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Phenotypic Plasticity of Cutaneous Squamous Cell Carcinoma Mediated by Cyclooxygenase-2

Hyeongsun Moon[1], **Dahihm Kim**[2], **Leanne R. Donahue**[2], **Andrew C. White**[2,*]

[1]Center for Comparative Medicine, University of California, Davis, California, USA

[2]Department of Biomedical Sciences, Cornell University, Ithaca, New York, USA

TO THE EDITOR

Squamous cell carcinomas are the most common type of cancers that are capable of metastasis (Yan et al., 2011). The enzymatic activity of cyclooxygenase-2 (Cox-2 also known as Ptgs2) contributes to the synthesis of prostanoids and is upregulated in several types of cancers, including cutaneous squamous cell carcinomas (cSCCs) (Hua et al., 2015; Sobolewski et al., 2010; Subbaramaiah and Dannenberg, 2003;). Cox-2 is an important regulator of tumor development and progression in the UV and chemical carcinogenesis models of cSCC (Elmets et al., 2014; Jiao et al. 2014a, 2014b). In addition to tumorigenesis, Cox-2 may also have the potential to mediate the formation of a mesenchymal-like spindle cell form of cSCC as previous studies have also shown that Cox-2 can also regulate epithelial–mesenchymal transition (Bocca et al., 2014).

Hair follicle stem cells (HFSCs) can act as the cancer cells-of-origin for cSCCs upon the expression of $Kras^{G12D}$ and loss of function in the tumor suppressor *p53* (Lapouge et al., 2011; White et al., 2011). Moreover, HFSC-originating cSCCs are primed to form mesenchymal-like cSCCs with morphological and gene expression characteristics of epithelial–mesenchymal transition in vivo (Figure 1a) (Latil et al., 2017; Pastushenko et al., 2018; White et al., 2014). Upon global transcriptional analysis, *Ptgs2* was found to be significantly overexpressed in HFSC-originating cSCCs (White et al., 2011), which was

*Corresponding author: acw93@cornell.edu.

AUTHOR CONTRIBUTIONS

Conceptualization: ACW, HM; Formal Analysis: HM; Funding Acquisition: ACW; Investigation: DK, HM, LRD; Methodology: ACW, HM; Project Administration: ACW; Validation: DK; Writing - original draft preparation: ACW, HM; Writing - reviewing and editing: ACW, DK, HM, LRD.

Ethics statement

All animals were properly kept and maintained following a protocol approved by the Institutional Animal Care and Use Committee and the Animal Research Committee at Cornell University.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2019.12.028

confirmed by quantitative real-time reverse transcriptase–PCR (Figure 1b, Supplementary Table S1).

In order to determine whether Cox-2 is required for the development of HFSC-originating cSCCs, we crossed *Ptgs2*$^{flox/flox}$ mice to *Krt15-CrePR*; ^{LSL}*Kras*^{G12D}; *p53*$^{flox/flox}$; *Rosa-*^{LSL}*TdTomato* mice (Figure 1c and d) (Supplementary Figure S1). Similar to other Cox-2 studies, using 7, 12-dimethylbenz[*a*]anthracene and 12-*O*-tetradecanoylphorbol-13-acetate- or UVB-induced cutaneous tumorigenesis in SKH-1 or 129S1+C57BL/6 mice, cutaneous tumors appeared in the conditional knockout mice; however, tumor-free survival was significantly improved (Figure 1f) (Jiao et al., 2014a, 2014b; Tiano et al., 2002). Gene deletion was confirmed in knockout tumor tissues, and the protein levels of Cox-2 were found to be strongly suppressed as compared with *Ptgs2* wild-type tumors (Figure 1d and e).

Interestingly, there were strikingly distinct macroscopic features between *Ptgs2* wild-type and knockout tumors (Figure 2a and b). When macroscopic tumors are evident in this cSCC mouse model, they exhibit a smooth surface, rapid growth, and often ulceration within a relatively short period (Figure 2a). In contrast, *Ptgs2* knockout tumors grew slowly and developed a rough surface (Figure 2b). Similar to previous studies (Lapouge et al., 2011; White et al., 2011), histopathological examination demonstrated that HFSC-originating cSCCs show mesenchymal-like spindle cell carcinomas with minimal keratinization (11 of 15) more frequently than cSCCs with mixed mesenchymal-like and epithelial components (4 of 15) (Figure 2c and e). On the contrary, *Ptgs2* knockout tumors showed significantly different phenotypes than those expressing wild-type *Ptgs2* (Fisher-Freeman-Halton test, $*P < 0.002$), as they were frequently well-differentiated with significant hyperkeratosis or papillomatous growths (6 of 11), and less often, mixed (3 of 11) or mesenchymal-like only tumors (2 of 11) (Figure 2d and e). Lineage tracing by using the ^{LSL}*TdTomato* allele demonstrated that tumor cells in both *Ptgs2* wild-type and knockout animals originated from *Krt15-CrePR*$^{+}$ HFSCs (Figure 2c and d) (Madisen et al., 2010).

Consistent with the characteristics of epithelial–mesenchymal transition, *Ptgs2* wild-type mesenchymal-like cSCCs express high levels of Vimentin, low or absent levels of E-Cadherin, and a lack of clear borders between stromal Pdgfr-α^{+} fibroblasts and tumor cells (Figure 2f) (Supplementary Figure S2). On the contrary, *Ptgs2* knockout tumor cells show an absence of Vimentin staining, high levels of E-cadherin, and distinct borders between the stromal and tumor cell compartments (Figure 2g) (Supplementary Figure S2).

In vitro, TdTomato^{+} positive tumor cells from *Ptgs2* wild-type animals showed mesenchymal phenotypes with elongated cell bodies, thereby indicating an invasive potential (Figure 2h and i) (Latil et al., 2017; Pastushenko et al., 2018; White et al., 2014). In contrast, *Ptgs2* conditional knockout tumors demonstrated round cell bodies and exhibited a relatively reduced expression of mesenchymal markers (N-Cadherin, Vimentin) at the protein level (Figure 2h and i). To understand the potential role of Cox-2 activity on the mesenchymal-like phenotypes of *Krt15-CrePR*; ^{LSL}*Kras*^{G12D}; *p53*$^{flox/flox}$ cSCCs cells during tumor growth, we established primary cell lines (Supplementary Figure S3a and b). Pharmacological suppression of Cox-2 by a selective Cox-2 inhibitor (Celecoxib) in vitro was confirmed by PGE_2 ELISA assay and Cox-2 immunoblot (Supplementary

Figure S3c and d), which resulted in a small and transient increase in E-cadherin, but no apparent changes in N-cadherin expression was observed (Supplementary Figure S3e). Additionally, celecoxib treatment demonstrates a change in cell morphology in vitro from elongated spindle-shaped to large, often multi-nucleated cells, thereby suggesting the potential induction of cellular senescence (Supplementary Figure S3f). These data indicate that primary cSCC cell lines do not exhibit a full mesenchymal to epithelial transition when Cox-2 is suppressed in vitro, but future experiments will be needed to further explore this finding because this system does not faithfully recapitulate the microenvironment of cSCC in vivo.

Taken together, oncogenic *Ras/p53* expression in *Krt15-CrePR*$^{+}$ HFSCs can induce the formation of advanced cSCCs with mesenchymal characteristics; however, the loss of Cox-2 function significantly suppresses epithelial–mesenchymal transition–like characteristics in HFSC-originating tumors and causes a conversion to tumors that possess epithelial characteristics at a high frequency (Figure 2j). While Cox-2 is required for an advanced squamous cell carcinoma formation from tumor-prone HFSCs, this phenotype may not be reversible solely by Cox-2 inhibition or may primarily be useful at the early stages of spindle cell cSCC initiation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability statement

No datasets were generated or analyzed during the current study.

Abbreviations:

cSCC	cutaneous squamous cell carcinoma
Cox-2	cyclooxygenase 2
HFSC	hair follicle stem cell

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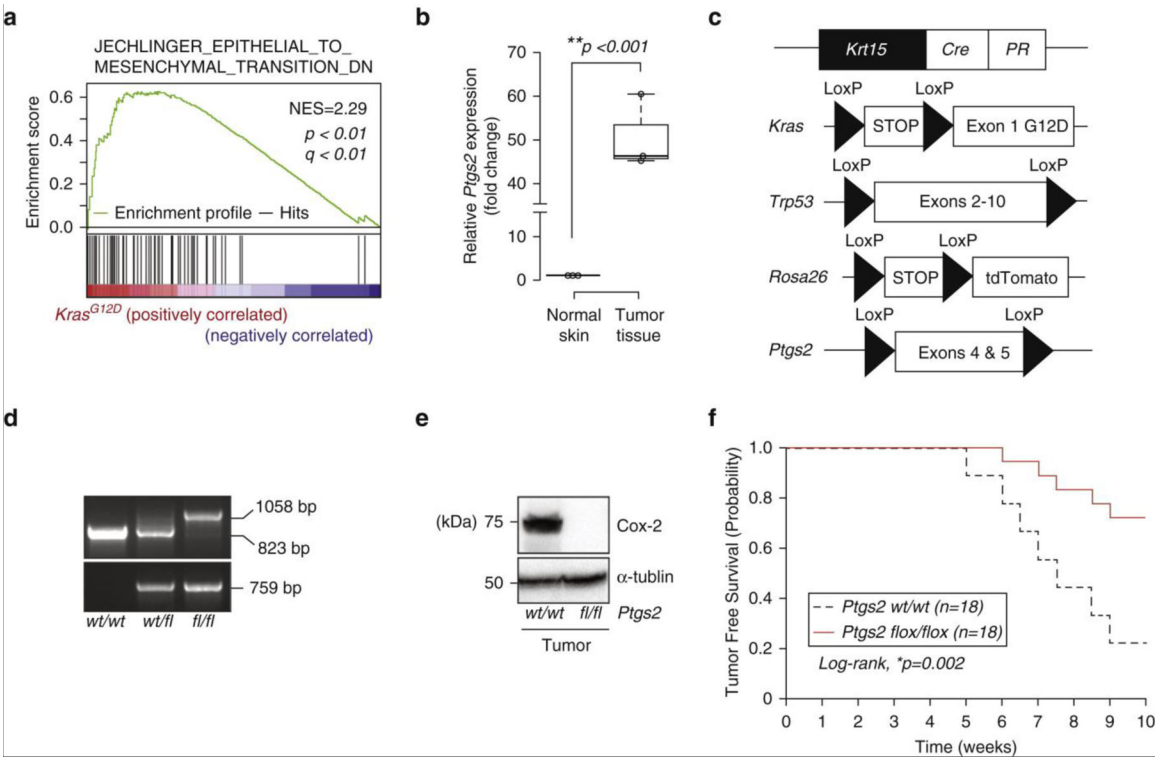

Figure 1. Cox-2 is required for efficient tumor formation.

(**a**) Gene set enrichment analysis shows the correlation between *Kras*^{G12D} and EMT markers. Original transcriptomics data were derived from the research of White et al. (2014). (**b**) *Ptgs2* gene expression was compared between the normal mouse skin and cSCCs collected from *Krt15-CrePR*; ^{LSL}*Kras*^{G12D}; *p53*$^{flox/flox}$ mice. n = 3/each. Error bar, SEM. (**c**) Experimental scheme. *Krt15-CrePR*; ^{LSL}*Kras*^{G12D}; *p53*$^{flox/flox}$; *Rosa*^{LSL}*TdTomato* mice were bred to animals harboring *Ptgs2*$^{flox/flox}$ genetic alleles. (**d**) Animal genotypes were confirmed by PCR, and (**e**) Cox-2 expression at the protein level in tumors originating from animals with or without *Ptgs2* conditional knockout was confirmed by using western blots. (**f**) The Kaplan–Meier curve demonstrates a significantly longer tumor-free survival period in *Ptgs2* conditional knockout animals. n = 18/group. Statistical significance: $*P < 0.01$, $**P < 0.001$. Bar = 100 μm. bp, base pairs; cSCC, cutaneous SCC; Cox-2, cyclooxygenase 2; EMT, epithelial–mesenchymal transition; fl, flox; NES, normalized enrichment score; wt, wild-type.

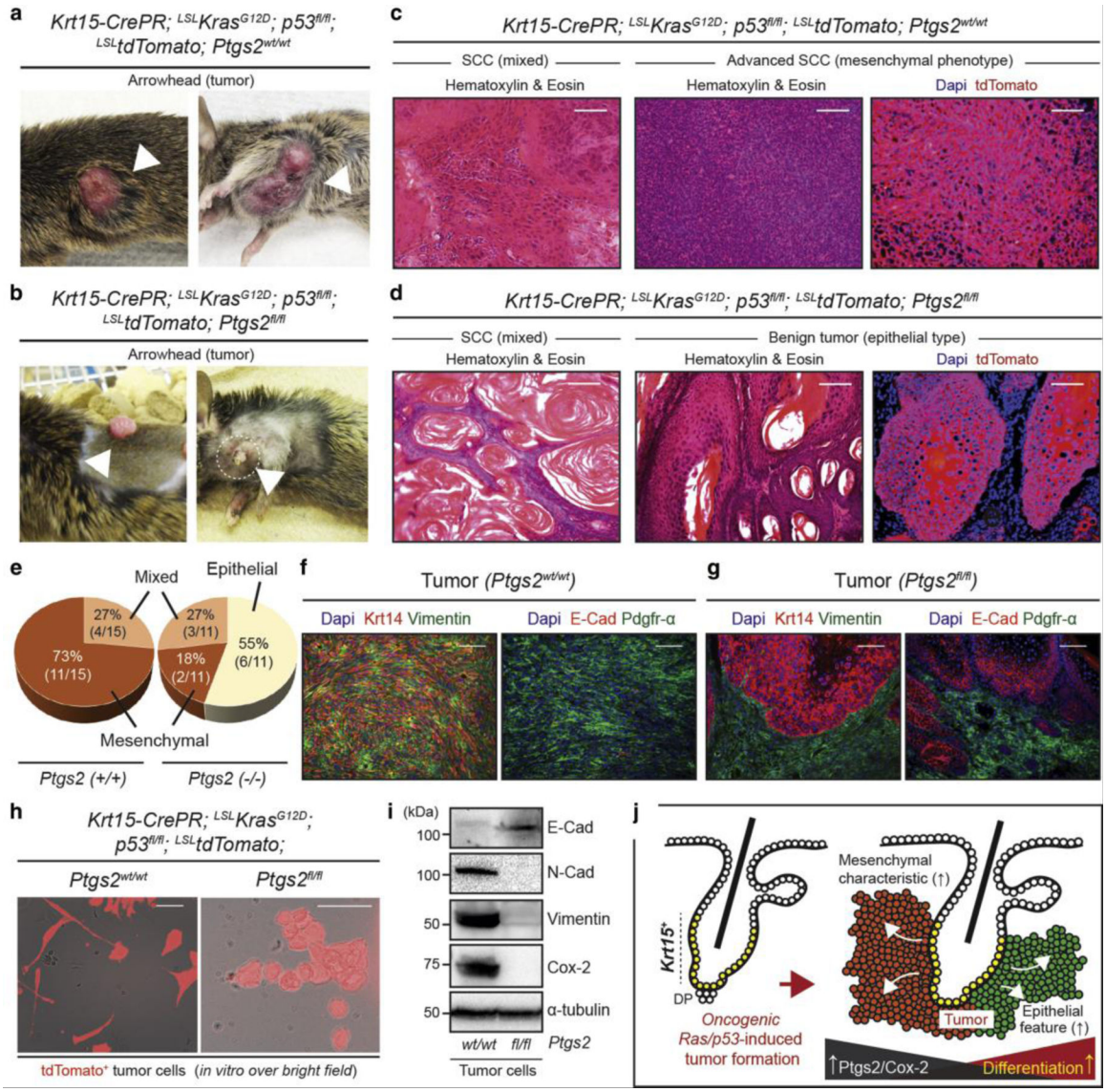

Figure 2. Cox-2 expression correlates with aggressive cSCC phenotypes.

(**a, b**) Macroscopic phenotypes of oncogenic *Ras/p53*-mediated cutaneous tumors originating from murine skin with or without the conditional knockout of *Ptgs2*. (**c, d**) Histological differences between cutaneous tumors with or without Cox-2 expression. TdTomato expression represents lineage tracing. (**e**) The histological phenotypic differences between cutaneous tumors ± Cox-2 expression. n = 15 for wild-type Cox-2 and 11 for Cox-2 knockout. (**f, g**) The expression pattern of cSCC (*Krt14*), mesenchymal (*Vim*), epithelial (*E-Cad*), and stromal fibroblast (*Pdgfr-α*) markers in cutaneous tumors. (**h**) The phenotype of primary cells isolated from cutaneous tumors were observed 16 hours after culture in vitro. (**i**) Relative expression levels of epithelial (*E-Cad*) and mesenchymal (*N-Cad* and *Vim*) markers, and Cox-2 of primary cells was examined by immunoblots. Loading control: α-tubulin. (**j**) Summary. Oncogenic *Ras/p53*-mediated cSCCs originating from *Krt15*$^{+}$ hair follicle stem cells can be more mesenchymal-like upon the expression of cell-type specific

expression of Cox-2. Bar = 100 μm. cSCC, cutaneous SCC; Cox-2, cyclooxygenase 2; fl, flox; SCC, squamous cell carcinoma; wt, wild-type.