Cancer Metastasis Rev (2013) 32:123–128
DOI 10.1007/s10555-012-9410-8

Reconstructing skin cancers using animal models

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Published online: 20 November 2012

Abstract The American Cancer Society estimates that skin cancer is the most prevalent of all cancers with over 2 million cases of nonmelanoma skin cancer each year and 75,000 melanoma cases in 2012. Representative animal cancer models are important for understanding the underlying molecular pathogenesis of these cancers and the development of novel targeted anticancer therapeutics. In this review, we will discuss some of the important animal models that have been useful to identify important pathways involved in basal cell carcinoma, squamous cell carcinoma, and melanoma.

Keywords Skin cancer · Animal model · Molecular pathogenesis

1 Basal cell carcinoma

Cutaneous basal cell carcinoma (BCC) is the most common human cancer overall, accounting for approximately 80 % of nonmelanoma skin cancers [1]. It typically affects fair-skinned individuals and is associated with ultraviolet light exposure [1]. The incidence of BCC continues to rise likely related to long-term sun exposure habits as well as the use of indoor tanning devices [1, 2]. While BCC rarely metastasizes, if it is left untreated, the tumor can become locally invasive leading to deformity and significant morbidity. Creation of adequate mouse models to study BCC in the past has been challenging due to the inability of UV light or chemical carcinogens to induce BCC on murine skin that mimics the human condition. Genetically modified mice represented an excellent option for development of animal models to study the pathogenesis of basal cell carcinoma and to evaluate novel therapeutics.

A genetic etiology for BCC was first suggested in the late 19th century by Jarisch and White and later by Robert Gorlin with the description of basal cell nevus syndrome, also known as Gorlin syndrome [3, 4]. Patients with this autosomally inherited syndrome develop multiple BCCs from a very early age. However, the identification of the causative gene was not discovered until almost 100 years later when two groups identified Patched 1 gene (PTCH1) mutations in these patients [5, 6]. Since this initial discovery, other groups have confirmed that mutations in PTCH1 as well as other genes in the Hedgehog (Hh) signaling pathway are crucial for the development of BCC in these patients as well as spontaneously forming BCCs [7, 8].

PTCH1 is a member of the canonical Hh signal transduction pathway that was first characterized in the fruit fly, *Drosophila melanogaster* [9]. The Hh pathway is a highly conserved cross species and is important for various aspects of normal embryonic development [10]. Activation of this signaling pathway begins with Hh binding to its cell surface receptor PTCH1. This interaction relieves the PTCH1-induced repression of another cell surface transmembrane protein, Smoothened (SMO), freeing SMO to process Gli family members into their active form. Gli proteins are zinc-finger-containing proteins that bind DNA leading to transcription of genes involved in embryonic development and proliferation [10] (Fig. 1). Although this

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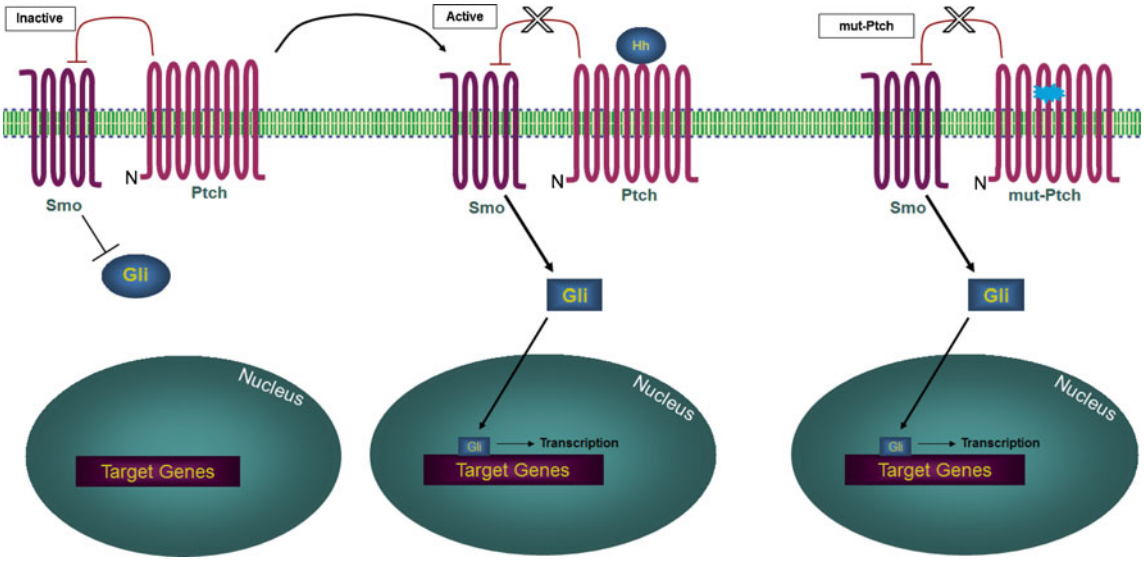

Fig. 1 Schematic of the hedgehog signaling pathway. Patched (Ptch) inhibits smoothened under physiologic conditions (*left*). Hedgehog (Hh) binding to ptch dissociates Ptch from smoothened, thereby activating smoothened (Smo), allowing for Gli activation and transcription of target genes (*middle*). Mutant ptch does not bind and repress Smoothened; this leads to persistent activation of Gli transcription factors (*right*). *Arrows* represent activation and *T-bars* represent inhibitory correlation

pathway is crucial for normal development, activation of this pathway has been seen in a number of tumors including BCC and medulloblastoma.

Current mouse models of BCC involve activation of some component of the Hh signal transduction pathway. The first genetic animal model involved overexpression of Sonic hedgehog (Shh, one of the human and murine homologs of Hh originally discovered in the fruit fly) under the control of the keratin 14 (K14) promoter [11]. K14 along with keratin 5 (K5) is naturally expressed in murine and human basal keratinocytes as well as BCC tumors. The K14-Shh transgenic mice developed cutaneous BCC-like tumors within 4 days of embryonic skin development [11]. Similar spontaneous BCC-like tumors were found in mice overexpressing a mutant variant of SMO (SMO-M2) under the control of the K5 promoter [12]. The SMO missense mutations rendered the protein unresponsive to PTCH repression resulting in continuous activation of the pathway similar to what is seen with the overexpression of Shh [12]. Overexpression of Gli1 and Gli2 also resulted in spontaneous BCC development [13, 14]. PTCH1 heterozygous mice ($PTCH1^{+/-}$) spontaneously develop microscopic BCC, and after chronic UV exposure, the $PTCH1^{+/-}$ mice develop rapidly growing BCC-like tumors after 4 months [15].

Since the mouse models that overexpress either Shh or the activating mutant SMO genes resulted in a lethal phenotype at a very young age, a mouse expressing mutant SMO under the control of a truncated K5 promoter (ΔK5) was created with the goal of studying the effect of the constitutively active SMO mutant, SMO-M2, on adult mouse skin [16]. The SMO-M2 gene mutation was identified in spontaneously formed BCC from adult human skin supporting its use in mouse models of adult BCC [12]. The lethal phenotype was avoided because the ΔK5 promoter is expressed in only a subset of K5-expressing cells [17]. The ΔK5-SMO-M2 mice exhibited upregulation of Shh signaling pathway genes, however in lower overall quantities compared to the mice using the full-length K5 promoter [16]. Interestingly, ΔK5-SMO mice developed basaloid follicular hamartomas but not BCC as seen when using the full-length K5 promoter suggesting that the level of Shh pathway is critical for driving the formation of basaloid epithelial neoplasms ranging from benign follicular hamartomas to infiltrative BCC [16].

Mouse models of BCC have also been created to help address the question of the cell of origin for BCC. Identifying the cells that can give rise to BCC tumors as well as the cells incapable of initiating tumorigenesis may have important clinical applications as new therapeutics are developed in the future. Initial studies indicated that mice expressing a SMO-M2 in the interfollicular epidermis developed BCC tumors. However, when SMO-M2 was expressed preferentially in the lower portions of the hair follicle (below the infundibulum), smaller basaloid lesions developed, but BCC did not develop [18]. However, K15-SmoM2 transgenic mice, which target expression of SmoM2 to stem cells in the bulge region of the hair follicle, demonstrated mobilization of the SmoM2-expressing cells to the interfollicular epithelium where they were capable of triggering BCC tumor formation [19]. Similar studies using Ptch1 conditional knockout mice in which Ptch1 is deleted in hair follicle stem cells resulted in basaloid proliferations. However, after wounding, these cells migrated to the interfollicular epithelium at the

site of injury and triggered BCC tumors [20]. This suggests that the local microenvironment around the hair follicle plays a role in suppressing the oncogenic activity of the Hh pathway. Understanding the molecular mechanism of this inhibition may lead to a novel class of antitumor therapeutics.

2 Squamous cell carcinoma

Cutaneous squamous cell carcinoma (cSCC) is the second most common malignancy in fair-skinned individuals behind BCC [21]. Similar to BCC, the development of animal models that recapitulate human cSCC is crucial for understanding the molecular pathogenesis of these tumors. The canonical carcinogenesis model that cSCC development is a multistep progression starting from the precancerous actinic keratosis (AK) in which keratinocyte atypia is confined to only a portion of the epidermis leading to abnormal differentiation and stratum corneum thickening with retained nuclei. When keratinocyte atypia progresses to include the entire epidermis, the lesion is defined as cSCC *in situ* (cSCCis) [22]. Approximately 26 % of AK/SCCis will spontaneously regress in 1 year; however, in patients with greater than 20 lesions, approximately 20 % will develop invasive histologic features and become cSCC [23, 24]. Mutations in p53, an important tumor suppressor whose inactivation has been implicated in a variety of tumors, have been identified in UV-exposed skin as well as the majority of AK/SCCis [25–27]. Inactivating mutations in this tumor suppressor can lead to genomic instability and loss of cell cycle regulation resulting in a greater propensity for development of additional mutations in oncogenes that drive carcinogenesis [28, 29]. Characterization of p53 null mice ($p53^{-/-}$) demonstrated an increased propensity for lymphoma and sarcoma but not primary cutaneous tumors [30, 31]. However, in the presence of chronic UV exposure, $p53^{-/-}$ mice form AK and SCC-like tumors supporting the role of UV in the pathogenesis of cSCC [31].

Mutations in p53, while common in AK and cSCC, are not the only mechanism for p53 inactivation. Downregulation or mutational inactivation of p53 alone is not sufficient to drive spontaneous cSCC tumor formation suggesting that other oncogenic signaling pathways are activated in these transgenic mice [31]. Activating mutations in epidermal growth factor receptor (EGFR) and Src family tyrosine kinases (SFK), such as Fyn, lead to downregulation of p53 expression [32]. Transgenic mice expressing Src, Fyn, or ErbB2, a member of the EGFR family, in the epidermis develop cSCC tumors [32–34]. Activation of EGFR and SFK family members classically leads to activation of the oncogenic Ras/MEK/ERK signaling pathway (Fig. 2). Indeed, mice that express a constitutively active form of Fyn

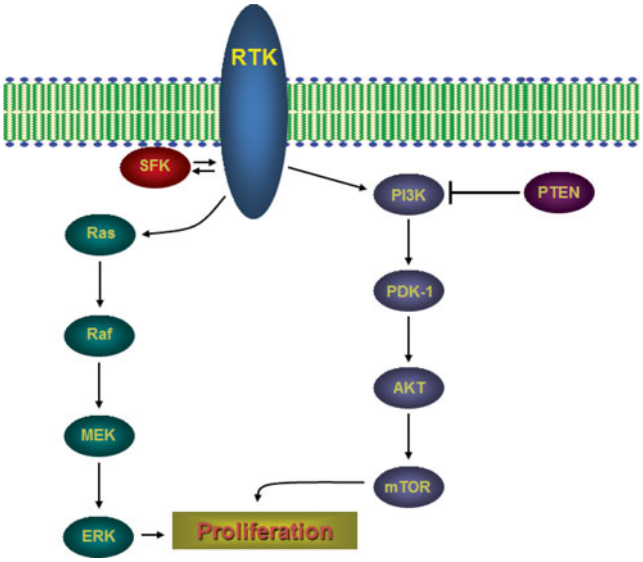

Fig. 2 Schematic of the receptor tyrosine kinase signaling pathways that drive cSCC and melanoma. *Arrows* denote activation, and *T-bars* indicate inhibition

under the control of the K14 promoter (K14-Fyn Y528F) exhibit constitutive activation of the Ras/MEK/ERK signaling pathway [34]. Mutations in Ras family members are the most common overall mutated gene in human cancers, and activating and amplifying mutations in Ras have been found in human AK and cSCC lesions [35–37]. Indeed, 21 % of human cSCC possess activating mutations in Ras with many located at pyrimidine dimer sites, one of the classic signatures of UVB-induced DNA damage [36, 38]. Transgenic mice, which possess a tamoxifen-inducible mutant Ras (H-Ras G12V) in the epidermis, exhibit features consistent with SCCis [39]. Overexpression of activated MEK1 in the epidermis leads to cSCC formation [40].

In addition to kinase activation, transcription factor activation is another gene target important in cSCC pathogenesis. Expression of phosphorylated STAT3, a transcription factor important in cell cycle regulation, apoptosis, and angiogenesis, is upregulated in human SCC [41]. Furthermore, phospho-STAT3 correlated with increased depth of invasion and risk of metastasis [42]. Overexpression of constitutively active STAT3 in the epidermis (K5-Stat3C) resulted in increased SCC after UV exposure [43]. Mice that are deficient in epidermal STAT3 were protected from UV-induced cSCC, supporting the role of STAT3 in UV-induced cutaneous carcinogenesis [43]. STAT3 has also been implicated in the two-step chemical carcinogenesis model. In this model, cSCC are induced after exposing the murine skin to 7,12-dimethylbenz[a]anthracene (DMBA) as the tumor initiator followed by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) as the tumor promoter. STAT3 is required for tumor formation using this DMBA/TPA tumor model [44]. Furthermore, transgenic mice overexpressing STAT3 developed

cSCC tumors in shorter latency and increased frequency using this two-stage chemical carcinogenesis model [44].

3 Melanoma

Melanoma is less common overall compared to nonmelanoma skin cancers; however, it has the highest mortality rate with 5-year survival rate of 15 % in patients with distant metastatic disease [45]. The development of animal models that closely replicate human melanoma is important for understanding the pathogenesis of this aggressive tumor and developing novel targeted therapeutics. Unlike BCC and cSCC in which the cell of origin is the keratinocyte, melanoma is derived from melanocytes. In humans, melanocytes are located within hair follicles, in the bulge, in sebaceous glands, and at the dermal–epidermal junction. However, in hair-bearing areas of adult mice, melanocytes in the skin are primarily located in the hair follicles and occasionally in the dermis [46]. A transgenic mouse that expresses a non-cleavable form of the c-kit ligand, also known as stem cell factor (SCF), under the control of the K14 promoter (K14-SCF) stimulated migration of melanocytes from the hair follicle to the interfollicular epithelium mimicking the distribution in human skin [47]. The K14-SCF transgenic mice do not spontaneously form melanoma; however, when expressed in mice lacking the xeroderma pigmentosa A complement group ($XPA^{-/-}$), they formed metastatic melanocytic skin tumors after UV exposure in approximately 30 % of animals [48].

Similar to the K14-SCF; $XPA^{-/-}$ mouse model, many early mouse models of melanoma used UV irradiation or two-step chemical carcinogenesis; however, these models were hampered by inconsistency and the long time latency to develop melanoma tumors [49]. Spontaneous melanoma in transgenic mice can be generated by targeted expression of SV40 T-antigen, a well-known inhibitor of p53-mediated cell cycle regulation, to melanocytes using the tyrosinase promoter [50]. Expression of activated mutant HRas ($HRas^{G12V}$) under the control of the tyrosinase promoter in the context of deleted cell cycle regulators such as p53, p16, Cdk4, or Cdkn2a increases the penetrance and shortens the latency for spontaneous melanoma formation [51–54]. The melanoma animal models are enhanced with regard to tumor penetrance and latency by exposing the mice to additional mutagens such as UV light or DMBA/TPA [53, 55]. Interestingly, transgenic mice that express only activated Ras mutant ($HRas^{G12V}$) under the control of the tyrosinase promoter do not spontaneously develop melanoma but rather melanocyte hyperplasia supporting the conclusion that activation of Ras signaling pathways alone is not sufficient to drive melanoma [56].

Recent genetic analysis of human melanoma indicates that over 50 % of melanomas have activating mutations in B-RAF, most commonly $B\text{-}RAF^{V600E}$ which leads to MEK/ERK activation [57]. Overexpression of $B\text{-}RAF^{V600E}$ in mice led to the formation of both benign nevi and melanoma [58–60]. Over 80 % of benign human nevi harbor mutations in B-RAF [61]. Overexpression of $B\text{-}RAF^{V600E}$ in zebrafish led to clustering of melanocytes mimicking benign nevi. However, in this zebrafish model, melanoma formation is triggered after inactivation of p53 [62]. This and other studies support the conclusion that $B\text{-}RAF^{V600E}$ alone results in melanocyte senesce, and therefore a second mutation in a tumor suppressor such as PTEN or Cdkn2A is needed for carcinogenesis [63].

Human melanoma frequently exhibits Cdkn2a gene mutations leading to inactivation of its tumor-suppressing gene product p16ink4a [64–66]. However, expression of $B\text{-}RAF^{V600E}$ in a p16ink4a null background did not lead to increased metastatic melanoma formation suggesting that inactivation of another tumor suppressor is critical for melanoma metastasis [59]. Since approximately 20 % of human melanomas have inactivating mutations in PTEN, a different animal model was generated to address the role of PTEN in the context of B-RAF activation [60]. Conditional expression of $B\text{-}RAF^{V600E}$ and conditional silencing of PTEN by deleting its phosphatase catalytic domains resulted in metastatic melanoma with 100 % penetrance [60]. These mice required euthanasia due to overwhelming tumor burden after only 25–50 days after gene induction. Most animal models prior to this often required 6 months for the melanomas to first appear or the addition of mutagens such as UV or DMBA/TPA [49]. Administration of mTOR inhibitor rapamycin improved survival as did administration of a MEK inhibitor that blocks ERK activation (Fig. 2). The data support the conclusion that metastatic melanoma can be generated through activation of B-RAF/MEK/ERK signaling pathway along with activation of PI3K/AKT/mTOR as a consequence of PTEN silencing [60]. The generation of this and other mouse models is important for identifying novel therapeutic targets to treat these potentially deadly tumors.

Conflict of interest The authors declare that no conflict of interest exists.

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