Hypoxia-inducible factor-1α plays a critical role in UVB-induced tumorigenesis by affecting DNA repair capacity and oxidative stress

Walid Mahfouf, Elodie Muzotte, Lea Dousset, François Moisan, Alain Taieb, Hamid Reza Rezvani

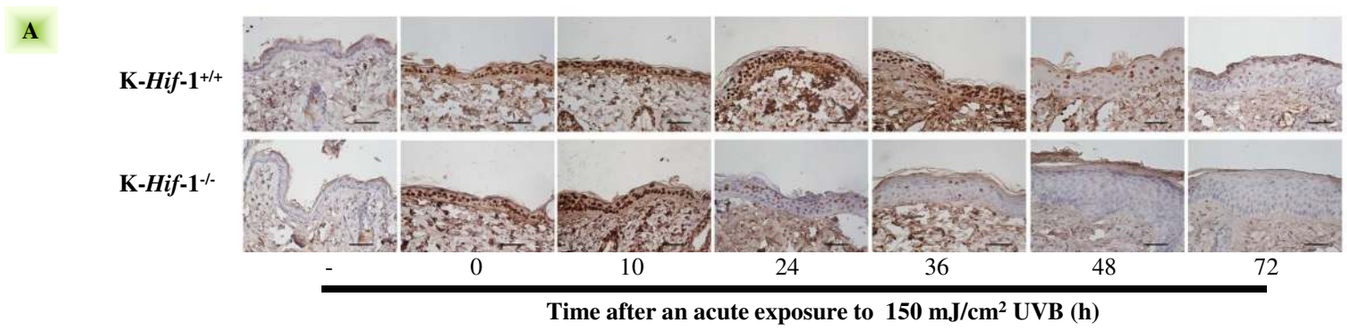
Univ. Bordeaux, Inserm, BMGIC, U1035, Bordeaux, France. Contact: Hamid-reza,rezvani@u-bordeaux.fr

Introduction

Hypoxia inducible factor (HIF) 1 is a heterodimeric transcription factor consisting of alpha and beta subunits. The beta subunit is constitutively expressed, while the expression of the alpha subunit is regulated by the relative concentration of oxygen in tissues. When stabilized, HIF-1α play a pivotal role in many biological processes by affecting the expression of hundreds of genes involved in angiogenesis, cell growth and apoptosis, cell adhesion and migration, the energy metabolism and DNA repair. Others and we showed that HIF-1α is constitutively expressed in mouse and human epidermis and that epidermal HIF-1α plays an important role in the skin, especially in local and systemic adaptation to environmental stresses including ultraviolet (UV) radiation. However, little information is available about its role in photocarcinogenesis. Using a multistage model of UVB radiation-induced skin cancer, we show that the knockout of *Hif*-1α in the epidermis prevents tumorigenesis, but at the same time triggers the formation of hyperkeratotic plaques. Our results indicate that the absence of oncogenic transformation in *Hif*-1α-ablated mice is related to increased DNA repair in keratinocytes. Indeed, *Hif*-1α ablation in the epidermis leads to more proficient removal of UV-induced DNA damage via boosting DNA repair machinery. Consequently, impairing the DNA repair machinery by ablating xeroderma pigmentosum C restores the UVB-induced neoplastic transformation of *Hif*-1α-ablated keratinocytes. Concerning the formation of hyperkeratotic plaques in irradiated K-*Hif*-$1^{-/-}$ mice, a causative role of the disturbance of the epidermal redox balance was found. Accordingly, the development of hyperkeratotic plaques is blocked by chronic antioxidant treatment.

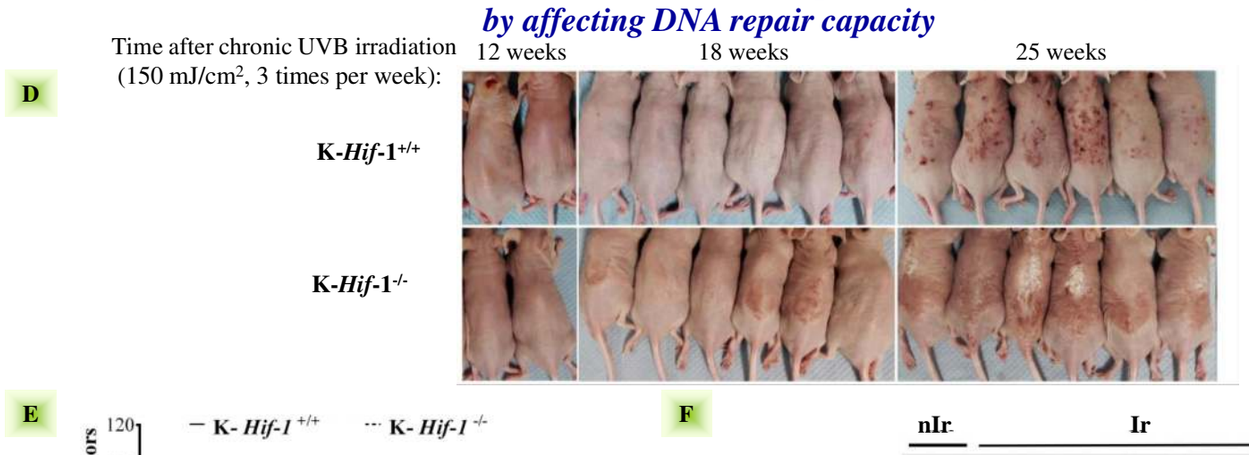
Results

1. Hif-1 knockout in mouse keratinocytes leads to increased DNA repair efficiency

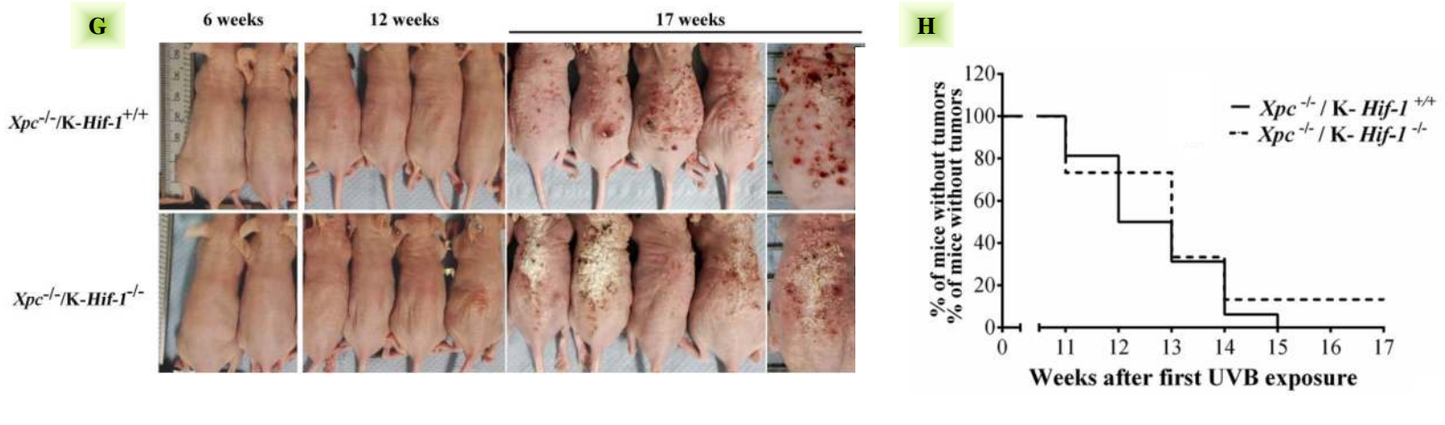


The DNA repair capacity was (**A**) assessed by anti-CPD staining of skin sections and (**B**) quantified by immuno-dot blot of the respective genomic DNA. SYBR green was used as loading control. (**C**) The number of CPDs was quantified at the indicated time points after irradiation by HPLC-MS/MS. CPD: cyclobutane pyrimidine dimers.

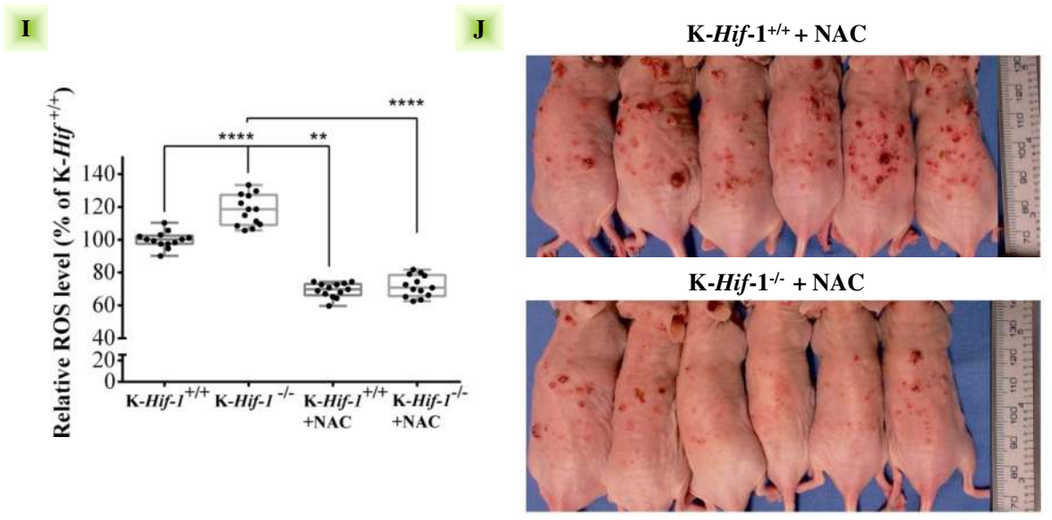
2. Keratinocyte-specific knockout of Hif-1α blocks neoplastic transformation of keratinocytes by affecting DNA repair capacity



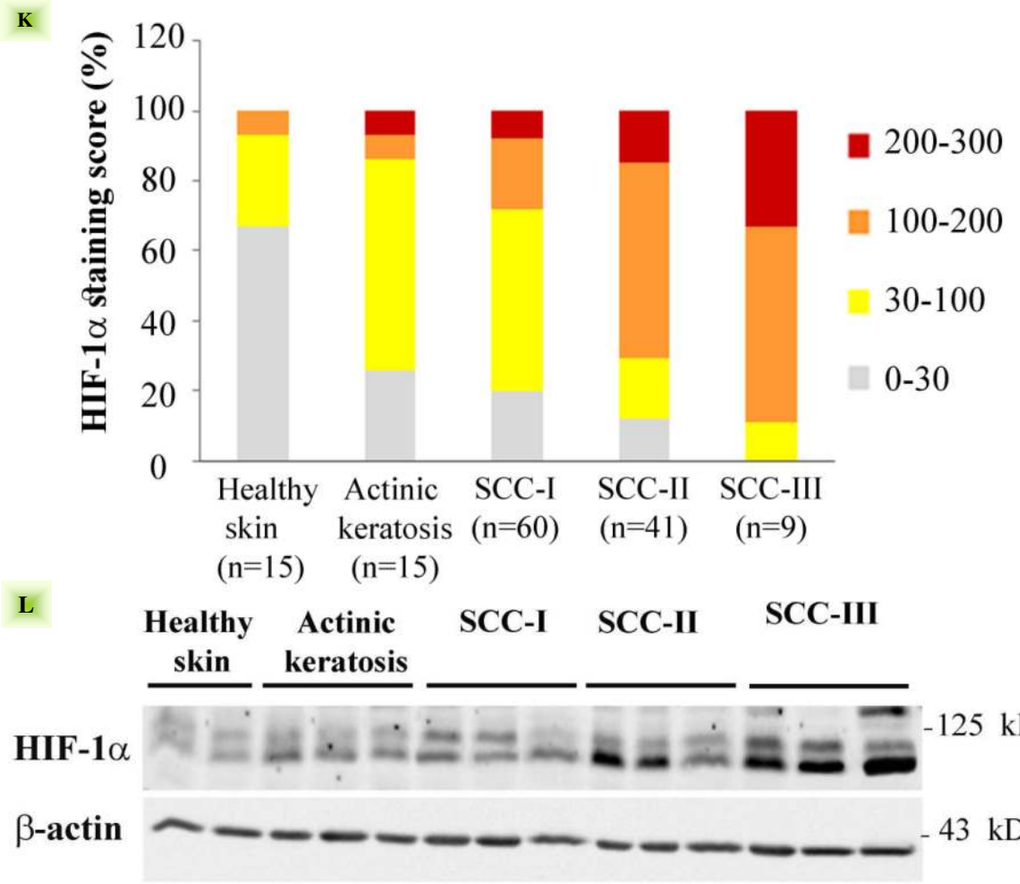
3. Ablation of Xpc in Hif-1α knockout mice restores UVB-induced neoplastic transformation in keratinocytes



4. Antioxidant (NAC) supplementation blocks completely chronic UVB irradiation-induced hyperkeratosis in Hif-1α ablated mice



5. Increase in HIF-1α expression during skin carcinogenesis



(**K**) Graph shows quantification of HIF-1α staining score (0-30 = no to very weak staining to 200-300 = high staining score) of the human skin sections at different stages of tumorigenesis.
(**L**) Western blot depicting the expression of HIF-1α in human skin specimens at different stages of tumorigenesis. ß-actin was used as a loading control.

Conclusion

The deletion of *Hif*-1α in keratinocytes has two profound effects on the skin's responses to chronic UVB exposure: it leads to decreased tumorigenesis by promoting DNA repair efficiency; and it causes skin hyper- and parakeratosis by affecting the redox regulation machinery in keratinocytes.