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The inflammasome adaptor ASC delays UV-induced skin tumorigenesis in Beta HPV38 E6 and E7 Transgenic mice.

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Short title: Protective role of ASC in UV/HPV38-skin cancer

Abbreviations

ASC: Apoptosis-associated speck-like protein containing a CARD

cSCC: Cutaneous squamous cell carcinoma

HPV: Human papilloma virus

NLR: nucleotide-binding domain and leucine-rich repeat containing

NLRP1: NLR family, pyrin domain containing 1

NMSC: Non-melanoma skin cancer

SCC: Squamous cell carcinoma

UV: Ultra violet

TO THE EDITOR

Non-melanoma skin cancer (NMSC) is the most common cancer in fair-skinned adults and comprises basal-cell carcinoma (BCC) and squamous-cell carcinoma (SCC). Its incidence reaches 1/100 person-year in Australia (Lomas et al. 2012), and is still on the rise worldwide (Madan et al. 2010). Development of NMSC depends on genetic and environmental factors. Particularly, there is clear evidence that cumulative sun exposure contributes to the development of NMSC (Ananthaswamy et al. 1997; Madan et al. 2010). In addition, the fact that immuno-suppression strongly increases the susceptibility to NMSC, supports the role of infectious agents in this pathological condition. Indeed, a sub-group of cutaneous human papillomavirus (HPV) types belonging to genus beta of the HPV phylogenetic tree has been implicated as etiological factors of cSCC (Chahoud et al. 2016; Rollison et al. 2019).

Inflammation is a double-edged sword in cancer by promoting tumorigenesis and participating in anti-tumoral immune responses. Inflammasomes are pillars of skin inflammation in response to UV exposure (Fenini et al. 2018) and infections. Inflammasomes are multiprotein complexes leading to the activation of the inflammatory caspase-1, -4 and -5 in humans (caspase-1 and -11 in mice), resulting in the release of the pro-inflammatory cytokines IL-1 β and IL-18. In addition, through the cleavage of GasderminD, inflammasome activation leads to a pro-inflammatory cell death termed pyroptosis. In human keratinocytes, NLRP1 is the major inflammasome sensor and acts with ASC, the inflammasome adaptor, to promote UV-B-induced cytokine release (Fenini et al. 2018).

The role of inflammasomes in cSCC development is controversial and to our knowledge has been studied only in chemical-induced skin carcinogenesis (Drexler et al. 2012; Gasparoto et al. 2014). The role of the inflammasome molecules ASC and caspase-1 in the development of cSCC induced by UV-B remains unknown.

To investigate this issue, we used a transgenic mouse model that expresses the beta HPV38 E6 and E7 under the control of the K14 promoter that is active in the basal layer of the epidermis (K14-HPV38 E6/E7^{Tg}). HPV38 E6/E7 are two oncoproteins that block p53 action in several ways (Accardi et al. 2006; Wallace et al. 2014). K14-HPV38 E6/E7^{Tg} mice are highly susceptible to developing UV-mediated skin carcinogenesis (Viarisio et al. 2011). K14-HPV38 E6/E7^{Tg} mice were crossed with *PYCARD*^{KO} mice (*PYCARD* encodes ASC). Initially on a C57Bl/6 background, *PYCARD*^{KO} mice were first rendered albino (e.g. tyrosinase mutated) through crosses with B6(Cg)-Tyrc-2J/J mice to model fair-skinned populations. The resulting mice were referred to as *ASC*^{KO-alb}. Littermates, termed *ASC*^{WT-alb}, were used as controls. Similarly, K14-HPV38 E6/E7^{Tg} mice were generated on the *CASP1/11*^{DKO} albino background to assess the influence of the inflammatory caspases on tumorigenesis. All experiments were approved by the ethics committee (CECCAPP) of the University of Lyon (#7960-201611 0313531963) and performed in strict accordance with the European laws. Mice were irradiated with escalating doses of UV-B for 30 weeks (Fig. 1a). As previously reported (Viarisio et al. 2011), in the absence of K14-HPV38 E6/E7 transgene, WT mice did not develop any tumor following 30 weeks of UV-B irradiation (Fig. 1b, 1d). Similarly, no tumor was observed in *ASC*^{KO-alb} or *Casp1/11*^{DKO-alb} mice (Fig. 1b, 1d) indicating that inflammasome deficiency is not sufficient to promote tumorigenesis in response to chronic UV-B irradiation. Long-term UV-B exposure and K14-HPV38 E6/E7 transgene synergistically promoted skin lesions in K14-HPV38 E6/E7^{Tg} *ASC*^{WT-alb} (littermate controls) thus recapitulating previous findings (Viarisio et al. 2011). Interestingly, K14-HPV38 E6/E7^{Tg} *ASC*^{KO-alb} mice developed skin tumors significantly faster (median tumor-free time=22 weeks, n=15, p=0.021 by Mantel Cox Log Rank test) than K14-HPV38 E6/E7^{Tg} *ASC*^{WT-alb} (median tumor-free time=26 weeks, n=15). Appearance of skin tumor was preceded by reddish patches, which were characterized as precancerous actinic keratosis (Fig. 1e,

bottom left panel) (Viarisio et al. 2011). The skin lesions also appeared earlier in K14-HPV38 E6/E7^{Tg} ASC^{KO-alb} than in K14-HPV38 E6/E7^{Tg} ASC^{WT-alb} mice (Fig. 1c). These results demonstrate that, in a model mimicking the synergy between chronic sun exposure and HPV38 infection in fair-skinned individuals, ASC provides a protective advantage against tumor development. Histology performed at the end of the experiments on surviving mice did not demonstrate any obvious differences between mice of different genotypes. Various skin lesions ranging from actinic keratosis (Fig. 1e, bottom left panel) to papilloma (Fig. 1e, top right panel) and invasive squamous cell carcinoma (SCC, Fig. 1e, bottom right panel) were observed. These results suggest that ASC-deficiency affects tumor development kinetics but not the oncogenic process per se.

Surprisingly, although caspase-1 is the major inflammasome effector downstream of ASC, K14-HPV38 E6/E7^{Tg} *Casp1/11*^{DKO-alb} mice did not demonstrate any significant differences with the littermate controls in terms of tumor development (Fig 1d; p=0.66). Phenotypic differences between ASC^{KO} and *Casp1/11*^{DKO} mice have been reported previously (Drexler et al. 2012) and might be due to redundancy downstream of ASC. Indeed, ASC can also recruit and activate caspase-8 to trigger cell death, IL-1 β and IL-18 processing (Gringhuis et al. 2012; Pierini et al. 2013).

In contrast to human keratinocytes, murine keratinocytes express very low to undetectable levels of inflammasome components ((Sand et al. 2018) and Supplementary Figure S1a). As a consequence, and in contrast to macrophages, they do not produce IL-1 β in response to LPS + UVB or to various NLRP3 stimuli ((Sand et al. 2018) and Supplementary Figure S1b, c). Assessing the keratinocyte-intrinsic role of inflammasomes is thus not possible in the murine model. The role of ASC described in this study likely reflects a cross-talk between UV-B-exposed E6/E7-expressing keratinocytes and inflammasome-expressing myeloid cells

although at this stage we cannot rule out that the low level of ASC expression in keratinocytes allows keratinocyte-specific inflammasome-independent functions (Drexler et al. 2012).

Importantly, ASC, originally known as Target of Methylation Silencing 1, is down-regulated in numerous cancers, including SSC (Meier et al. 2016). Studying the impact of its loss is thus highly relevant.

Together, this study highlights the protective role of ASC against tumor development triggered by long term UV-B exposure in the presence of HPV 38 E6/E7. The role of p53 and of the individual inflammasome effectors, GasderminD, IL-1 β and IL-18 in this protection remains to be characterized to assess their therapeutic potentials in cSCC.

Data availability

No datasets were generated or analyzed during the current study.

Conflict of interest

The authors state no conflict of interest

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Figure legends

Figure 1: ASC delays UV-B-induced HPV38 E6/E7-dependent skin tumorigenesis.

Mice of the indicated genotypes were irradiated with escalating doses of UV-B three times a week for 30 weeks as indicated (a). (b, d) The frequency of tumor-free mice and (c) the average of individual macroscopic pathologic score were quantified three times a week for 30 weeks. (e) Haematoxylin and eosin staining of representative skin sections with different lesions after 30 weeks of UV-B irradiation are shown (scale bar 500 μm). Higher magnification images are presented in the insets (scale bar 50 μm).

Data information: (b, d) Results from two independent experiments are combined. (b) ASC^{WT-*alb*} n=10; ASC^{KO-*alb*} n=10; HPV38 E6/E7^{Tg} ASC^{WT-*alb*} n=14; HPV38 E6/E7^{Tg} ASC^{KO-*alb*} n=15; ***: p<0.0001; *: p=0.021 by Log rank (Mantel Cox) test. (d) Casp1/11^{WT-*alb*} n=10; Casp1/11^{DKO-*alb*} n=10; HPV38 E6/E7^{Tg} Casp1/11^{WT-*alb*} n=13; HPV38 E6/E7^{Tg} Casp1/11^{DKO-*alb*} n=14; *(1): p=0.0050; *(2): p=0.0073; ns: p=0.66 by Log rank (Mantel

Cox) test. (c). The mean macroscopic pathology score was calculated as follows: presence of at least one reddish zone: score 1, presence of at least one red patch: score 5, presence of at least one tumor: score 8. Each point represents the mean of individual score calculated for HPV38 E6/E7^{Tg} ASC^{WT-alb} n=10; HPV38 E6/E7^{Tg} ASC^{KO-alb} n=7 mice. (e) Representative skin sections were taken from HPV38 E6/E7^{Tg} ASC^{KO-alb}; HPV38 E6/E7^{Tg} ASC^{WT-alb}; HPV38 E6/E7^{Tg} ASC^{KO-alb}; HPV38 E6/E7^{Tg} Casp1/11^{KO-alb} (top left to bottom right, respectively).

Author contribution list

Conceptualization: MT, JM, TH; Formal Analysis: FM, SV, JM, TH; Funding Acquisition: MT, JM, TH; Investigation: FN, SV, PG, CF, PC, MW; Project Administration: MW, MT, JM, TH; Resources: DV; Supervision: DV, AV, MW, MT, JM, TH; Visualization: FM, SV, PG, TH; Writing - Original Draft Preparation: FM, TH; Writing-Review and Editing: FM, SV, PG, DV, AV, MW, MT, JM, TH.

