DHODH inhibition for chemoprevention and combination therapy of UVB-induced epithelial oncogenesis

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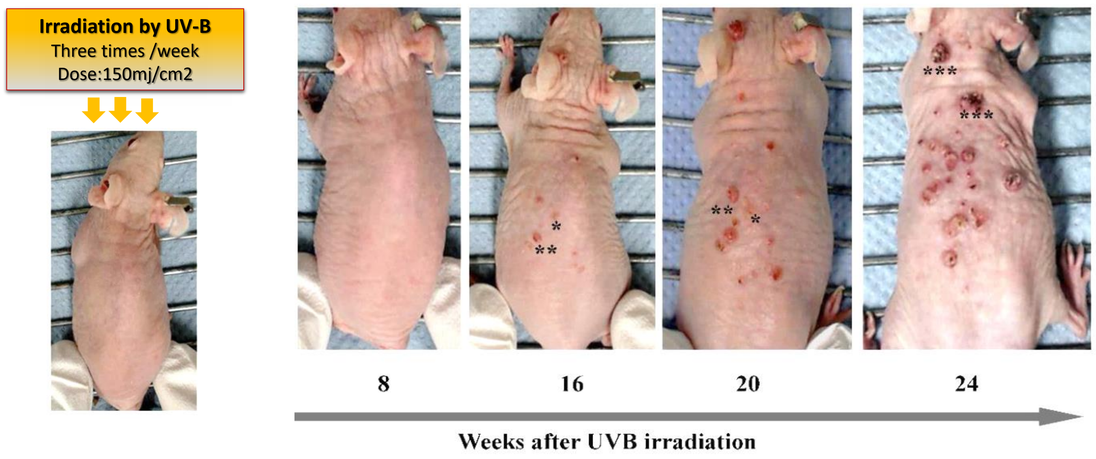
No conflics of interest

❑ Introduction

Exposure to ultraviolet (UV) radiation from the sun is the most significant risk factor resulting in non-melanoma skin cancers (NMSCs), including cutaneous squamous cell carcinomas (cSCCs) which their incidence rates are still on the rise.

* Actinic keratosis, ** Bowen's disease, *** Invasive SCC



When exposed to chronic UVB irradiation, SKH-1 hairless immunocompetent mice closely mimic photocarcinogenesis in humans[1,2]. Different stages (initiation, promotion, and progression) of skin carcinogenesis are easily evidenced at different times after chronic UVB irradiation.

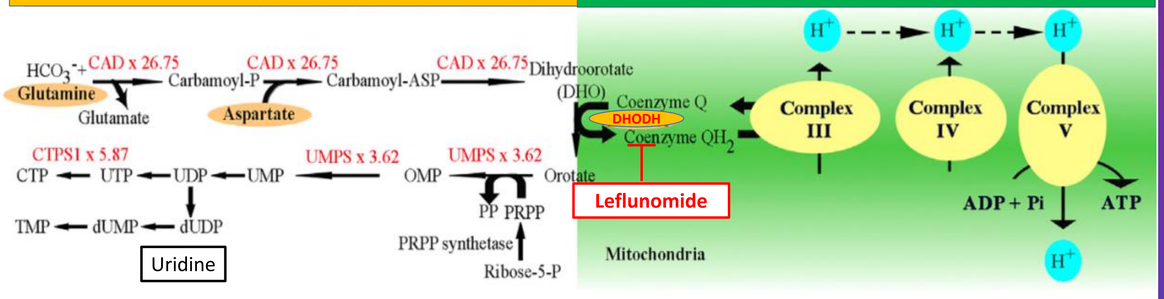
❑ Research Highlights

- **Chronic UV-B irradiation alters bioenergetic machinery in initial stage of carcinogenesis**[2]
- **Dihydroorotate Dehydrogenase (DHODH) overexpression play a pivotal role in UV-B induced tumorigenesis and its regulation control by *STAT3***[3]
- **Chemical or genetic inhibition of DHODH increases DNA damages and block tumor formation process**
- **Bioenergetic machinery could be exploited for tumor prevention or curative treatment**

Results

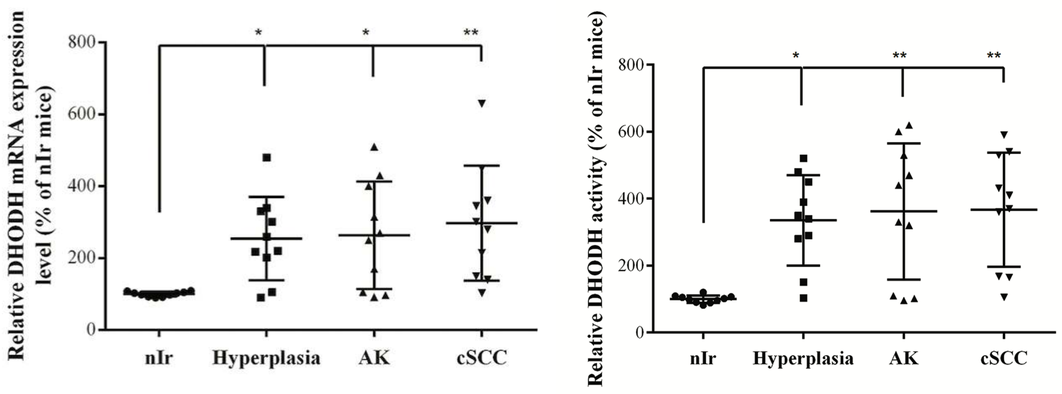
1- DHODH proteine is up-regulated at a early stage of tumorigenesis

Proteomic analysis between non-irradiated and Irradiated mouse skin biopsies



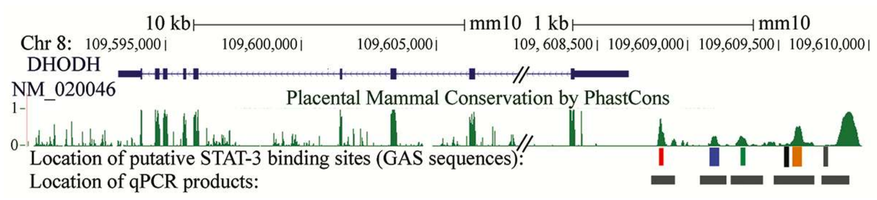
- DHODH is located in the inner mitochondrial membrane and catalyzes the fourth step of pyrimidine in the *de novo* pyrimidine synthesis pathway.
- UVB irradiation triggers activation of DHODH that
  a) drives increased electron transport chain activation and
  b) allows persistent nucleotide biosynthesis using glutamine as main carbon source

2- DHODH mRNA expression and its enzyme activity are upregulated at a very early phase of UVB-induced carcinogenesis



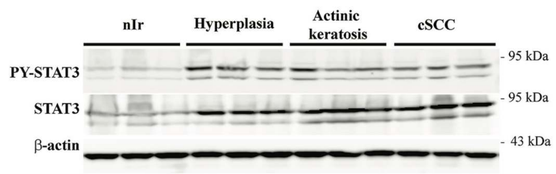
DHODH up-regulation persists during the subsequent steps of carcinogenesis

3- UVB-induced DHODH upregulation is mainly regulated transcriptionally by STAT3



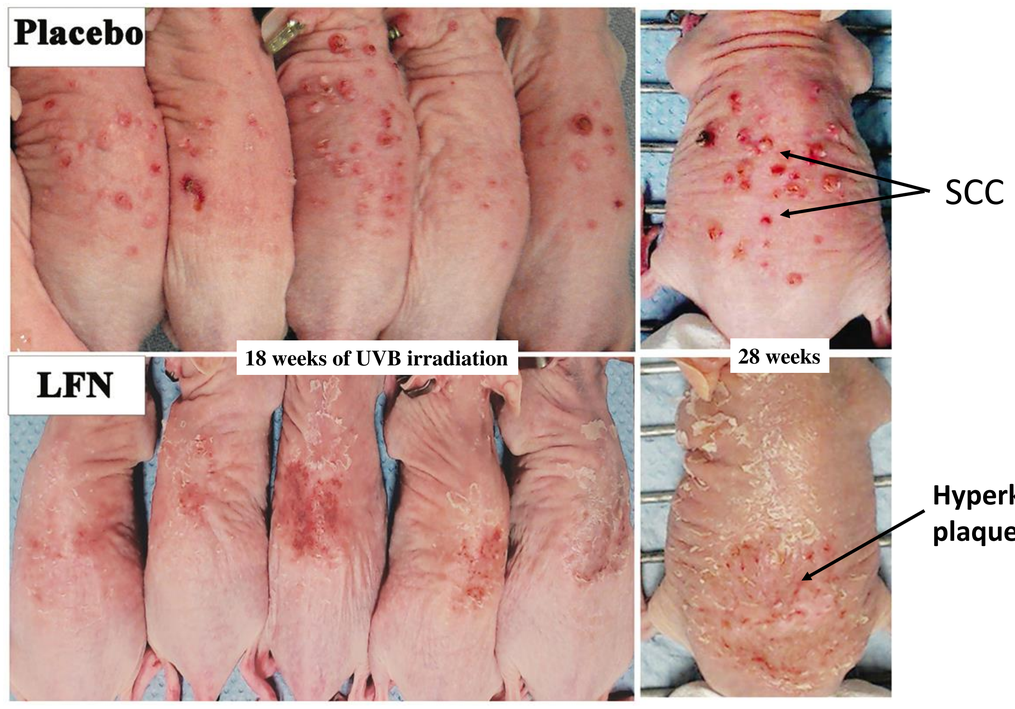
Analysis of the promoter region of DHODH gene

- 8 interferon- activated sequences (GAS) are located in the 1.4 kb region upstream from the ATG translation initiation codon.
- The protein family that can bind to GAS sequences is the signal transducer and activator of transcription (STAT).

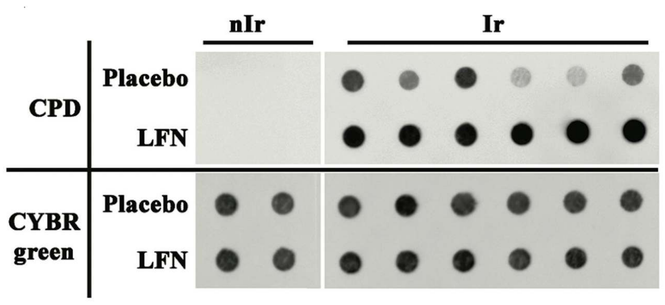


- **STAT3** and its phosphorylated form at tyrosine-705 (**pY-STAT3**) are **up-regulated** at early stage of carcinogenesis
- AND **persists at different stages of carcinogenesis**.

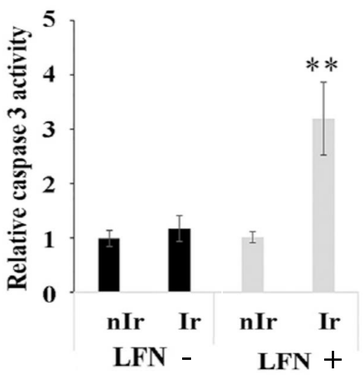
4- Inhibition of DHODH by leflunomide (LFN) blocks UVB-induced tumor formation



5- Decreased DNA repair capacity could explain the absence of tumor in LFN treated mice



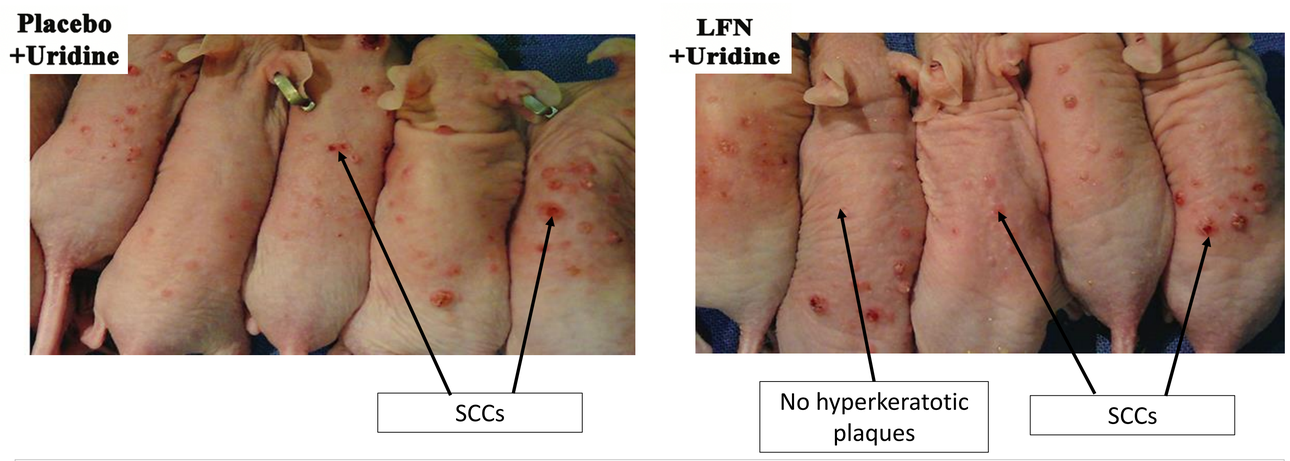
Cyclobutane Pyrimidine Dimers (CPD) level increases in epidermal DNA of LFN-treated mice



Increased apoptosis in LFN-treated mice

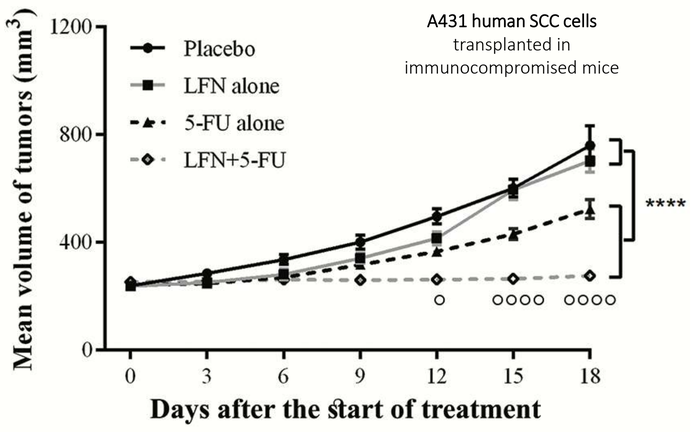
The paradoxical absence of tumor formation in LFN-treated mice despite the early aggressive UVB exposure phenotype could be related to the **decreased pyrimidine biosynthesis** and **subsequently decreased DNA repair capacity** and **increased apoptotic cell death**.

6- Pyrimidine supplementation (Uridine) restores UVB-induced tumor formation in LFN treated mice.



No significant differences for number, growth rate, incidence between mice treated with uridine alone and the group receiving LFN and Uridine.

7- Synergic anti-tumoral effect of LFN and 5-FU on established human tumors



	Placebo	LFN alone	5-FU alone	LFN+ 5-FU
Doubling time	10.48	9.7	14.42	167.9

Conclusion

✓**DHODH** is upregulated at a very early phase of UVB-induced carcinogenesis and its inhibition **blocked the tumorigenic transformation of damaged keratinocytes**.

✓When tumors are subjected to **LFN in combination with a genotoxic agent** such as 5-FU, a significant synergistic anti-tumor effect occurs with less cytotoxicity.

✓Given that STAT3 could have pro- or anti-tumorigenic activities depending on the several conditions, targeting the downstream effectors of STAT3 with more narrow activities might increase the likelihood of developing an efficient anti-cancer drug.

✓Our data suggest that **DHODH can be targeted for both skin tumor prevention and curative combination therapy**.

[1] Di Giovanni J. Multistage carcinogenesis in mouse skin. Pharmacol. Ther. 1992. 54: 63–128.
[2] Hosseini M, Dousset L, et al. Energy Metabolism Rewiring Precedes UVB-Induced Primary Skin Tumor Formation. Cell Rep. 2018. 23(12):3621-3634
[3] Hosseini M, Dousset L, et al. UVB-induced DHODH upregulation, which is driven by STAT3, is a promising target for chemoprevention and combination therapy of photocarcinogenesis. Oncogenesis 2019.