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 \textit{in vitro} and \textit{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 \textit{in vitro} and in mouse models with the pure protein.
Signaling pathways of IL-24/MDA-7 induced apoptosis

Ad-IL-24/MDA-7

↑ adhesion
↓ metastasis

LEGEND

- IL-20R2
- IL-22R1
- IL-20R1

Secreted IL-24/MDA-7

TUMOR CELLS

APOPTOSIS

STAT3

NFκB

G1 arrest

BAX

IL-20R Type I

IL-20R Type II

NFκB

iNOS

APOPTOSIS

MELANOCYTES

KERATINOCYTE

IMMUNE CELLS

SECRETED IL-24/MDA-7

PKR

UPR stress response

[Ca2+] release

Effectors Caspases

Effector Caspases

Caspase 12

mitochondria

PERK eIF2α

Bcl family

Nucleus

NORMAL CELLS

NO CELL DEATH

STAT3

OTHER?

MELANOCYTES

KERATINOCYTE

IMMUNE CELLS

↑ adhesion
↓ metastasis

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

NFκB

iNOS

APOPTOSIS

G1 arrest

↑ BAX

↑ STAT3

NO CELL DEATH
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

<table>
<thead>
<tr>
<th>Patient</th>
<th>Day 2</th>
<th>Day 6</th>
<th>Day 10</th>
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<tbody>
<tr>
<td>1</td>
<td>+++</td>
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<tr>
<td>2</td>
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<tr>
<td>4</td>
<td>+</td>
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+ 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?
A. IL-1β activation of keratinocytes
B. TNFα maintains activated state
C. Growth factor ligation of EGFR results in proliferation and migration
D. IFNγ contractions of newly formed extracellular matrix and stops proliferation of keratinocytes
Cytokine Induced Expression of IL-24 in Keratinocytes

![Graph showing IL-24 expression in keratinocytes induced by various cytokines.](image-url)
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

Koivisto L et al. HaCaT keratinocyte migration is dependent on EGFR signaling. Exp Cell Research 2006
NHEK *in vitro* Wounding Assay

+ 0 + TGF alpha 100 ng/ml + TGF alpha 50 ng/ml

+ 0 + TGF alpha 100 ng/ml + TGF alpha 50 ng/ml

+ IL-24 25 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
Effect of TGFα 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
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

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)

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.