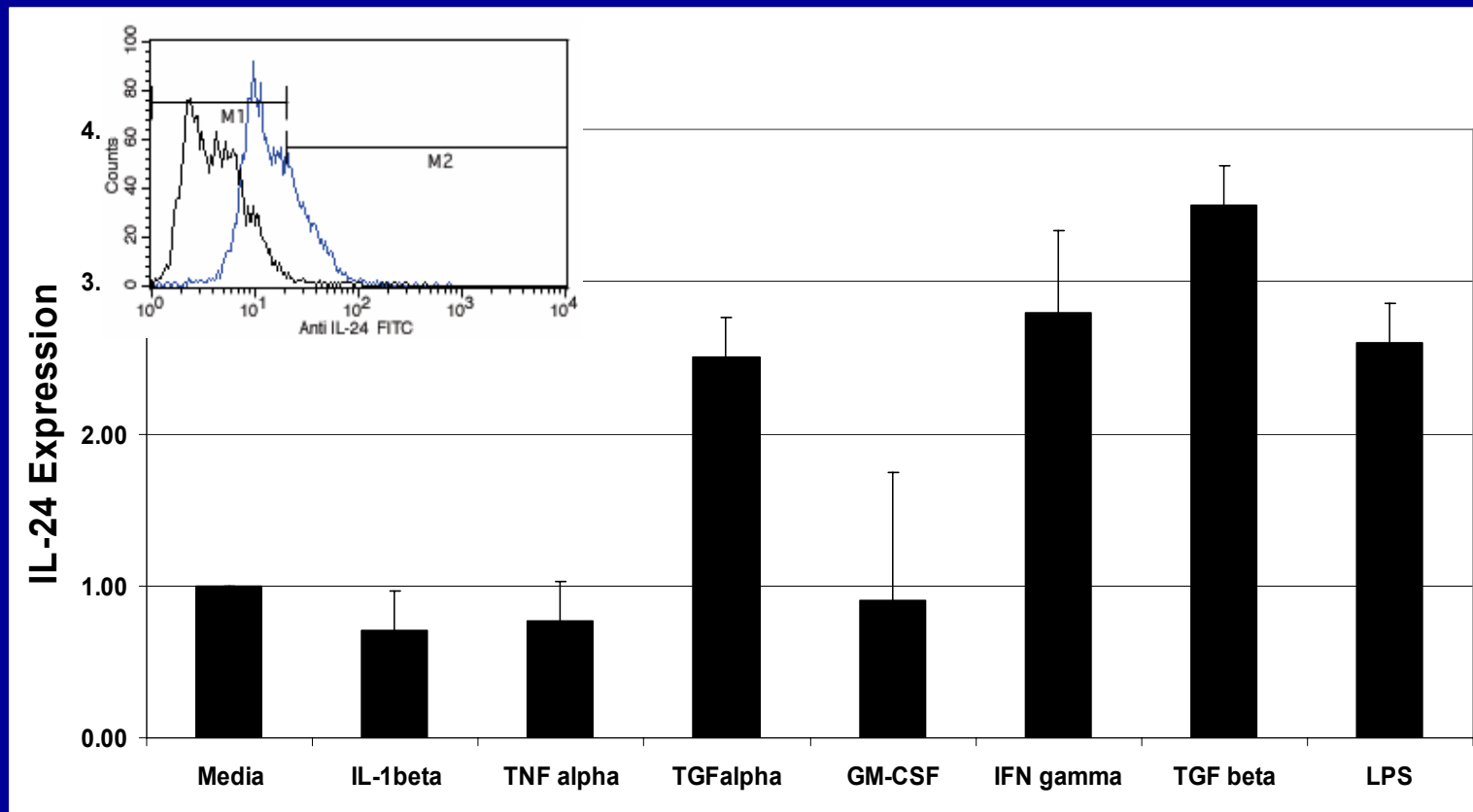
- Is IL-24 involved in inflammatory responses in the skin?
- What stimulates the expression of IL-24 ?
- What is the function of IL-24 in skin inflammation?

# Signaling Pathways in Keratinocytes



- IL-1 $\beta$  activation of keratinocytes
- TNF $\alpha$  maintains activated state
- Growth factor ligation of EGFR results in proliferation and migration
- IFN $\gamma$  contractions of newly formed extracellular matrix and stops proliferation of keratinocytes

# Cytokine Induced Expression of IL-24 in Keratinocytes



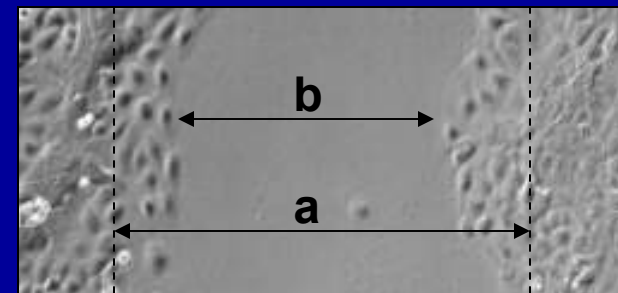
# IL-24 in the Skin

- Is IL-24 involved in inflammatory responses in the skin?
- What cells express IL-24 and its receptors?
- What is the function of IL-24 in skin inflammation?



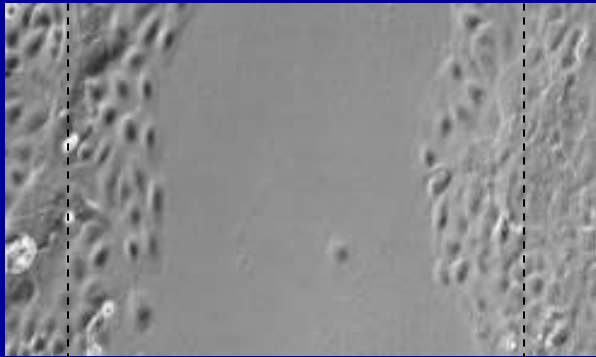
# *in vitro* Wounding Assay "Scratch Test"

- Grow confluent monolayer of keratinocytes (NHEK)
- +/- Mitomycin C
- Wash well
- Scratch monolayer
  
- Incubate 18 + hr
- Measure relative growth  
= width of scratch at 0hr -  
width at 18 hr

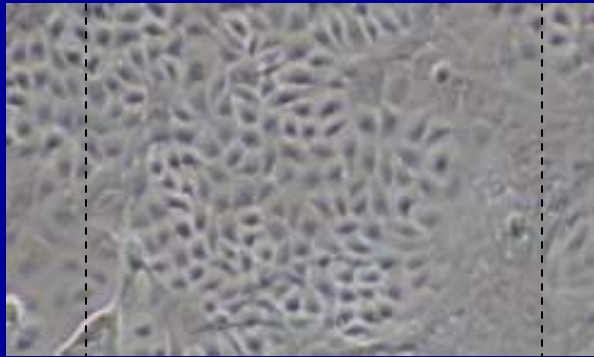


$a-b=\text{growth}$

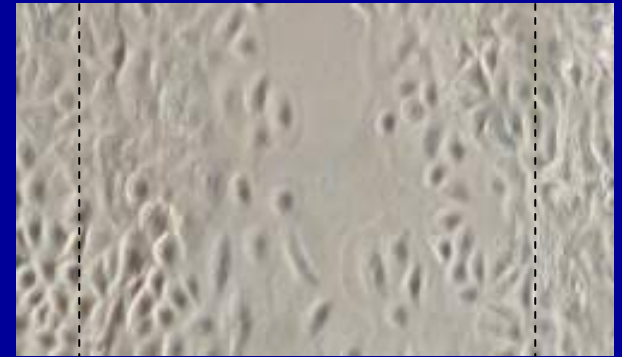
# NHEK *in vitro* Wounding Assay



+ 0



+ TGF alpha 100 ng/ml



+ TGF alpha 50 ng/ml

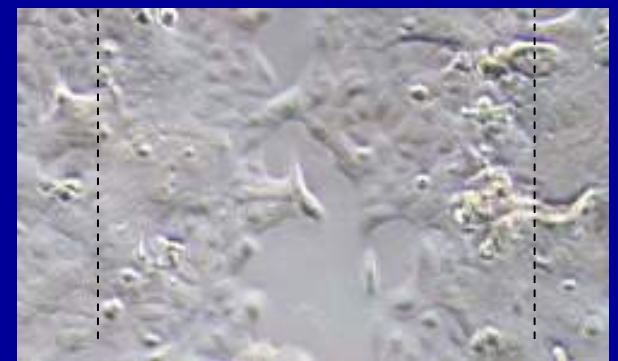


+ 0

+IL-24 25 ng/ml



+ TGF alpha 100 ng/ml

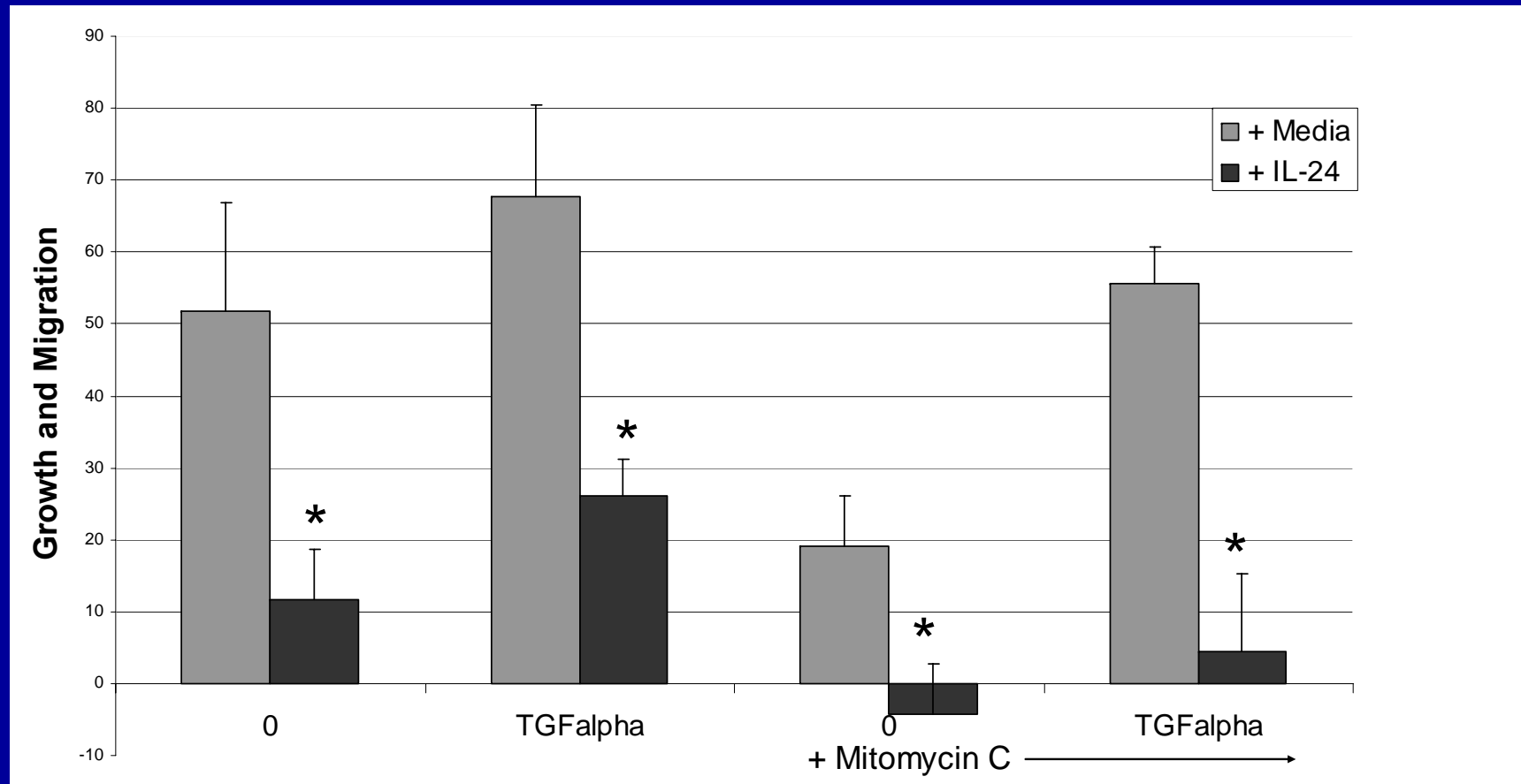


+ TGF alpha 50 ng/ml



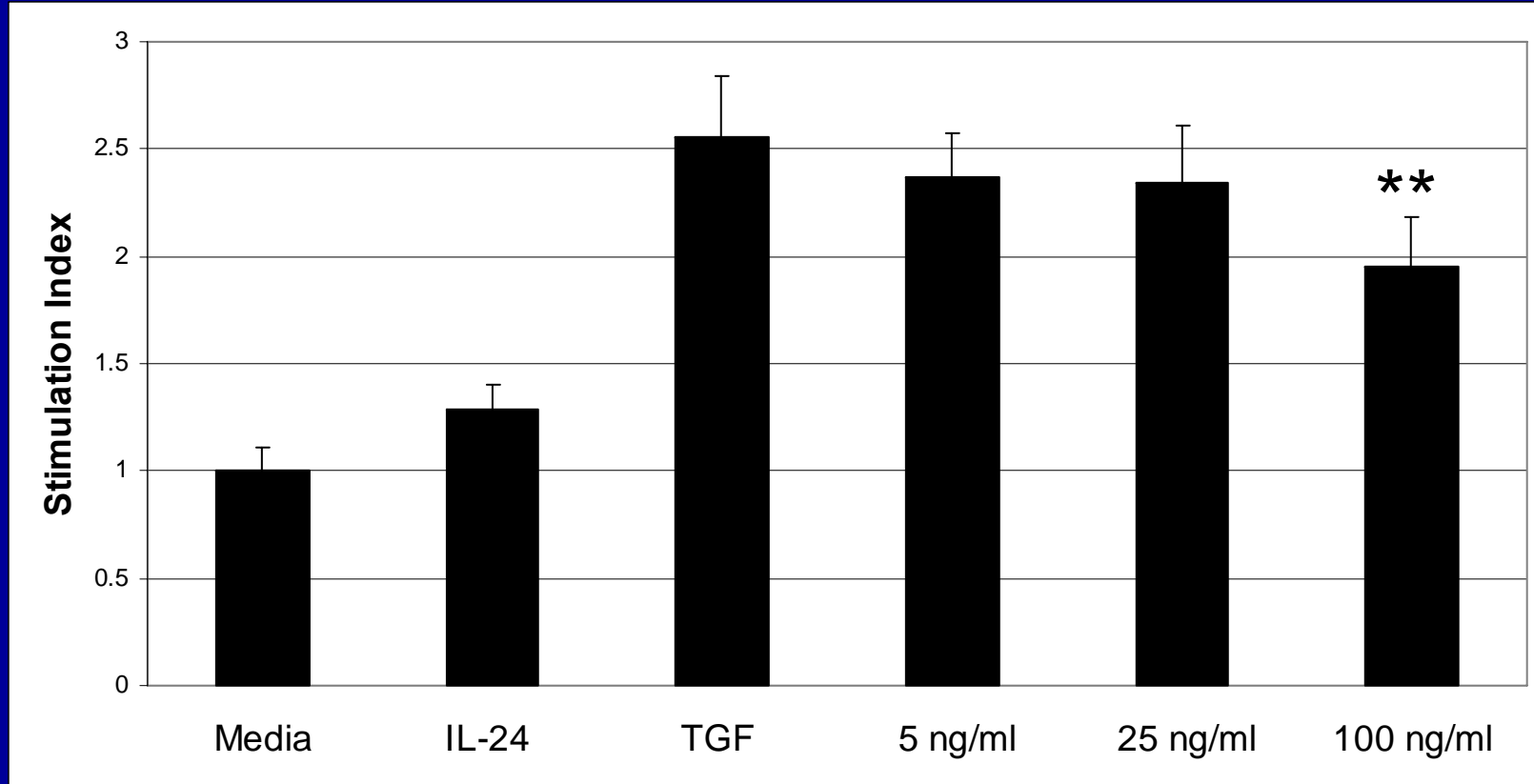


# Effect of IL-24 on TGF $\alpha$ stimulated Proliferation and Migration



\*p<0.05 (media vs IL-24)

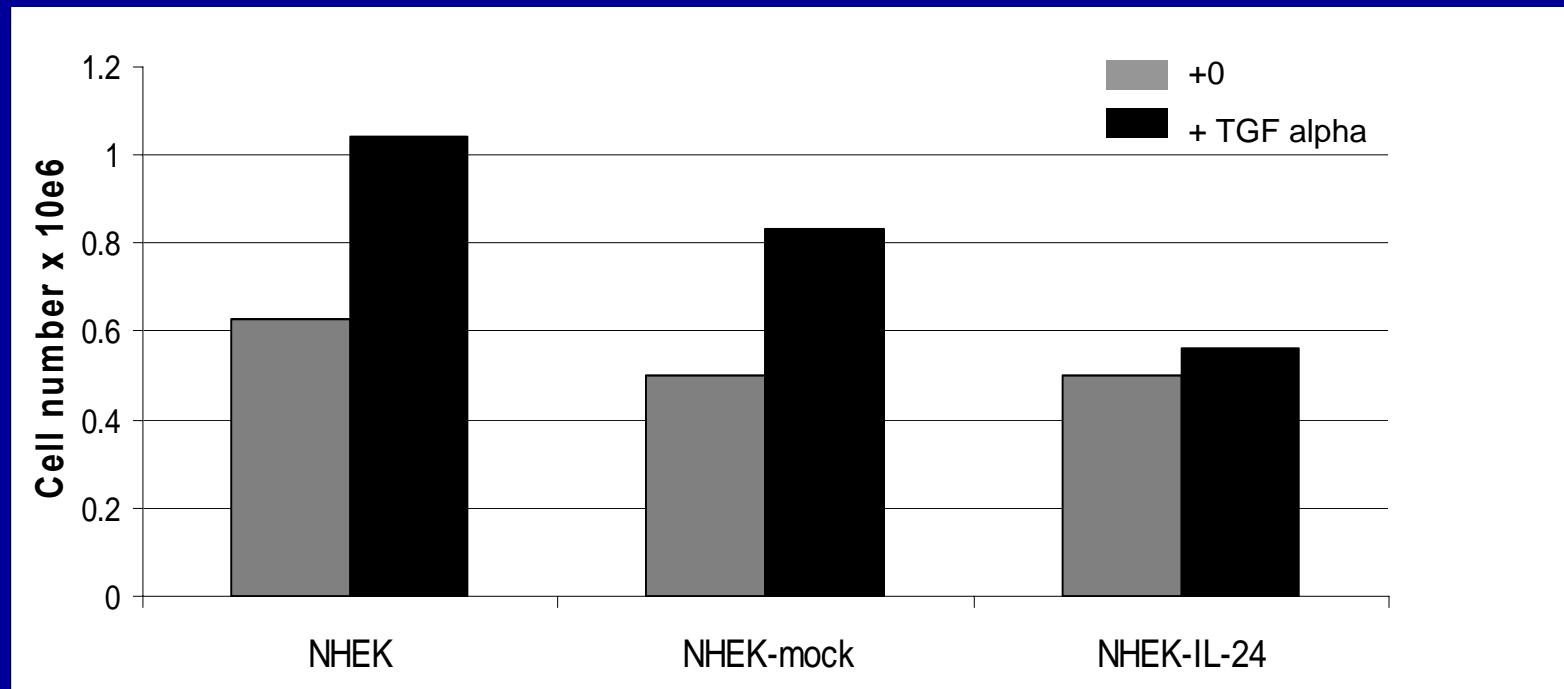
# Effect of IL-24 on Keratinocytes Proliferation



\*\*  
p<0.05

IL-24 →  
&  
TGF $\alpha$  50 ng/ml →

# Effect of TGF $\alpha$ on NHEK expressing IL-24



All cells were > 90% viable @ 48 hr

# Conclusions

- IL-24 is expressed during the later stages of wound healing in proliferating keratinocytes
- Cytokines involved in wound healing including  $TGF\alpha$ ,  $TGF\beta$  and  $IFN\gamma$ , induce the expression of IL-24 in keratinocytes.
- IL-24 protein inhibits growth factor stimulated proliferation and migration of keratinocytes.
- NHEK forced to express IL-24 do not proliferate in response to growth factors

# Working Hypothesis

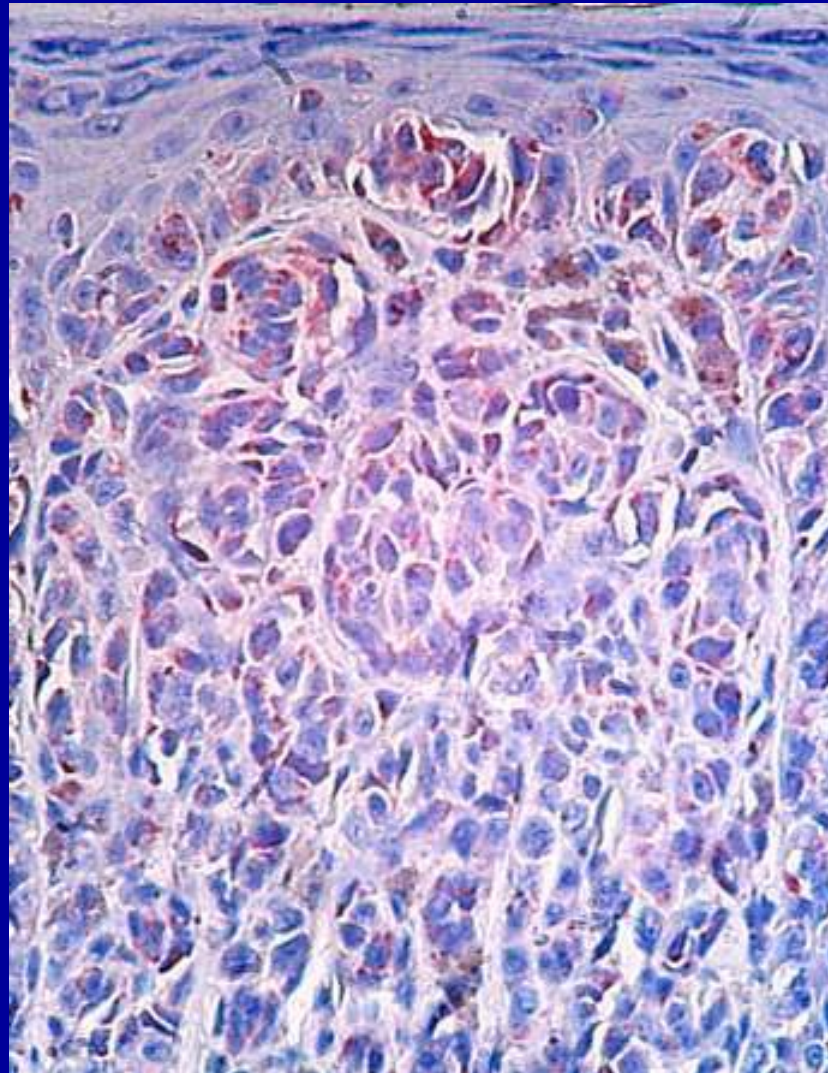
- We propose that the pro-inflammatory cytokine IL-24 functions in wound repair. Expression of this cytokine is upregulated by  $TGF\alpha$ ,  $TGF\beta$  and  $IFN\gamma$ , factors involved in the later stages of wound healing.
- IL-24, produced by keratinocytes and immune cells, acts to inhibit proliferation and migration causing the contraction of the wound and return of keratinocytes to their normal differentiating processes.
- Loss of IL-24 expression will lead to uncontrolled keratinocyte proliferation abnormal healing.

# Loss of IL-24 in the invasive front of a Primary melanoma

superficial



deep



MDA-7 IHC of the Clark Level III and IV primary tumors shows significantly less staining in the deep portions of superficial layer

n=84 tumors

(p = 0.003)

## IL-24 and Melanoma Progression

- Cancer and wound healing are both characterized by cell proliferation, cell migration and invasion, and angiogenesis.
- We propose that IL-24 inhibits growth factor-induced proliferation and invasion in both wound repair and melanoma progression.
- Loss of IL-24 expression in invasive melanoma cells results in the loss of growth control and subsequent tumor progression.

