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Hmga2 is dispensable for cutaneous squamous cell carcinoma

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Abstract

Hmga2 functions as a chromatin associated factor during development, but is not expressed in most adult tissues. Expression of Hmga2 in adult tissues has been associated with a variety of human cancers. Numerous studies have implicated Hmga2 in epithelial to mesenchymal transition (EMT) and cancer progression through gain of function studies, but it is unclear whether Hmga2 is necessary for EMT, tumor formation and tumor progression. We deleted Hmga2 in two mouse models of squamous cell carcinoma and found this gene to be dispensable. In fact, EMT, tumor initiation and progression all appeared to be mostly unaffected by the absence of Hmga2. Tumors lacking the ability to induce Hmga2 proceeded to initiate cutaneous spindle cell and squamous cell carcinomas with all the typical pathological and molecular hallmarks of these cancers.

Keywords

Squamous Cell Carcinoma; Hmga2; Tumor progression; Hair Follicle Stem Cells

For over two decades, HMGA2 has been implicated as a player in human cancer(1), however, the question of whether HMGA2 is required for tumor initiation or progression has not been addressed(2). Our lab and others have determined that cutaneous SCC can be initiated by induction of constitutively active K-ras and loss of p53 specifically in hair follicle stem cells(3, 4). Here we studied the role of HMGA2 in EMT, tumor initiation and progression using both molecular genetics and chemical carcinogenic tumor models. Surprisingly, we did not detect any dramatic effect of the loss of Hmga2 in EMT, initiation or progression of SCC.

Shown in Figure 1A are the top 30 upregulated genes from hyperplasia towards the high grade tumor. Interestingly, many of the genes (8 out of 30) strongly induced in this

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tumorigenesis model are also known to be targets of the let-7 family of miRNAs (8 out of 30), including Hmga2, which was strongly induced in this model as shown at the protein level by immunostaining (Fig 1B). Furthermore, the induction of Hmga2 in these tumors was present in cells that were formerly hair follicle cells as shown by co-localization and lineage tracing (Fig S1). Hmga2 is not typically expressed in most post-natal tissues, but is instead induced relatively late in tumor progression, as predicted by the gene expression profiling.

Because of the remarkable induction of Hmga2 in our SCC model, we sought to determine whether induction of this gene is required for tumor initiation, tumor progression, or induction of the mesenchymal state found in cutaneous spindle cell carcinomas. We obtained Hmga2^{+/-} mice and mated them into the background of our two-hit tumor model for SCC(3). Remarkably, even with loss of either one or both alleles of Hmga2, hair follicle stem cells were still able to serve as cancer cells of origin and produced bona fide SCC (Fig 1C). Tumor frequency, severity, and pathology were not markedly affected by loss of Hmga2 (Fig 1D). Most mice in this model presented with 1–3 macroscopically visible tumors (as quantified in Fig 1D), and full penetrance of more subtle phenotypes upon microscopic examination across all follicles ($n > 7$) (Fig 1D). Quantification of the timing of tumor onset suggested that tumors appeared with a longer latency in the absence of Hmga2 (Fig 1E). This observation could have been due to differences in tumor initiation or even hair cycle timing, as we previously showed that tumor initiation by HFSCs depends on activation of the hair cycle (5). Regardless, the longer latency observed in the absence of Hmga2 did not correlate with severity of phenotype. Western blotting with two independent antibodies confirmed the absence of Hmga2 protein in the tumors and validated the specificity of the immunostainings shown in Fig 1B and 1G (Fig 1F). Immunostaining confirmed the fact that Hmga2 protein was dispensable for acquisition of high grade SCC in this model (Figure 1G).

To determine whether Hmga2 could play a role in SCC driven by an alternative method, we employed the DMBA/TPA protocol on WT and Hmga2 null skin. 16 weeks of the DMBA/TPA protocol, papillomas and keratoacanthomas began to appear on both WT and Hmga2-null mice, some which progressed towards SCC (Fig 1H). These experiments suggested that neither the rate of initiation nor the frequency of tumor formation by DMBA/TPA was affected by the loss of Hmga2 (Fig 1I) ($n > 7$).

We previously described some key molecular cornerstones of tumor initiation and progression from hair follicle stem cells in this model(3), including induction of signaling through Tgfbeta (pSMAD2), Map Kinase (pErk), Creb (pCreb), or Akt (pS6). We failed to detect any significant change in the activation of each of these pathways in Hmga2-null tumors (Fig S2). In addition, Igf2bp1 and Keratin 8, markers of dedifferentiation, were induced in tumors produced in the absence of Hmga2, while another, Sox2, was diminished (Fig S3). These results suggest that while the loss of Hmga2 does have some molecular effects on tumor formation, these are not significant enough to strongly affect tumor initiation or progression (Fig S3).

Numerous studies have shown that Hmga2 is important in regulating factors that determine whether epithelial cells undergo an EMT during tumorigenesis (6, 7). The SCC model used here adopts features of a typical EMT. Immunostaining for both epithelial (E-cad, K14, Integrin α 6) and mesenchymal (Ncam, TnC, CD34) hallmarks, or regulators of EMT (Slug and Twist) demonstrated that loss of Hmga2 expression appeared to have no effect on EMT (Fig 2). An analysis of independent samples by RT-PCR showed the same effect, namely EMT genes were still induced in tumors made without Hmga2 (Fig S4).

The lack of substantial phenotypic effect in the absence of Hmga2 raises many questions as to the role of this gene in cancer, and whether the role for this protein in SCC is typical of that of other cancers as well. It is important to note that cutaneous SCC is only rarely metastatic and thus it is difficult to assign a role for Hmga2 in metastases of SCC as has been done for numerous other cancers. In addition, we cannot rule out the fact that Hmga2 could play an important role in tumor physiology once expressed, even in SCC.

It is certainly possible that the loss of Hmga2 could be compensated for by Hmga1. Hmga1 shares significant homology with Hmga2, but has typically been ascribed to different roles in the cell. The best evidence that these genes may compensate for each other is a recent report demonstrating that while deletion of Hmga2 leads to the pygmy phenotype, deletion of both Hmga1 and 2 generates what were called super-pygmy mice that were significantly smaller still(8). It is worth noting that, according to our RNA profiling, Hmga1 is expressed throughout the epidermis at a significant level and does not change over the course of tumorigenesis, or in the absence of Hmga2 (data not shown). Regardless, we cannot rule out the possibility that the loss of Hmga2 was compensated for by Hmga1 in our experiments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Defining the role for Hmga2 in SCC

A, gene expression profiling by microarray identified many genes induced FACS sorted, lineage traced cancer cells as HFSCs progress from a high grade SCC. Shown are the top 30 genes induced in high grade tumors, many of which have been implicated in EMT. Numbers indicate the average, normalized signal intensity on the microarray from at least two replicates. Shown in RED are those that are predicted targets of the let-7 family of miRNAs. **B** Immunostaining with Hmga2 confirms the expression pattern seen by transcriptional profiling. **C**, Hematoxylin and Eosin staining shows SCC induction upon induction of KrasG12D and loss of p53 (second panel). Loss of one or two alleles of Hmga2 generates

tumors of a similar phenotype (third and fourth panels). **D**quantification of visible tumors across genotypes 10 weeks after induction is provided (bottom panels). **E**, Quantification of the timing of tumor onset as measured by visual inspection is shown either from the point of induction with mifepristone (left) or the start of anagen (right). p -value was calculated with two-tailed student t-test with unequal variance and $n > 4$; error bars represent variation over replicate animals. **F**western blotting with cell extract from tumors formed either in the absence or presence of Hmga2, and confirms the deletion. Note that Hmga2 antibody (ab97276) was used for the immunostainings in **B**, **G** and **H**. **G**immunostaining for Hmga2 shows a typical pattern expression during SCC formation, and the absence of Hmga2 protein in tumors made from mutant mice is shown. **H**mice treated with DMBA/TPA over the course of 18 weeks, and all developed tumors. **I**, across $n > 7$ experiments, neither tumor initiation nor frequency was significantly affected by the loss of Hmga2 as measured by visual inspection. p -value was calculated with two-tailed student t-test with unequal variance and $n > 7$; error bars represent variation over replicate animals.

Figure 2. Loss of Hmga2 does not affect EMT in SCC

Immunostaining for a variety of EMT markers or for regulators of EMT shows that in general the epithelial program is suppressed and a mesenchymal program is induced in SCC, even in the absence of Hmga2 (E-cad, Vim, TnC, Krt5, Krt14, Ncam, Cd49f, Slug, Twist, and Zeb1). Shown are two panels for each marker from both genotypes to indicate the diversity of staining patterns across individual tumors both in the WT and the Hmga2 KO.