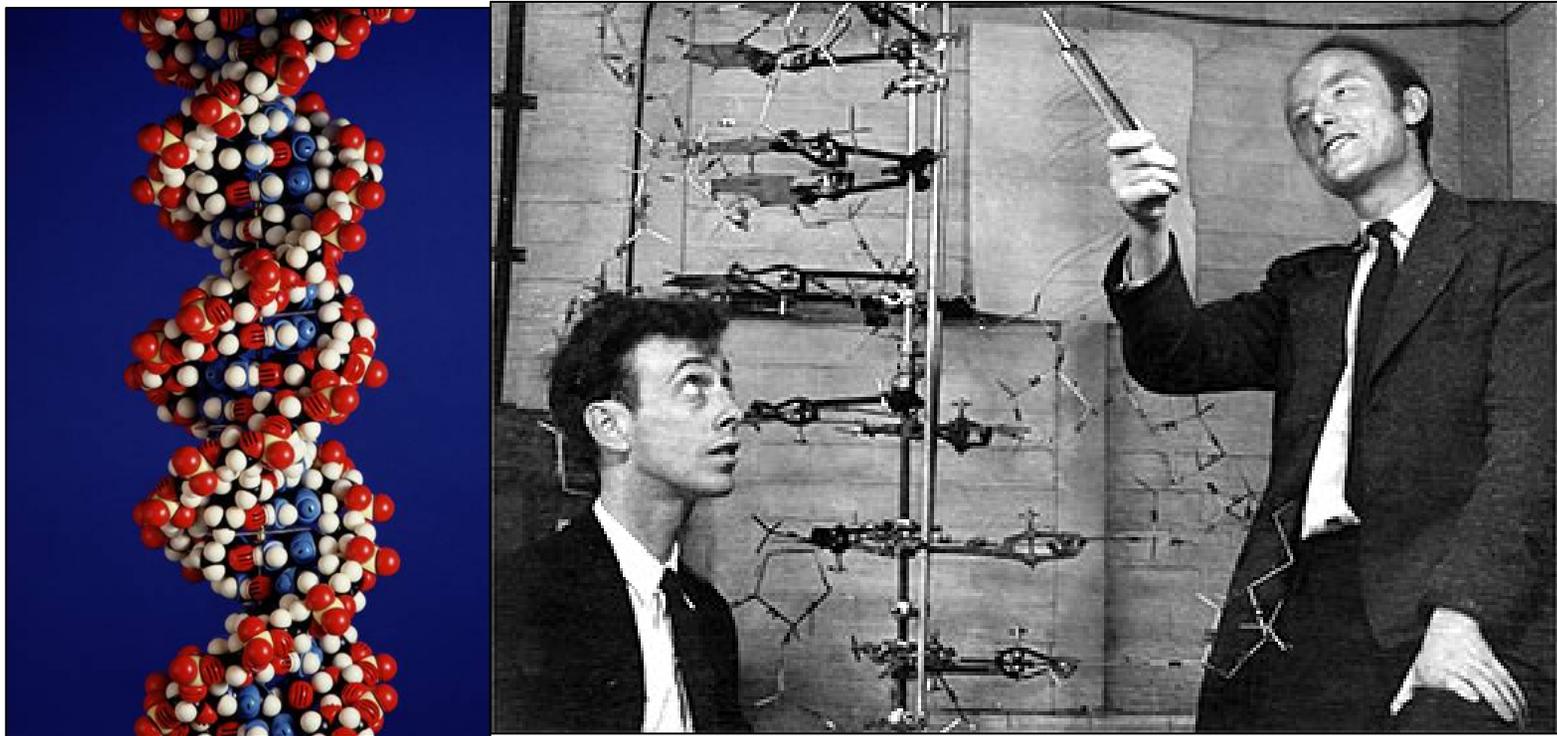


DNA Repair and Intracellular Communication

Daniel B. Yarosh, Ph.D.
SVP, Basic Science Research
Estee Lauder Co.

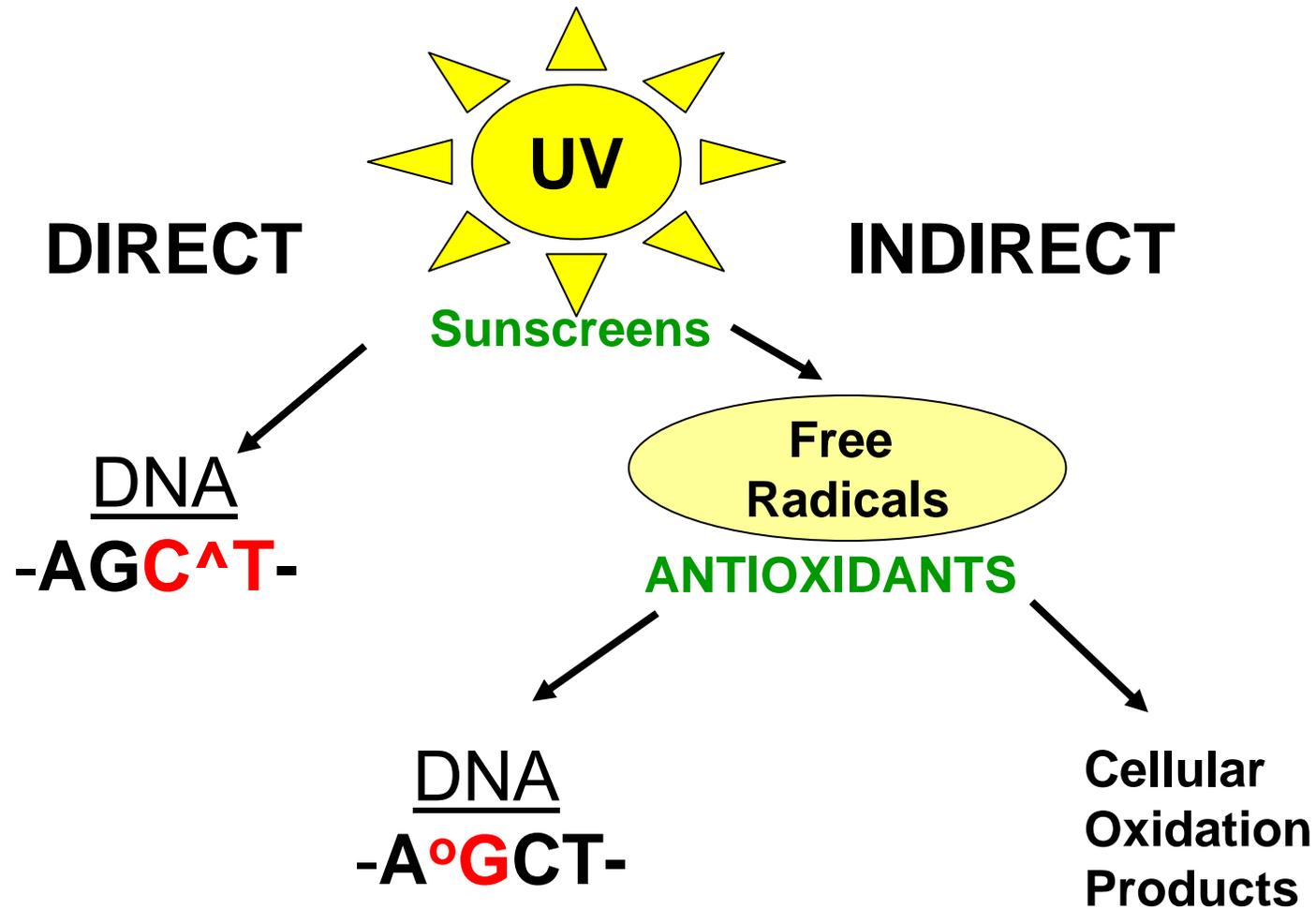
1953: Birth of Molecular Genetics

Watson and Crick

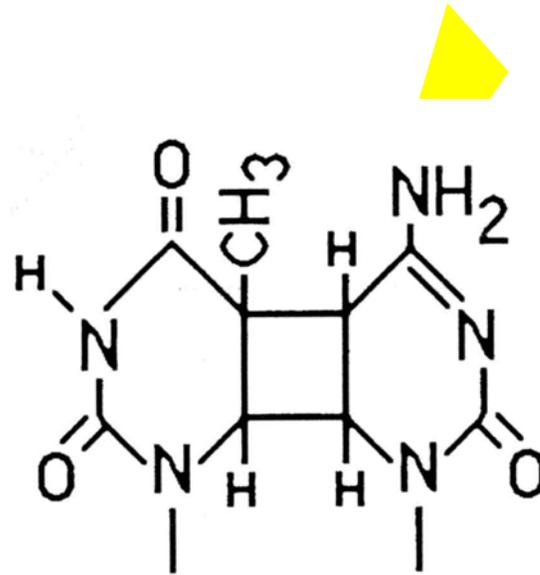


Heredity / Control of Cell Function
DNA is a sensor for cell damage

UV DAMAGE TO DNA



Direct DNA Damage



Cyclobutane pyrimidine dimer

Can Antioxidants Prevent CPD?

15% Vitamin C, 1% Vitamin E and 0.5% Ferulic Acid (CEFer)

CEFer: Thymine Dimers

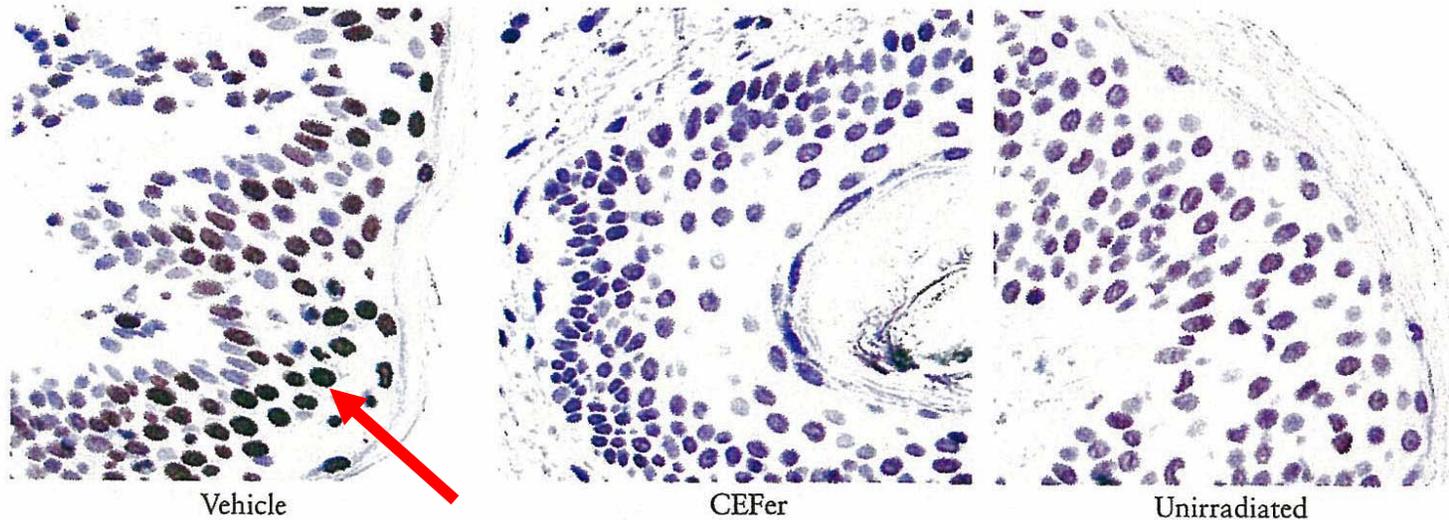
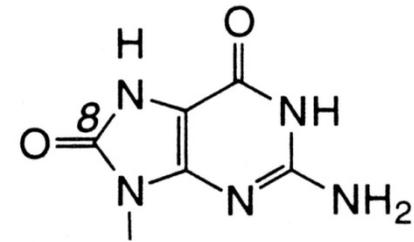
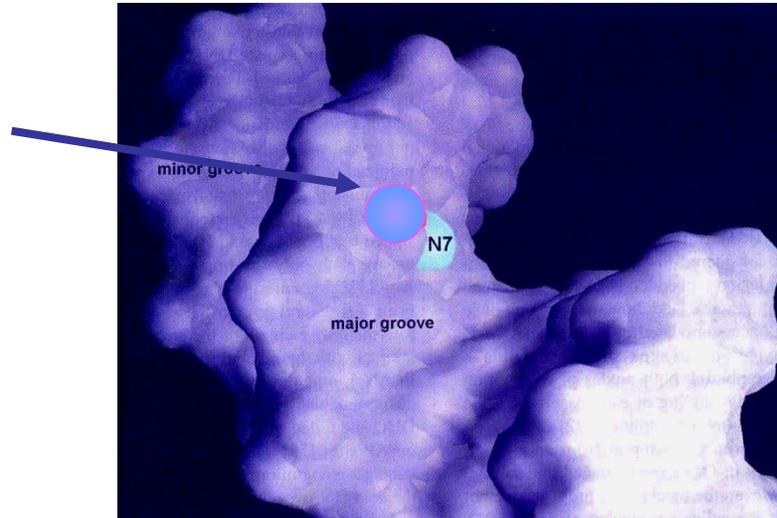
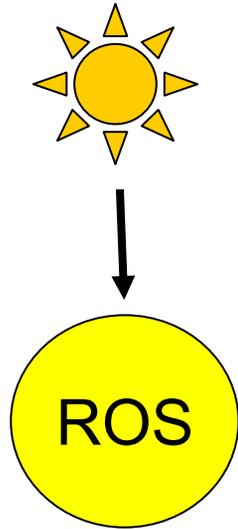


Fig 4. CEFer inhibits ultraviolet (UV)-induced thymine dimer formation. CEFer and vehicle were applied to back skin (2 mg/cm^2) daily for 4 days. Skin was irradiated with solar-simulated UV radiation, $1\times$ to $5\times$ minimal erythema doses (MED) at $1\times$ -MED intervals. Skin biopsy specimens of $2\times$ MED-treated skin and unirradiated, untreated skin were taken 1 day later and formalin-fixed tissue was stained for immunohistochemistry using mouse monoclonal antibody to thymine dimers (clone KTM53 Kamiya Biomedical, Seattle, Wash). UV generation of cellular thymine dimers was almost completely protected by CEFer application. (Original magnification: $\times 40$.)

Indirect DNA Damage



8-oxo-Guanine

UV DAMAGE TO DNA

Type of Damage

DNA Repair Enzyme

Direct:

Cyclobutane pyrimidine
dimer

T4 endonuclease V
UV Endonuclease
Photolyase

Indirect:

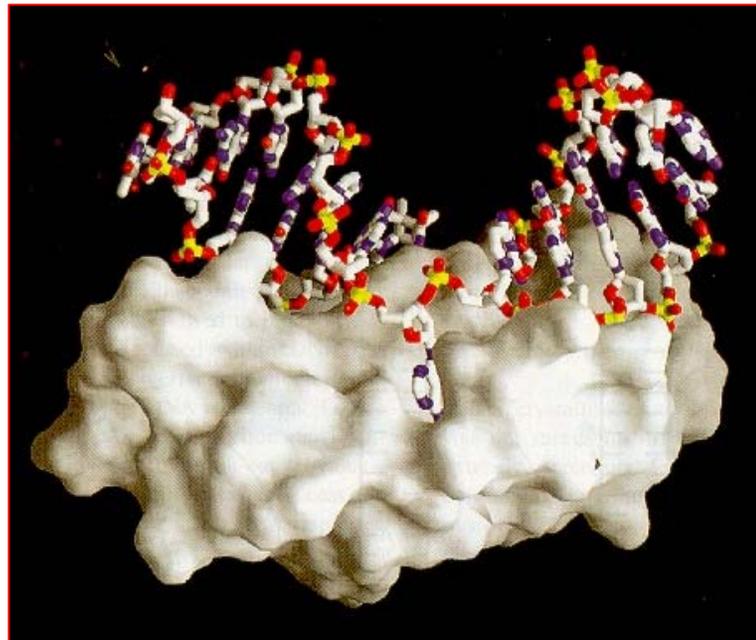
ROS → 8-oxo-Gua

OGG1

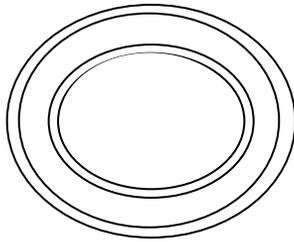
T4 Endonuclease V

Bacteriophage T4
recombinant
protein

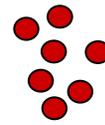
Recognition and
incision in one
polypeptide



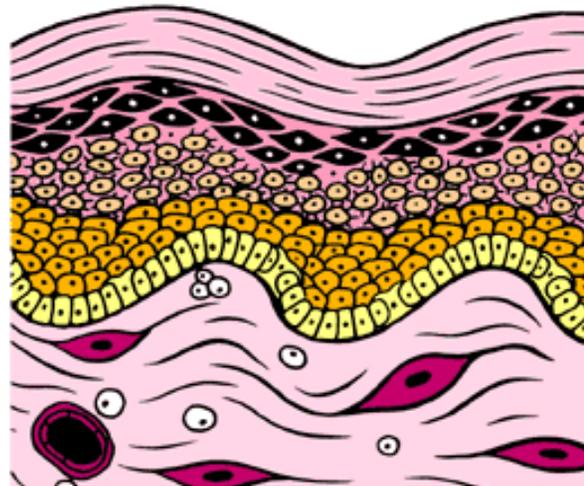
Liposome Delivery



LIPOSOME



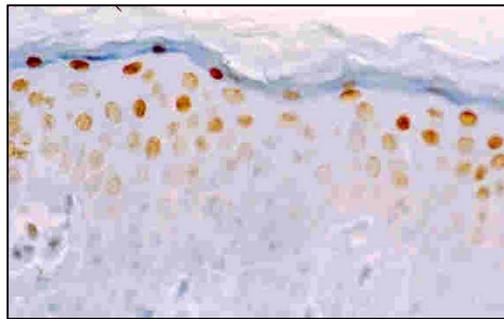
DNA repair enzymes



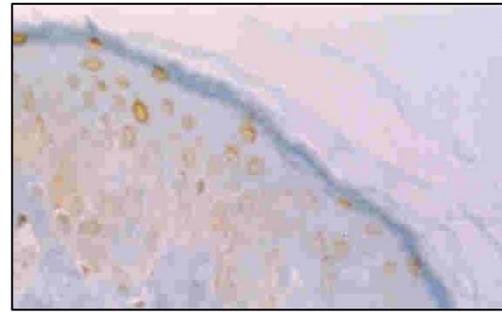
DNA Repair in Human Skin

Single UVB dose (1,500J/m²)

6h treatment with the DNA Repair Lotion (2mg/cm²)



Untreated



DNA Repair Lotion

DNA Repair in Human Skin



UV

Human skin: UVB

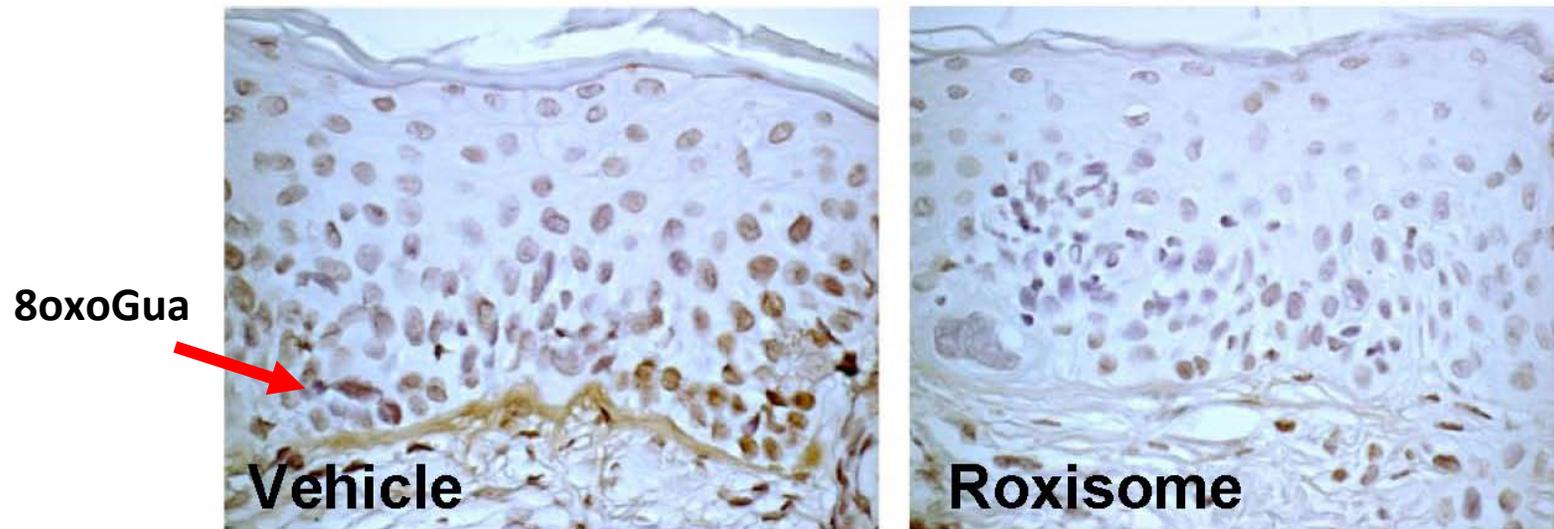
CPD (green)

**Photosomes (1h) reduce
CPD by 45%**

(Stege et al., PNAS 97:1790, 2000)

UV+Photosomes

OGG1 Repair of 8-oxo-Gua



SKH-1 mice: UV followed by OGG1 liposomes

Wulff et al., Photochem. Photobiol. 84:317, 2008

Organ Transplant Patients and Skin Cancer

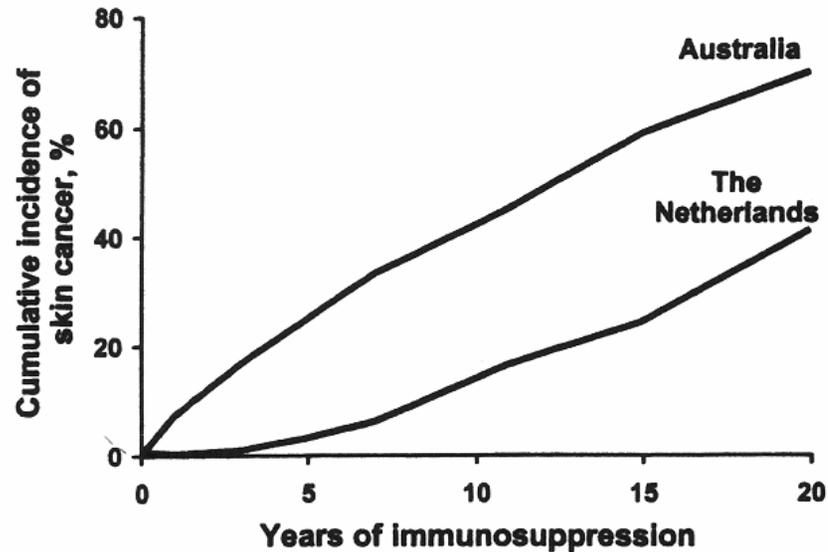
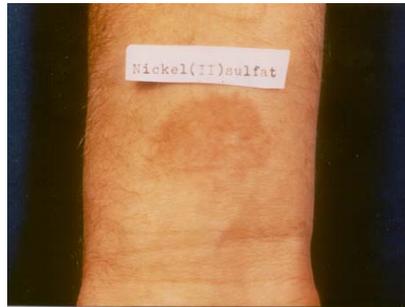


Figure 2. A squamous cell carcinoma (arrow) occurring on a background of widespread epidermal dysplasia on the forehead of a renal transplant patient.

180,000 living transplant patients

DNA Damage and Immunity



***Nickel sulfate:
wheal and flare
immune response***



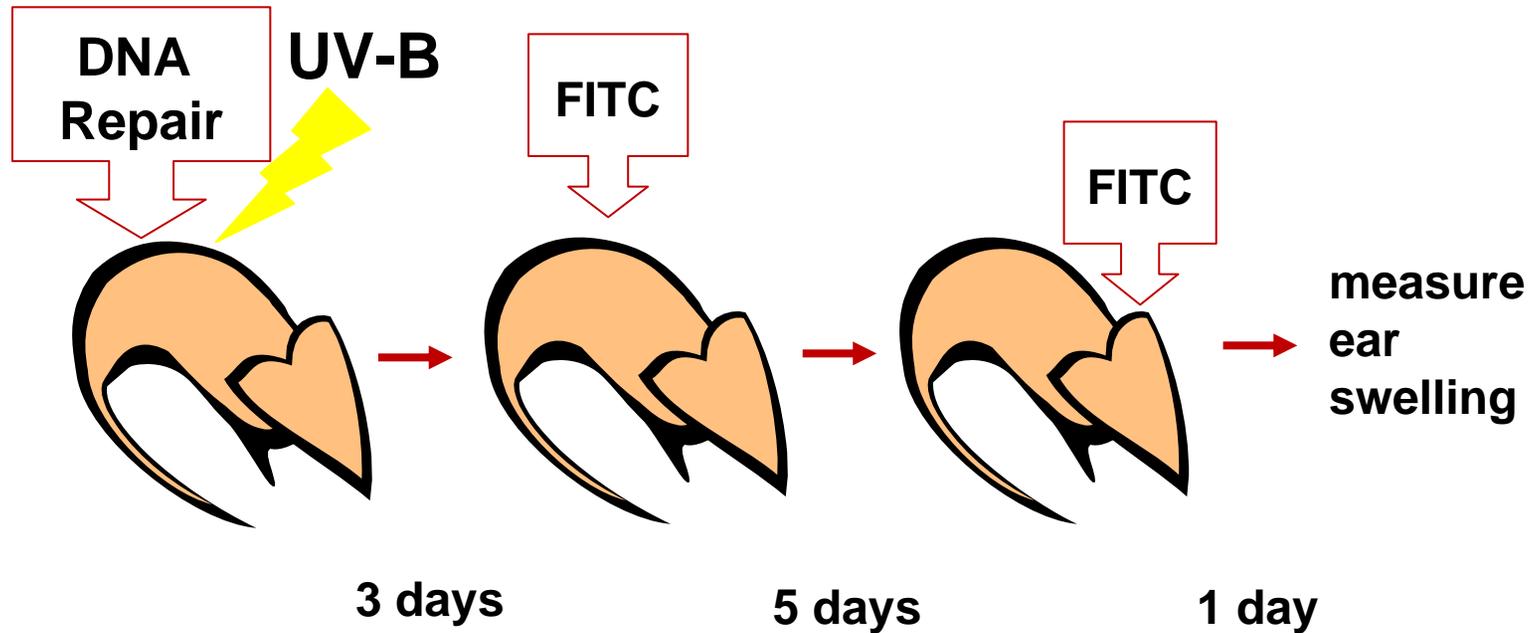
***UV erythema and
suppression
of immune response***



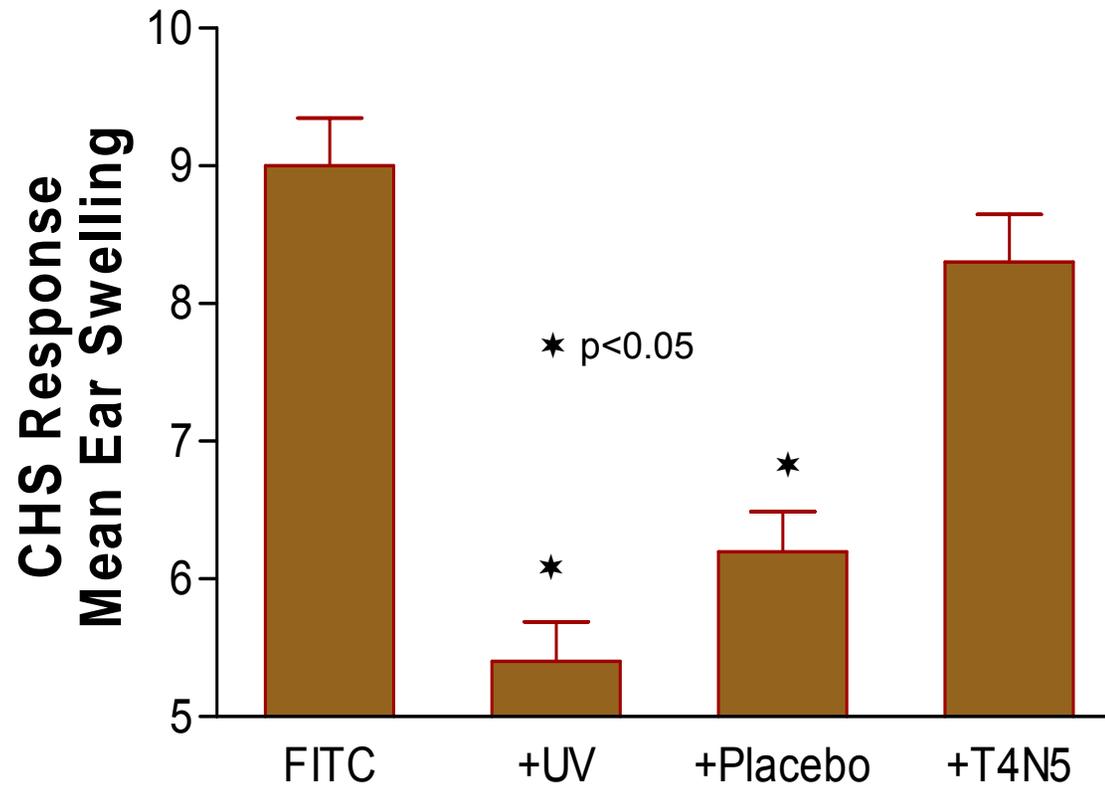
***Photosomes prevent
UV-B erythema
and restore immune
response***

Stege et al., PNAS 97:1790, 2000

Local Contact Hypersensitivity



DNA Repair Prevents Immunosuppression at Remote Sites

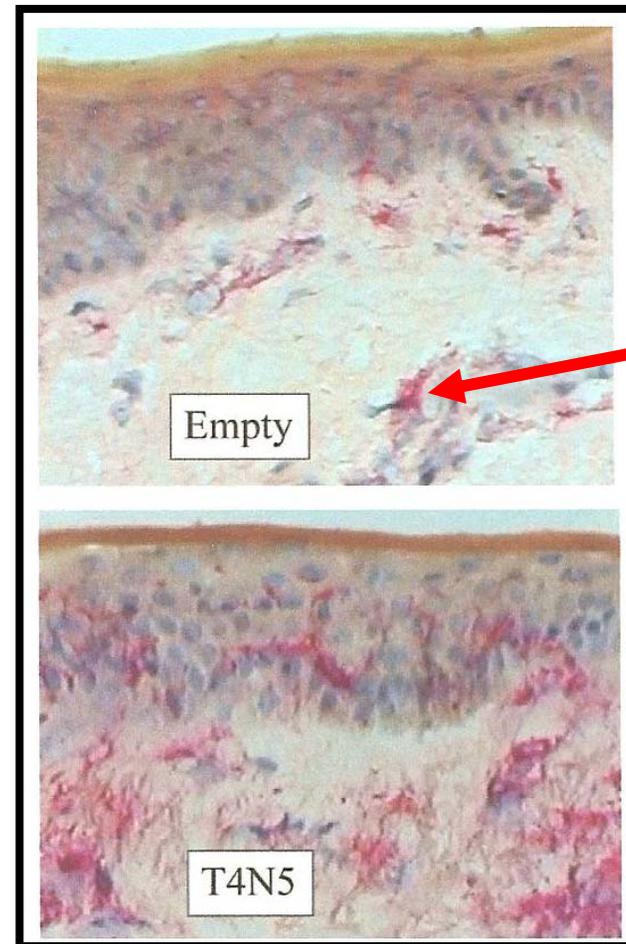


Kripke et al. Exp Derm 5:173, 1996

Langerhans Cells Flee Skin

Dendritic cells in human skin
(red)
After 4 days of solar
simulating light

Treated with Placebo
liposomes (Empty)
or
T4N5 Liposome Lotion



Kuchel et al. PPS 4:577, 2005

T-cells in Lymph Node

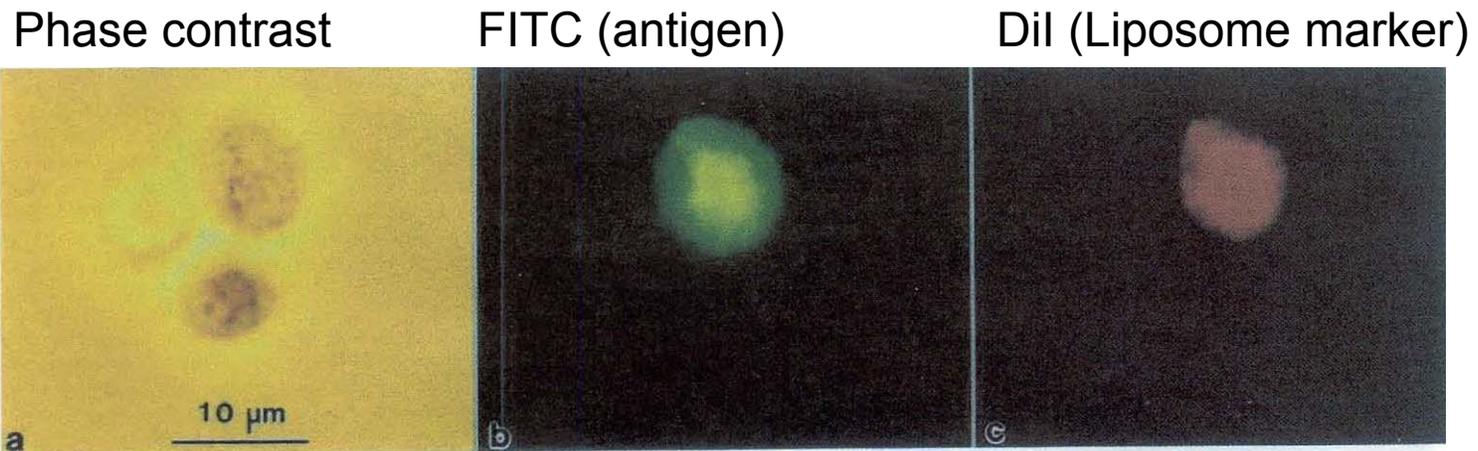


Figure 8. Cluster of draining lymph node cells contain both antigen and liposomes. Cells from the draining lymph nodes were collected from mice 18 h after application of Dil-T4N5 liposomes followed by epicutaneous sensitization with FITC, and were examined by phase-contrast microscopy (a). One cell in the cluster contained the contact sensitizing hapten (FITC), as detected by fluorescence microscopy with the green filter/barrier set (b); the same cell contained Dil-T4N5 liposomes, as detected with the red filter/barrier (c).

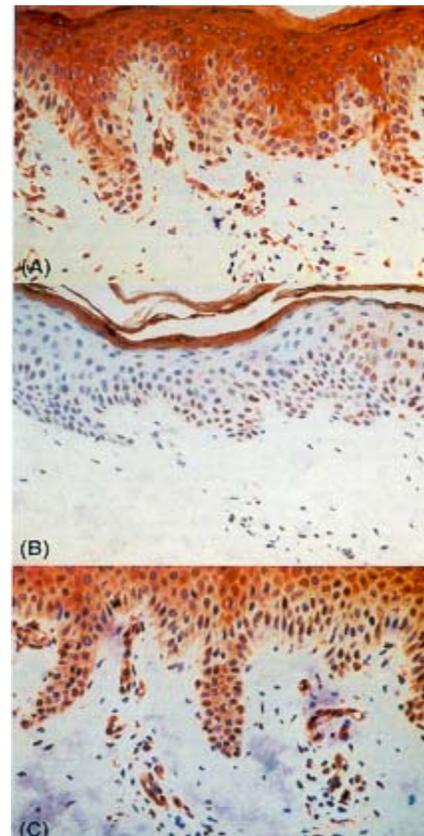
Yarosh et al, JID 103:461, 1994

Cell to Cell Communication

DNA Damage induces IL-10

DNA repair reduces expression of IL-10

Human Skin



2 MED (UVA,B)
6 hours

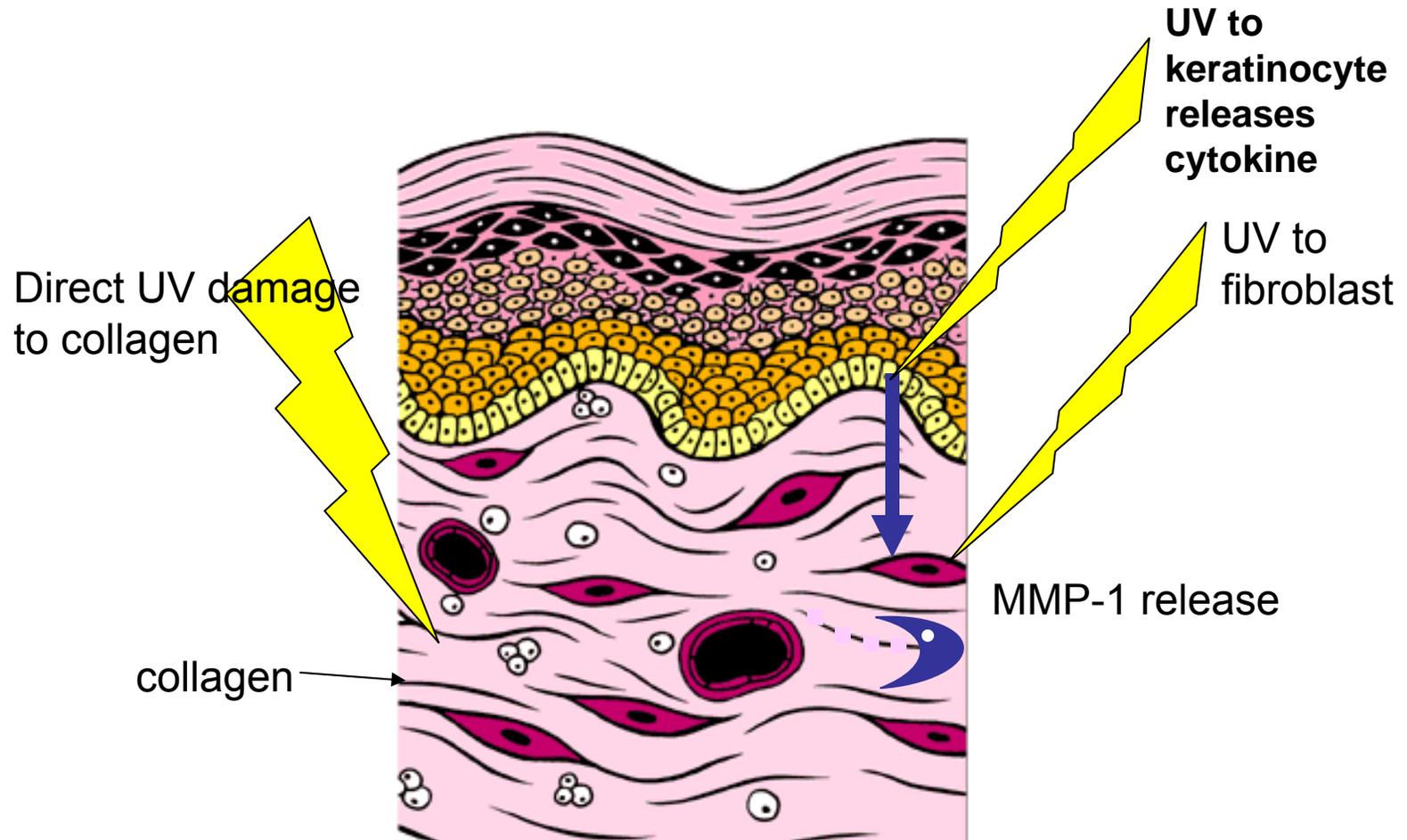
anti-IL-10 antibody
staining

T4N5 Liposome
Lotion

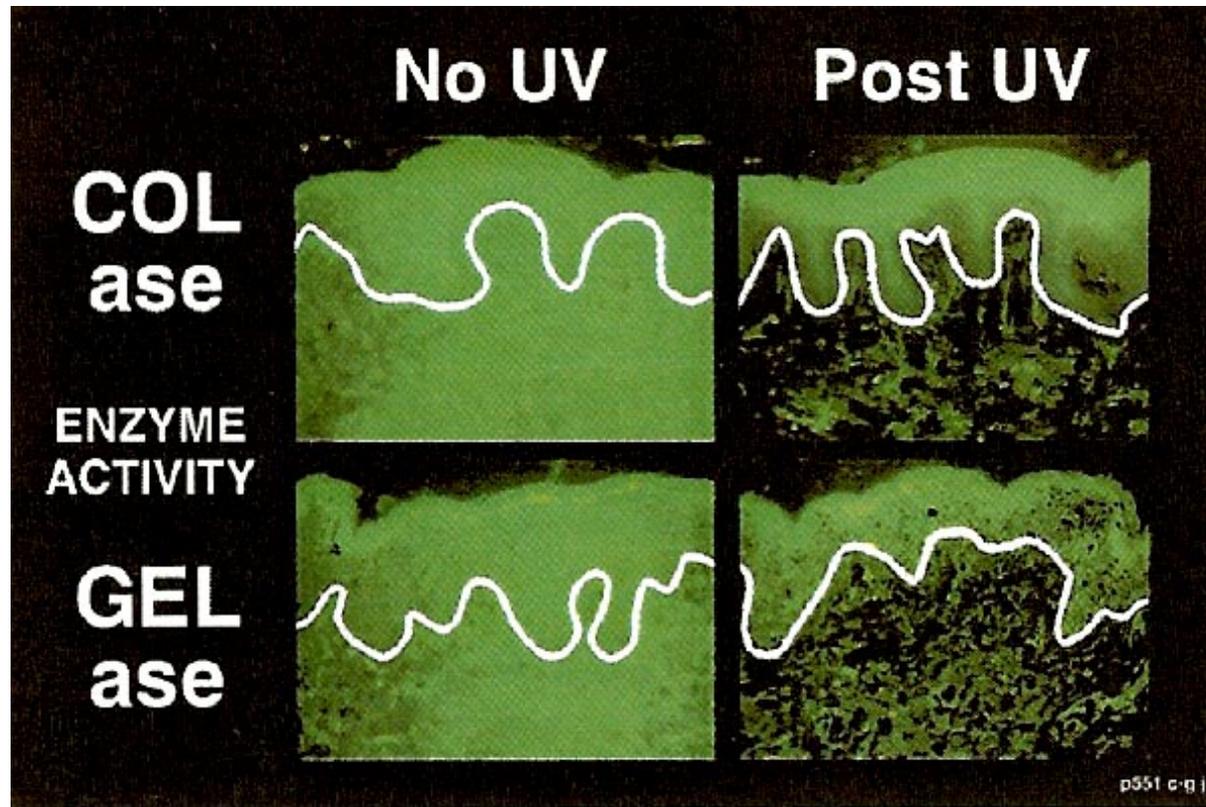
Placebo

Wolf et al. JID 114:149-156, 2000

Wound Healing Protease: MMP-1

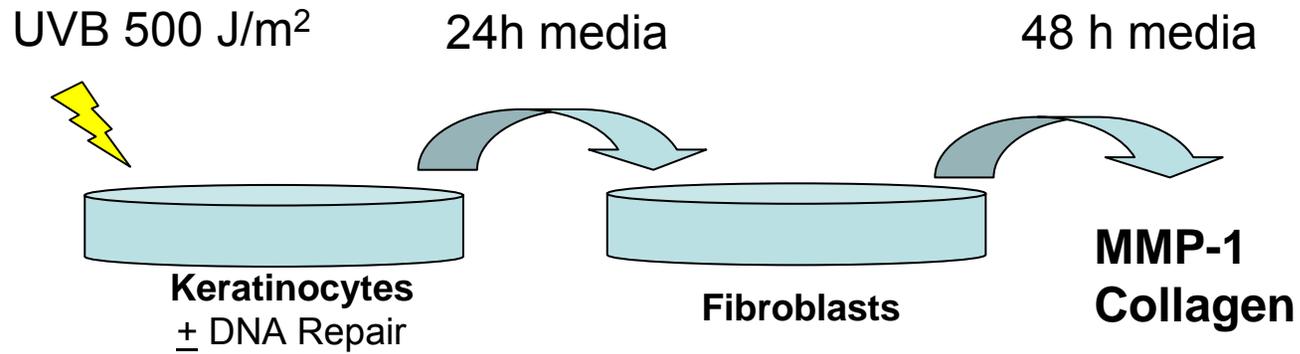


Induction of MMP-1 in Skin



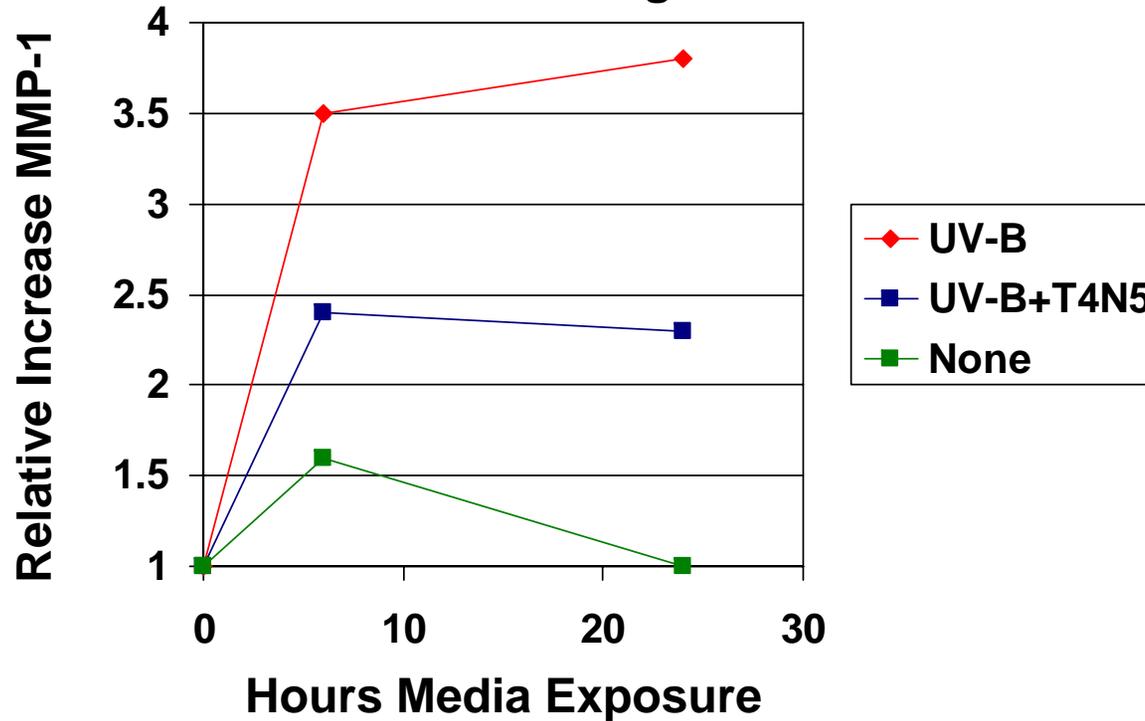
Modified from: Fisher et al., New England Journal of Medicine, 337:1419, 1997

Cell to Cell Communication



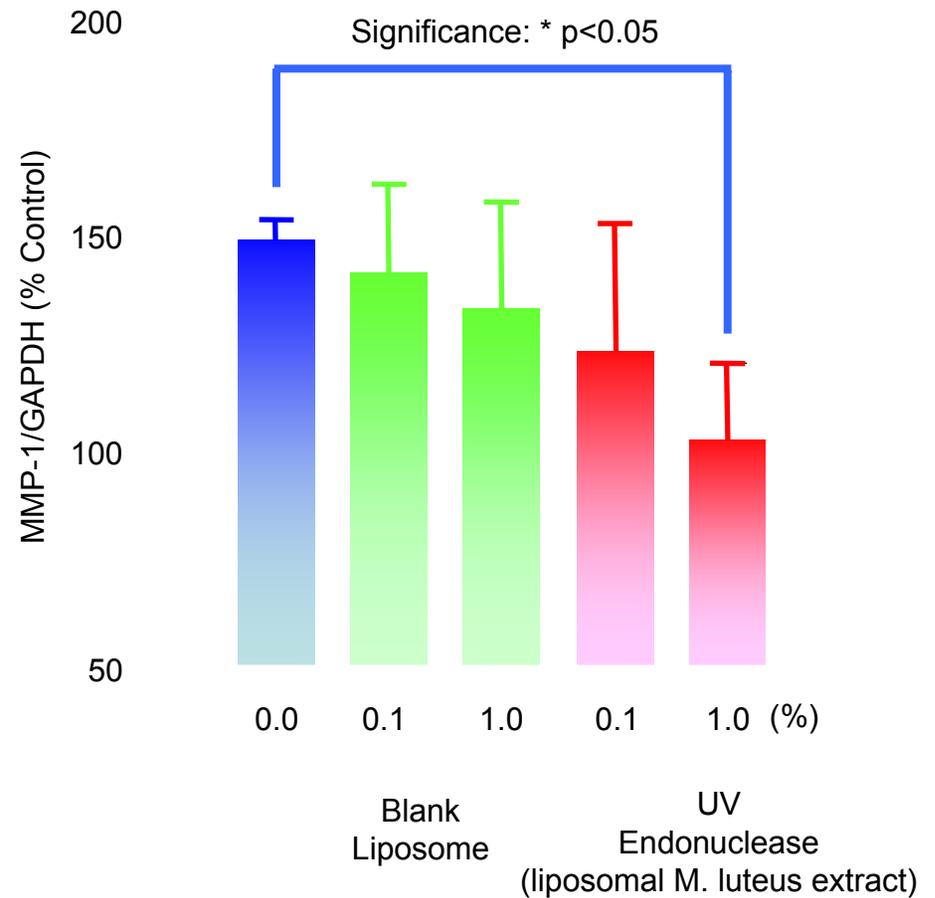
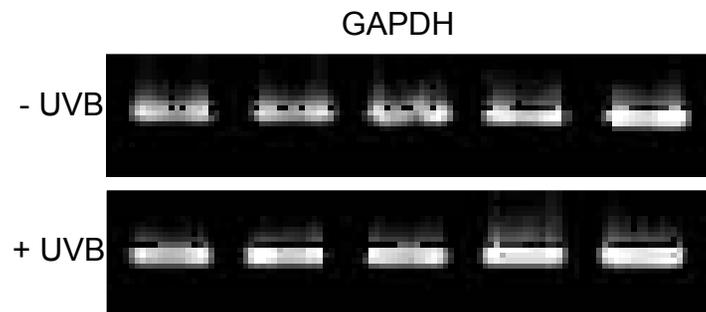
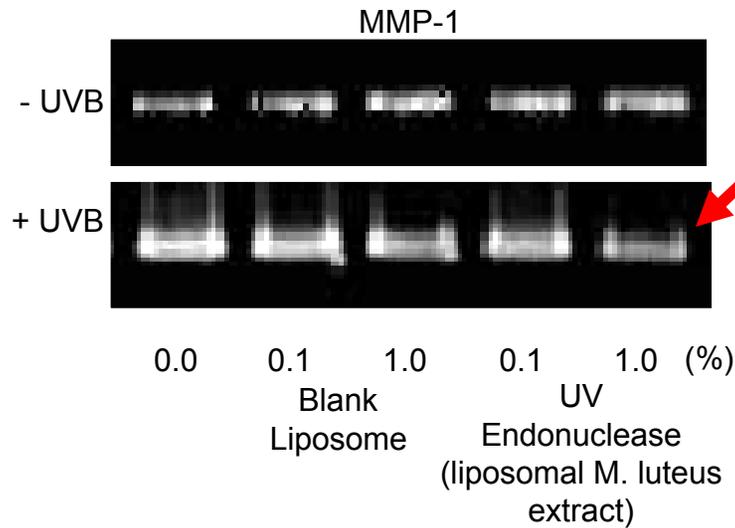
MMP-1 Gene Expression

Human Fibroblasts Receiving NHEK Media

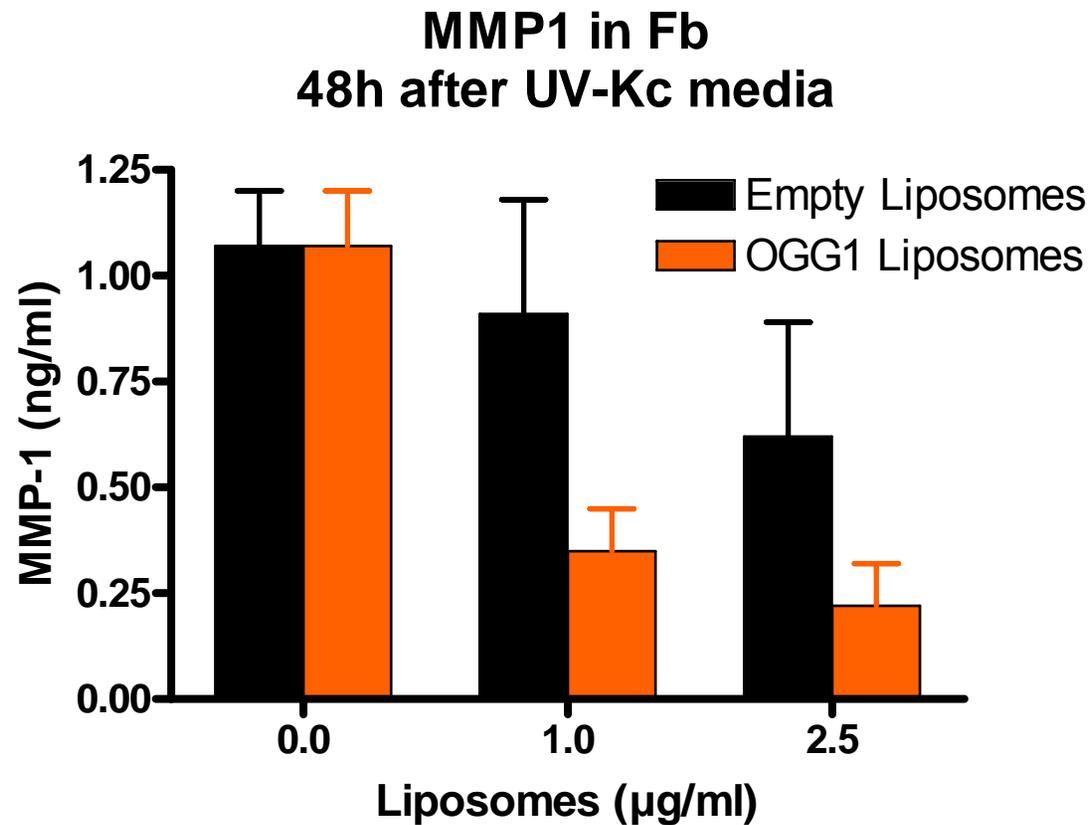


Normal human fibroblasts treated with media from NHEK
NHEK: 500 J/m² UV-B + T4N5 Liposomes (1 µg/ml) for 1 hr post-UV.
MMP-1 mRNA expression measured by Real-Time PCR (RT-PCR)

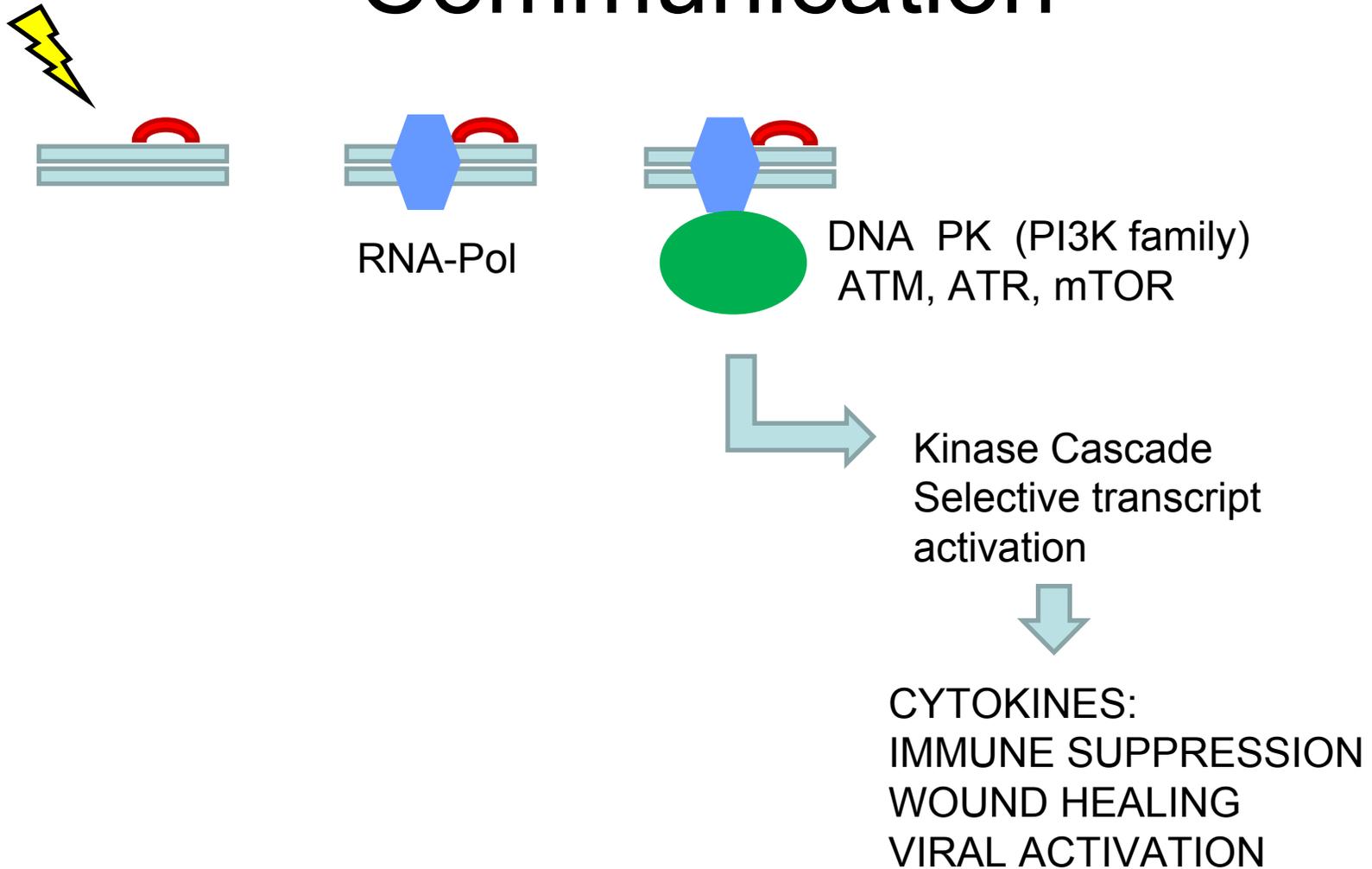
UV endonuclease significantly suppressed the Kc-induced MMP-1 expression in Fb



OGG1 Inhibition of MMP-1



DNA Damage and Cell Communication



The New Synthesis

- DNA damage injures a cell
- Injured cells communicate
 - Cytokines release
 - Immune suppression
 - Wound healing response
 - Viral activation
- Undamaged cells respond to DNA damage
- Extracellular signals contribute to skin cancer

Acknowledgements

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