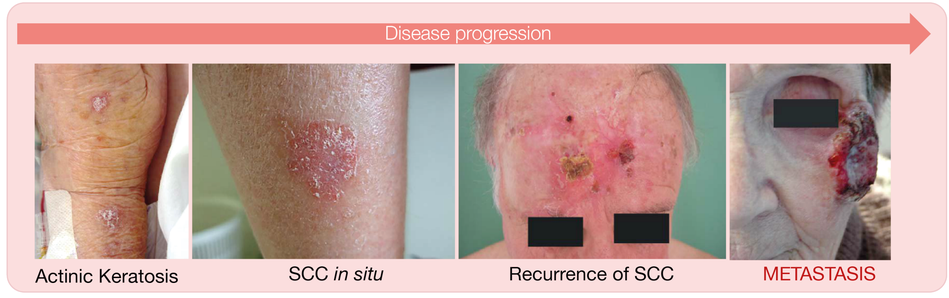
Metabolic Vulnerabilities as Predictive Biomarkers and Therapeutic Targets in Skin Carcinogenesis

Pauline MICHON[1], Léa DOUSSET[1], Walid MAHFOUF[1], Elodie MUZOTTE[1], François MOISAN [1] ,Rodrigue ROSSIGNOL[2], Hamid-Reza REZVANI[1]

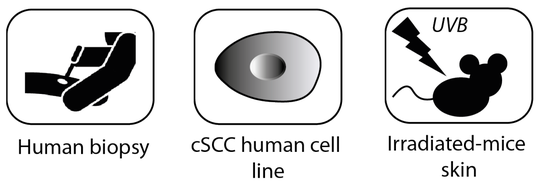
[1] Université de Bordeaux, *INSERM, BGMIC, UMR1035,* Bordeaux, France
[2] Université de Bordeaux, *INSERM U1211 MRGM,* Bordeaux, France

Introduction

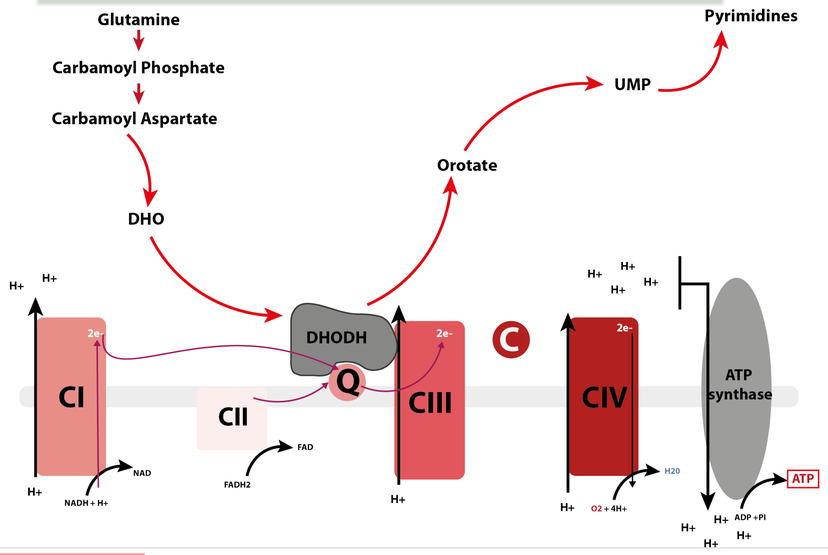
- **Ultraviolet B (UVB) is the main factor** of cutaneous cancers development like **cutaneous squamous cell carcinoma (cSCC)** a non-melanoma skin cancers (NMSCs).
- **cSCCs can progress** from precursor Actinic Keratosis (AK) to in situ, invasive and finally metastasis.
- Despite its low distant metastatic potential, **the presence of metastasis is associated with a dismal prognosis** and a median survival of less than 2 years.
- **Metabolic reprogramming plays** an important role in the **initiation and progression** of many type of human tumor.
- Our main aim in this project is to **understand metabolism rewiring in cSCC** and its molecular inter-patients and intra-tumor heterogeneity in skin cancer in order to **improve the level of prevention and protection for skin cancer and the evalution of new therapeutic strategies.**



Material & Methods

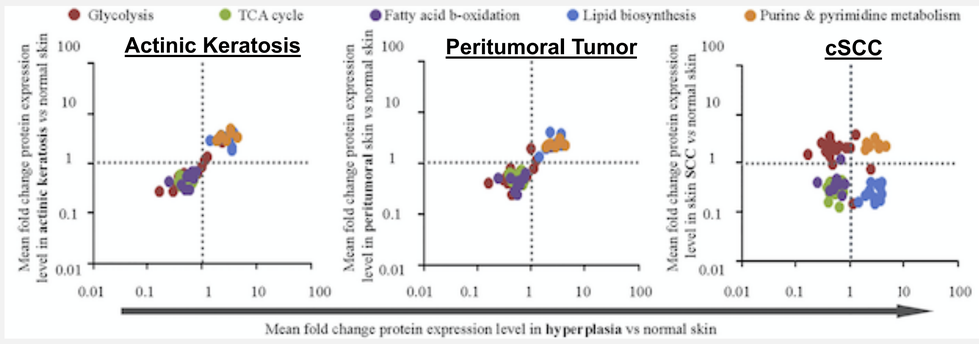


DHODH pathway



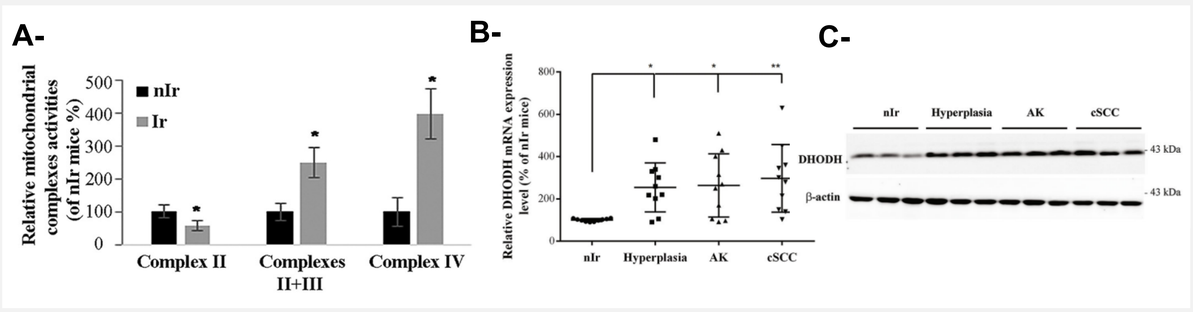
Results

Figure 1: Metabolic Profiles Are Very Similar among Epidermal Hyperplasia, Actinic Keratosis, and Peritumoral Tissue



Skin biopsies were subjected to proteomic analysis. Scatterplots show comparison of fold change of protein expression levels between hyperplasia and actinic keratosis (left), hyperplasia and peritumoral tissue (middle), and hyperplasia and full-blown SCC (right). Each color dot represents an individual protein. N = 20 samples per group. Red, green, purple, blue, and orange points indicate the proteins involved in glycolysis, TCA cycle, fatty acid b-oxidation, lipid biosynthesis, and purine and pyrimidine metabolism, respectively.

Figure 2: UVB Irradiation Results in Overactivation of Distal Part of OXPHOS and in DHODH overactivation SKH-1 mice

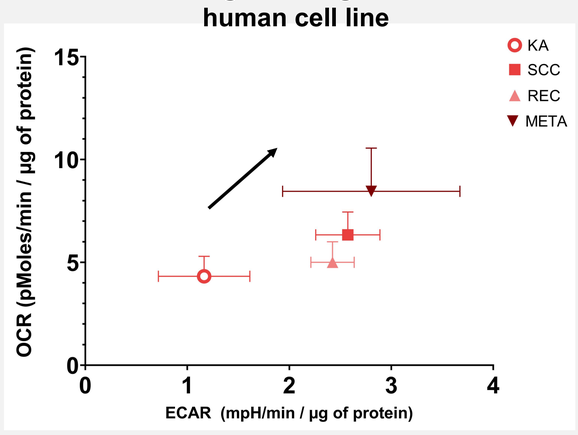


A- The maximal activities of mitochondrial complexes II, III, and IV were measured in irradiated and non-irradiated mice. UVB irradiation results in over-activation of complexes III and IV

B- The relative levels of DHODH mRNA were quantified by quantitative reverse transcription PCR

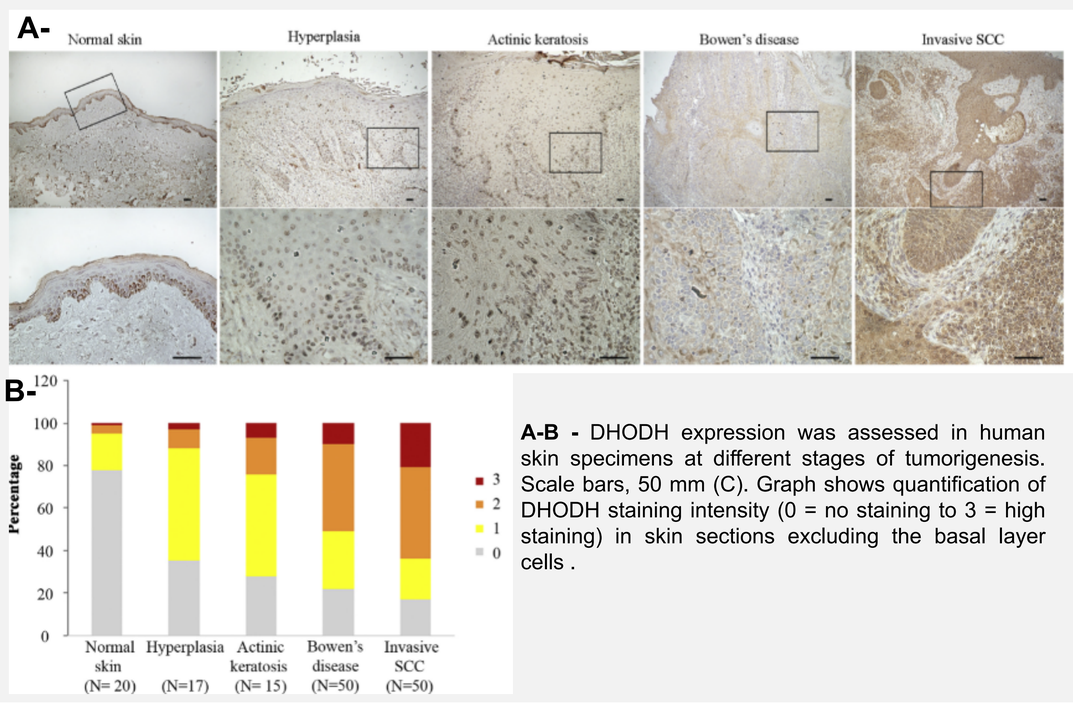
C- Total protein extracts of skin biopsies at different stages of tumorigenesis were assessed for expression of DHODH by western blot. β-actin was used as a loading control. Full-length

Figure3 : A metabolic reprogramming occurs during carcinogeneis in cSCC human cell line



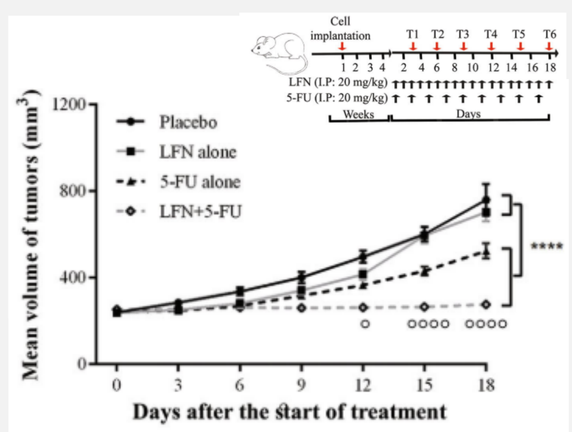
Biochemical functional analysis on human cell lines at different stages of carcinogenesis revealed a positive correlation between stages of tumor development with following features: oxygen consumption rate (OCR) and extracellular acidification rate (ECAR).

Figure 4 : DHODH Upregulation Persists at Different Stages of Skin Carcinogenesis



A-B - DHODH expression was assessed in human skin specimens at different stages of tumorigenesis. Scale bars, 50 mm (C). Graph shows quantification of DHODH staining intensity (0 = no staining to 3 = high staining) in skin sections excluding the basal layer cells .

Figure 5 : The combination of LFN and 5-FU reduces tumor growth in vivo



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

The combination of LFN and 5-FU reduced the average A431 tumor volume greater than either drug alone. Data is presented as the mean ± SEM of one independent experiment. Statistical analysis (two-way ANOVA with Bonferroni's post-hoc test) compares the combination therapy versus each drug alone.

Conclusion

- **These results suggest that DHODH is a promising target for chemoprevention and combination therapy of UVB-induced cSCCs.**

References

1- *Hosseini, M., Dousset, L., Mahfouf, W., Serrano-Sanchez, M., Redonnet-Vernhet, I., Mesli, S., Kasraian, Z., Obre, E., Bonneu, M., Claverol, S., et al. (2018).* ***Energy Metabolism Rewiring Precedes UVB-Induced Primary Skin Tumor Formation****. Cell Rep 23, 3621–3634.*

2- *Hosseini, M., Dousset, L., Michon, P., Mahfouf, W., Muzotte, E., Bergeron, V., Bortolotto, D., Rossignol, R., Moisan, F., Taieb, A., et al. (2019).* ***UVB-induced DHODH upregulation, which is driven by STAT3, is a promising target for chemoprevention and combination therapy of photocarcinogenesis****. Oncogenesis 8, 52.*