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Biomarker function of HMGA2 in ultraviolet-induced skin cancer development

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Abstract

The high mobility group AT-hook 2 (HMGA2) gene encodes a transcription factor that is expressed during embryonic development but down-regulated in adult tissues. Its re-expression in adult tissues is often associated with tumorigenesis. In this study, we found that HMGA2 is highly expressed in human cutaneous squamous cell carcinoma (SCC) cell lines and primary SCC tumors, but not in adjacent normal skin. In non-ultraviolet (UV)-irradiated mouse skin, baseline Hmga2 expression was detected in the epidermis but not in hair follicles. Following chronic UV exposure, we found activation of Hmga2 in hair follicles. UV-induced mouse skin SCC tumors displayed a ubiquitous increase in Hmga2 expression compared to non-tumor bearing adjacent skin. In human SCC cells, decreased HMGA2 expression was linked with reduced cell proliferation following depletion of FOXM1 and TRIP13, two UV master regulator genes. Taken together, these findings highlight an important biomarker function of HMGA2 expression in UV-induced skin tumorigenesis and cell proliferation.

Keywords

HMGA2; keratinocytes; skin cancer; UV radiation; biomarker

1. BACKGROUND

Non-melanoma skin cancer (NMSC) is the most common malignancy in the United States [1]. As the second most common form of NMSC, cutaneous squamous cell carcinoma (SCC) is often more aggressive than basal cell carcinoma with a higher propensity for metastasis and significant disease-related morbidity and mortality. Its incidence continues to rise and over 700,000 new patients are diagnosed in the U.S. each year [2–4]. The high mobility group AT-hook 2 (HMGA2) gene encodes a transcription factor that is expressed during embryonic development but down-regulated in adult tissues [5–10]. Aberrant activation of HMGA2 in

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Author Contributions

L.L. conceived and designed the study. W.H., L.X., A.H. and L.L. performed the research. W.H. and L.L. analyzed and interpreted the data. L.X., M.T. and L.L. wrote and revised the manuscript. All authors read the manuscript and approved the submission.

Conflict of interest

The authors state no conflict of interest.

adult tissues is linked to oncogenesis including breast, lung, and ovarian cancers [11–13]. HMGA2 has been identified as a biomarker of melanoma progression and prognosis in previous transcriptome profiling studies [14], but its role in NMSC development has yet to be defined. White et al. reported that Hmga2 is not required for cutaneous SCC development, as ablation of Hmga2 in a genetic mouse model of SCC does not prevent SCC development [15]. In a separate study, Li et al. showed that Hmga2 expression is induced self-activation during DMBA/TPA-induced mouse SCC tumorigenesis [16].

2. QUESTIONS ADDRESSED

In this study, we investigated the role of HMGA2 activation in UV-induced NMSC development.

3. EXPERIMENTAL DESIGN

3.1 Cells, tissues, and UV irradiation (UVR)

Adult human keratinocytes were purchased from ATCC. Human SCC cells and neonatal keratinocytes were obtained from Columbia University Skin Disease Research Center and were cultured using CnT-07 medium (Zen-Bio) [19]. Human SCC tumors with matched adjacent normal skin tissues were obtained from the Molecular Pathology Shared Resource at Columbia University as described previously [19].

3.2 Animals and chronic UV irradiation

The SKH-1 hairless mice were purchased from Charles River Laboratory (Kingston, NY). TCRdelta-knockout ($Tcrd^{-/-}$) mouse and B6.Cg-Tyr^{c-2J}-Hr^{hr}/J were purchased from The Jackson Laboratory (Bar Harbor, ME). $Tcrd$ -SKH1 mice were produced by repeated backcrossing of the $Tcrd^{-/-}$ mouse with the SKH1-elite mouse (Charles River) to generate $Tcrd^{+/-}$ -SKH1 hairless and haired pups on the albino SKH1 background. Hairless and haired $Tcrd$ -SKH1 and B6.Cg-Tyr^{c-2J}-Hr^{hr}/J mice were subjected to a standard UV irradiation protocol where all animals were irradiated twice weekly with 180 mJ/cm² of UVB. This study was performed in strict accordance with a protocol approved by the Institutional Animal Care and Use Committee of University of Minnesota.

3.3 Gene expression analysis and immunofluorescence staining

Total RNA was isolated from cultured keratinocytes, primary SCC tumors or adjacent normal skin tissues using the RNeasy kit. For qRT-PCR, 2 ug of RNA was converted to cDNA. qRT-PCR was performed using RT² SYBR Green qPCR Mastermix (Qiagen) and results were analyzed using the Bio-Rad CFX 96 Touch system (Bio-Rad). Anti-HMGA2 antibody (D1A7) was purchased from Cell signaling Technology (Catalogue #8179S) and used in immunofluorescence staining as we previously reported [19]. Briefly, frozen human or mouse skin tissue or tumor tissue sections (5 μ m thickness) were fixed in 3% paraformaldehyde for 15 minutes and washed with Tris-buffered saline (TBS). Following blocking of non-specific binding, tissue sections were incubated at 4 °C with specific primary antibody for overnight, followed with incubations of secondary antibodies for 1 h at room temperature before being washed with TBS and mounted with gelvatol mounting

media. Images were acquired using a fluorescence confocal microscope (Zeiss, Thornwood, NY, USA).

3.4 CRISPR/Cas9-mediated deletion of TRIP13 and FOXM1 in SCC cells

To deplete FOXM1 and TRIP13 in human SCC cells, single-guide RNA (sgRNA) sequences were designed to target the human FOXM1 gene or TRIP13 gene^[19]. Each sgRNA was cloned into the pXPR-based LentiCRISPR lentivirus vector expression system. Lentivirus were produced using HEK293FT cells and used to transduce and generate TRIP13- or FOXM1-depleted SCC cells following puromycin selection. Proliferation of control SCC cells and TRIP13- or FOXM1-depleted SCC13 cells was assessed using IncuCyte® live cell analysis systems.

3.5 Statistics

Statistical analysis of the results from each experimental group and control group was performed using t-test. Differences were considered significant if p value was lesser than 0.05.

4. RESULTS

Ultraviolet radiation (UVR) is a primary risk factor for skin cancer, but the role of HMGA2 activation in UV-induced NMSC carcinogenesis remains to be investigated. We posited that HMGA2 activation occurs in response to UVR and UV-induced SCC development. Through transcriptomic profiling studies by RNA-seq, we found drastic upregulation of HMGA2 in 19 human primary SCCs compared to matched adjacent normal skin from 21 SCCs in total (Figure 1A). Immunofluorescence staining confirmed that HMGA2 protein was highly expressed in SCC tumor tissue but not detectable in the adjacent normal skin (Figure 1B and Supplementary Figure 1). These results are consistent with the general observation that HMGA2 is elevated in human cancers. By real-time RT-PCR, we further demonstrated that HMGA2 expression was significantly down-regulated in adult keratinocytes compared to neonatal keratinocytes from foreskin (Figure 1C), but reactivated in two different SCC cell lines (SCC13 and SCC39) as well as the transformed HaCaT cells (Figure 1D). These findings confirm a down-regulation of HMGA2 in normal adult skin and its aberrant upregulation in human skin SCCs. To test the impact of UVR on HMGA2 expression, we exposed neonatal human keratinocytes to 20 mJ/cm² of UVB irradiation for different times. We found that multiple UVR exposures consistently down-regulated HMGA2 expression (Figure 1E). Similarly, UVR reduced HMGA2 expression in human SCC cells as well (Figure 1F) coupled with reduced cell proliferation (data not shown).

Following these observations in human tissues and keratinocytes, we further investigated the in vivo connection between UVR and Hmga2 regulation during skin tumorigenesis using two hairless mouse models, Tcrd-SKH1 and B6.Cg-Tyr^{c-2J}-Hr^{hr}/J, both of which harbor mutations in the hairless gene. Homozygous mutation in the hairless gene leads to complete hair loss and predisposes hairless mice to UV-induced skin tumorigenesis^[17,18]. We subjected hairless and haired Tcrd-SKH1 and B6.Cg-Tyr^{c-2J}-Hr^{hr}/J littermates to a standard UV irradiation protocol where all animals were irradiated twice weekly with 180 mJ/cm² of

UVB. Papillomas emerged in hairless Tcrd-SKH1 and B6.Cg-Tyr^{c-2J}-Hr^{hr}/J mice after approximately 20 weeks of UVR, which progressed into SCC tumors, but not in their haired littermates (Figure 2A). Both tumor and adjacent non-tumor bearing skin were collected for Hmga2 expression analysis by RT-PCR and immunofluorescence. Notably, unlike adult human skin, non-irradiated mouse skin contains a baseline level of Hmga2 expression in the epidermis but not in the hair follicles in haired mice (Figure 2B, upper panel). Following chronic UVR exposure (>20 weeks), Hmga2 expression was elevated in both the epidermis and hair follicles (Figure 2B, lower panel). In UV-irradiated Tcrd-SKH1 hairless mice, UVR significantly increased Hmga2 expression in cutaneous SCCs compared to non-tumor bearing adjacent skin (Figure 2C and 2D). While a baseline Hmga2 expression was also detected in the epidermis of hairless mice without UVR, UVR increased the expression of Hmga2 as well as the thickness of epidermis in hairless mouse (left two panels in Figure 2D). Notably, no Hmga2 expression was detected in the dermal compartment in haired or hairless mice, indicating a tissue-specific regulation of Hmga2 expression. Taken together, these findings demonstrate that chronic UVR increased the expression of Hmga2 in the epidermis of both haired and hairless mouse, indicating that Hmga2 upregulation is not specifically associated with UV-induced skin tumor formation. Upregulation of Hmga2 is more likely associated with the increased epidermal proliferation in hairless skin. Consistent with this idea, we found that decreased HMGA2 expression in human SCC cells was coupled with reduced cell proliferation following depletion of key UV master regulators TRIP13 and FOXM1 in SCC39 cells [19] (Figure 2E & 2F), suggesting that HMGA2 expression might serve as a biomarker of tumor cell proliferation.

5. CONCLUSIONS

In addition to the prevalent view that HMGA2 is a proto-oncogene whose activation plays a major role in malignant transformation [20], there is emerging evidence that HMGA2 is functionally dispensable for in vivo malignant transformation and progression of skin or pancreatic cancers based on studies in mouse models [15,21]. Despite the ubiquitous upregulation of HMGA2 in human SCC tumors and cells, we found that short-term UVR decreased HMGA2 expression in vitro in cultured human keratinocytes and SCC cells (Figure 1E & 1F). In contrast, long-term chronic UVR augmented Hmga2 expression in mouse epidermis (Figure 2B & 2D). Furthermore, HMGA2 expression is detectable in adult mouse epidermis but not in human epidermis, revealing species-specific Hmga2 gene regulation. The in vivo UVR experiments in mice demonstrated that Hmga2 expression was upregulated in both UV-induced SCC tumors and non-tumor bearing skin following chronic UVR, which corroborates the idea that Hmga2 is dispensable for tumorigenesis. Given the ubiquitous upregulation of HMGA2 in human SCC and UV-induced mouse SCC tissues, we propose that HMGA2 upregulation functions as a novel biomarker for UV-induced cell proliferation instead of as a driver of skin tumorigenesis. Future studies to confirm and validate the biomarker function of HMGA2 in skin carcinogenesis will have important clinical implications, particularly in developing biomarker-based assessments of skin UV exposure to risk stratify individuals for timely prevention or intervention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Quantitative analysis of HMGA2 gene expression in human cells and tissues.

A: Comparison of HMGA2 expression in cutaneous SCCs and matched adjacent normal skin from 21 patients. HMGA2 expression in each SCC and matched adjacent normal skin pair was measured by RNA-seq (n=21). **B:** Detection of HMGA2 expression in patient SCC tumor and adjacent normal skin tissue by immunofluorescence. Scale bar is 50 μ m. **C:** Quantification of HMGA2 expression by qRT-PCR showing that HMGA2 is drastically down-regulated in adult human keratinocytes compared to neonatal foreskin keratinocytes (n=4). **D:** qRT-PCR analysis showing that HMGA2 is upregulated in human SCC cells and HaCaT cells compared to adult keratinocytes. **E & F:** UVB irradiation decreased HMGA2 expression in neonatal foreskin keratinocytes and human SCC cells. UV-0.5: cells were irradiated once and collected at 6 h following UVR; UV-1: cells were irradiated once and collected two days following UVR; UV-nx: cells were irradiated multiple time as indicated

by each number and collected two days after the last UVR. *: $P < 0.05$; **: $P < 0.005$; ***: $p < 0.001$.

Figure 2. Hmga2 expression in UVB-induced SCCs in hairless mouse models.

A: UV-induced skin SCCs in Tcrd-SKH1 and B6.Cg-Tyr^{c-2J}-Hr^{hr}/J that emerged after approximately 25 weeks of UVR. **B:** Immunofluorescence detection of Hmga2 expression in haired B6.Cg-Tyr^{c-2J}-Hr^{hr}/J skin with or without UVR. A baseline expression of Hmga2 was detected in the epidermis without UVR (upper panel), which was augmented and spread into the hair follicles following 25 weeks of chronic UVR (lower panel). **C:** qRT-PCR confirmation of augmented Hmga2 expression in UV-induced skin SCCs when compared to non-tumor bearing skin. **D:** Immunofluorescence staining showing that UVR increased Hmga2 expression in UV-induced SCCs and non-tumor bearing adjacent skin in Tcrd-SKH1 mice. Scale bar is 50 μ m. **E & F:** Significantly decreased HMGA2 mRNA expression was associated with reduced cell proliferation following depletion of FOXM1 and TRIP13 by CRISPR/Cas9 in human SCC39 cells, concomitant with decreased cell proliferation. Cell

proliferation rate was measured by using an IncuCyte live cell analysis imaging system. *: $P < 0.05$; **: $P < 0.005$; ***: $p < 0.001$.

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