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Deciphering the cells of origin of squamous cell carcinomas

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Abstract

Squamous cell carcinomas (SCCs) are among the most prevalent human cancers. SCC comprises a wide range of tumours originated from diverse anatomical locations that share common genetic mutations and expression of squamous differentiation markers. SCCs arise from squamous and non-squamous epithelial tissues. Here, we discuss the different studies in which the cell of origin of SCCs has been uncovered by expressing oncogenes and/or deleting tumour suppressor genes in the different cell lineages that compose these epithelia. We present evidence showing that the squamous differentiation phenotype of the tumour depends on the type of mutated oncogene and the cell of origin, which dictate the competence of the cells to initiate SCC formation, as well as on the aggressiveness and invasive properties of these tumours.

Introduction

Squamous cell carcinomas (SCCs) are among the most frequent solid cancers in humans[1] and represent a major cause of death worldwide. Their incidence is sharply rising owing to increased exposure to carcinogens, such as ultraviolet radiation related to sun exposure, smoking, alcohol consumption or human papilloma virus (HPV) infection[1,2]. SCCs are classified according to the location where they appear, being frequently found in skin, head and neck, oesophagus, lung and cervix[2–6] and more rarely in pancreas, thyroid, bladder and prostate[7–10].

During the past decades, great efforts have been made to elucidate the cell of origin of different malignancies[11]. Lineage tracing studies allowed the identification of the cellular hierarchies and lineage segregation that mediate homeostasis and repair of the different tissues from which cancer arises[12] (FIG. 1). Many cancers arise from tissues maintained by the presence of stem cells and progenitors that self-renew and differentiate into the different cell lineages that compose these tissues. Depending on the turnover, differentiated cells and progenitors usually present a shorter lifespan, while stem cells reside long term, sometimes

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throughout the life of the animals. Upon tissue damage, the cellular hierarchy that governs epithelial tissue homeostasis can be altered, and more committed progenitors and even differentiated cells can acquire stem cell potential and contribute to tissue repair[13]. With oncogenic hits, both stem cells and progenitors can serve as the cells of origin in cancer (BOX 1).

The nature of the cells at the cancer origin has also been proposed to determine the differentiation characteristics and aggressiveness of tumours. Tumours arising from progenitors may show lineage-restricted differentiation, while tumours arising from stem cells could present multi-lineage differentiation potential. However, multipotent differentiation of a tumour could also result from plasticity of tumour cells induced by their oncogenic mutations or by their microenvironment or by neighbouring cells.

In this Review, we discuss recent studies that define the cell of origin of cutaneous, head and neck, oesophageal and lung SCCs, which represent the most common SCCs. We first describe the architecture and cellular hierarchy present in the different epithelia from which the different SCCs arise. Then, we discuss how lineage tracing strategies have been instrumental to identifying the cell of origin in these SCCs. Finally, we discuss how oncogenic mutations and the cell of origin cooperate in determining the differentiation, aggressiveness and metastatic potential of SCCs.

Architecture of tissues of SCC origin

SCCs arise from epithelial tissues that can be classified as stratified squamous epithelium (which includes epithelia of skin, oesophagus and oral cavity) and non-squamous epithelia (which include airway epithelium). The different types of SCCs have common histological features, such as the presence of squamous differentiation visible by the formation of keratin pearls.

Skin compartments are maintained by their own resident stem cells

The skin epidermis acts as the first defensive line to isolate and protect our bodies from the external environment. The mammalian epidermis is a stratified epithelium composed by the interfollicular epidermis (IFE), hair follicles, sebaceous glands and sweat glands[14]. Lineage tracing experiments in mice have shown that during homeostasis, the distinct skin compartments are maintained by their own pool of lineage-restricted stem cells[15–19] (FIG. 2). However, during tissue regeneration such as wound healing, epithelial cells acquire plasticity, and the different lineage-restricted stem cells, such as hair follicles and infundibulum, get activated, migrate towards the wounded region and differentiate into suprabasal cells of the IFE[19–22].

The IFE is a stratified squamous epithelium maintained by the existence of basal cells with high self-renewing capacities that balance proliferation and differentiation. How precisely the balance between renewal and differentiation is achieved to sustain the homeostasis of the epidermis remains a matter of discussion and may vary between the different parts of mouse skin (ear, paw, tail, ventral and dorsal skin)[23–29]. It was initially hypothesized that the epidermis is maintained by the existence of many small units of proliferation called

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epidermal proliferative units, which contain slow-cycling stem cells that generate transit-amplifying cells, which, after a defined number of cell divisions, give rise to terminally differentiated cells30. Lineage tracing using *Ah–Cre–ER*, a construct that combines the cytochrome P450, family 1, subfamily a, polypeptide 1 (*Cyp1a1*) promoter with a Cre recombinase fused to the oestrogen receptor (Cre–ER) and leads to expression in a largely ubiquitous manner following the administration of beta-naphthoflavone, has been used to assess the mode of epidermal homeostasis[28,29]. The Cre–ER allows control of the activity of Cre by the administration of tamoxifen, which mediates the translocation of Cre to the nucleus, the recombination of *loxP* sites and the expression of the reporter gene (FIG. 1). Clonal analysis of *Ah–Cre–ER* lineage tracing data in the IFE demonstrated that the clone size does not converge on a precise number of basal cells, suggesting that the unit of proliferation has no predefined fixed size. The clone size distribution could be explained by the presence of a single population of equipotent progenitors that balance renewal and differentiation in a stochastic manner[28,29]. More recent studies have demonstrated that epidermal cells are more heterogeneous than initially anticipated and have illustrated that different Cre–ER mice target different stem and progenitor cells with distinct proliferation rates and survival capacities, different long-term renewing capacities and different abilities to mediate long-term skin repair[24–27]. The population of progenitor cells targeted by the involucrin (*Ivl*)–*Cre–ER* in the mouse tail IFE is identical to the one targeted by the *Ah–Cre–ER*[25,28]. By contrast, keratin 14 (*K14*; also known as *Krt14*)–*Cre–ER* (*K14–Cre–ER*) targets progenitors and stem cells that have long-term survival and divide asymmetrically to give rise to progenitors[25,27](FIG. 2a,b).

Distinct anatomical regions compose the hair follicles, including the infundibulum, isthmus, sebaceous glands and lower hair follicle regions that produce the hair shaft. These different epidermal regions are maintained by their own pool of resident stem cells[15–19,31] (FIG. 2a). Mouse hair follicle stem cells can be analysed by using the *K15* promoter-driven, the *K19* promoter-driven or leucine-rich repeat-containing G protein-coupled receptor 5 (*Lgr5*) promoter-driven Cre-inducible constructs *K15–Cre–PR*[18], *K19–Cre–ER*[31] and *Lgr5–Cre–ER*[15], respectively. In Cre–PR constructs, Cre is fused to the progesterone receptor (PR), and administration of RU468, a PR ligand, promotes the translocation of Cre to the nucleus and its activity (FIG. 1).

The oral epithelium is maintained by a proliferative basal compartment

The oral epithelium contains keratinized areas composed of keratinized and non-keratinized stratified squamous epithelium (FIG. 2b). The oral cavity and pharynx are coated with non-keratinized stratified epithelium. The masticatory mucosa is keratinized. The larynx is lined by ciliated pseudostratified columnar epithelium, except the vocal cords, which are covered by stratified squamous epithelium. In all these epithelia, cell proliferation is restricted to the basal layer.

Very little is known concerning the identity of stem cells in head and neck epithelia, the location of stem cell niches or how stem cells and progenitors balance proliferation and differentiation. Pulse–chase tritiated thymidine and 5-bromodeoxyuridine (BrdU) experiments revealed the existence of slow-cycling label-retaining cells in mouse oral

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epithelia, suggesting the existence of a pool of slow-cycling basal stem cells[32]. Lineage-tracing experiments in mice using the SRY-box 2 (*Sox2*)*–Cre–ER* and *K14–Cre–ER* transgenic systems, which drive Cre-inducible reporter gene expression in SOX2-expressing or K14-expressing basal cells, showed that cells located in the basal compartment present long-term maintenance and can give rise to the different cell types that form the tongue and soft palate[33, 34].

The oesophagus is maintained by basal progenitors

The mouse and rat oesophagus has a keratinized squamous epithelium composed of 4–5 cell layers with a rapid turnover, which is maintained by the presence of proliferative basal cells that are able to self-renew, differentiate and migrate towards the lumen, giving rise to suprabasal layers of terminally differentiated cells[35] (FIG. 2b).

The cellular hierarchy that mediates the homeostasis in mouse oesophageal epithelium remains a matter of intense debate[33,36–42]. Clonal analysis using *Ah–Cre–ER* transgenic mice suggests that the mouse oesophageal epithelium is maintained by a single, equipotent, committed progenitor cell population that balances renewal and differentiation[37]. By contrast, lineage tracing studies using *Sox2–Cre–ER* or *K15–Cre–PR* transgenic mice labelled a subpopulation of mouse basal cells with long-term maintenance and the ability to give rise to the differentiated cells, suggesting that the cells targeted by *SOX2* promoter-inducible or K15 promoter-inducible Cre mark a progenitor and/or stem cell population with higher self-renewal potential than that of committed progenitors[33,41]. Other studies using fluorescence-activated cell sorting isolation, colony forming assays and 3D organoid assays further suggested the presence of heterogeneity within oesophageal epithelium in terms of marker expression, proliferation kinetics and ability to reform oesophageal epithelium[39,40]. Side by side comparison using the different Cre systems will be important to resolve this apparent discrepancy.

The human oesophagus has a non-keratinized squamous epithelium composed of several layers and two anatomical compartments, the papillae and the interpapillary region. A study in human oesophageal epithelium suggested the existence of two anatomically different compartments, one populated by a relatively quiescent stem cell population (interpapillary region) and the second populated by transit-amplifying cells that give rise to terminally differentiated cells (papillae)[38]. However, in another study, the quiescent cells expressing the stem cell marker CD34 were found at the tip of the papillae, and the highly proliferative cells were found at the interpapillary region[42].

Lung compartments are maintained by their own resident stem cells

Two main compartments constitute mammalian lungs: the airways and the alveoli. The respiratory system allows gas exchange and protects lung epithelia from microorganisms and dust particles that are constantly inhaled. All different lung compartments (trachea, bronchi, bronchioles and alveoli) are maintained by their own resident stem cells during homeostasis[43–47]. Upon tissue damage, differentiated cells present some plasticity and can revert back to a basal stem cell fate[48].

The trachea and bronchi are lined with a pseudostratified epithelium[49], which consists of basal cells, secretory cells, ciliated cells and rare neuro-endocrine cells. The murine bronchioles are lined with a simple columnar epithelium composed mainly of secretory and ciliated cells, containing some neuroendocrine cells and no basal cells (FIG. 2c).

The airway epithelium has low renewal activity under steady-state conditions. In the mouse trachea, lineage tracing experiments using *K5– Cre–ER*, which targets basal cells of the trachea, showed that these cells contain self-renewing multipotent stem cells that give rise to basal, secretory and ciliated cells during postnatal growth and homeostasis and upon injury[43]. Lineage tracing in secretoglobin, family 1A, member 1 (*Scgb1a1*)*–Cre–ER* transgenic mice, which drives the inducible Cre in secretory cells in the trachea, bronchi and bronchioles and in cells of the alveolar epithelium, showed that *Scgb1a1*$^{+}$ cells are progressively lost and replaced over time, suggesting that secretory cells in the trachea and bronchi represent a transit-amplifying cell population. However, in the bronchioles, *Scgb1a1*$^{+}$ cells self-renew for longer periods of time without being lost and generate ciliated cells, thus representing a population of bipotent stem cells[44]. Lineage tracing in forkhead box J1 (*Foxj1*)*–Cre–ER* transgenic mice, a system that drives Cre-inducible reporter gene expression in ciliated cells, showed that ciliated cells are terminally differentiated cells[45] that usually arise from secretory cells but upon injuries can arise from the direct differentiation of basal cells[50] (FIG. 2c).

The alveolar epithelium contains two cell types: thin type 1 cells (AT1 cells) and cuboidal type 2 cells (AT2 cells). Lineage tracing experiments using surfactant associated protein C (*Sftpc*)*–Cre–ER* transgenic mice, a system that drives Cre-inducible reporter gene expression in AT2 cells, demonstrated that AT2 cells can self-renew for long periods of time and give rise to AT1 cells, suggesting that they represent a bipotent population of alveolar stem cells[46,47] (FIG. 2c).

Common pathways in SCCs

SCCs are thought to result from a multistep process in which the sequential accumulation of genetic mutations leads to the generation of preneoplastic lesions that progress into invasive carcinomas[2–5]. The development of next-generation sequencing allowed the identification of new driver mutations responsible for tumour initiation and progression[51]. Recent studies have reported the mutational landscape of human SCCs, including cutaneous SCC (CSCC)[52–54], oesophageal SCC (ESCC)[55–58], head and neck SCC (HNSCC)[59–64], lung SCC (LSCC)[65–67] and cervical SCC[68,69]. These studies demonstrated that SCCs from different tissues present mutations in a common set of genes, suggesting that common mechanisms regulate SCC initiation across different tissues (FIG. 3).

Cell cycle regulators

The tumour suppressor gene *TP53*, which induces cell cycle arrest and apoptosis[70], is the most commonly mutated gene in SCCs originating from the different body locations (FIG. 3). Deletions and somatic mutations in cyclin-dependent kinase inhibitor 2A (*CDKN2A*), *CDKN2B* and *RB1* are also frequent in the different types of SCCs. *CDKN2A* and *CDKN2B* encode p16-INK4A, and p15-INK4B respectively, which both control cell cycle

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arrest and activate the tumour suppressor genes *TP53* and *RB1*. *RB1* regulates G1 to S phase progression[71]. Moreover, amplifications of the cyclin D1 (*CCND1*) and *MYC* genes are frequently found in HNSCCs[59–64] and ESCCs[55–58] (FIG. 3). CCND1 binds to cyclin-dependent kinase 4 (CDK4) as well as CDK6 and promotes G1 to S phase progression by inhibiting RB[72]. *MYC* overexpression promotes cell cycle progression and contributes to cell transformation[7]

RAS, AKT and receptor tyrosine kinase signalling

Alterations in components of the RAS, AKT and receptor tyrosine kinase (RTK) signalling pathways, which regulate cell proliferation and survival[74–76], are commonly found in SCCs (FIG. 3). *HRAS* was the first discovered oncogene associated with mouse and human CSCC[77,78]. Mutations in both *HRAS* and *KRAS* are frequently found in CSCC[52–54] and to a lesser extent in HNSCC[59–64] and LSCC[65–67] (FIG. 3). Activation of the AKT pathway via phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit-α (*PIK3CA*) gene mutation or amplification or *PTEN* gene mutation or deletion is found in SCCs from different body locations. HNSCCs[59–64], ESCCs[55–58] and LSCCs[65–67] have amplifications in epidermal growth factor receptor (*EGFR*) and fibroblast growth factor receptor 1 (*FGFR1*) (FIG. 3).

Squamous cell differentiation pathways

The NOTCH signalling pathway is involved in regulating cell fate decisions in various tissues during development and homeostasis including skin, oral, oesophageal and bronchial epithelia[79,80]. Four NOTCH receptors (NOTCH1–NOTCH4) exist in mammals. NOTCH1 is expressed in the skin in homeostatic conditions, predominantly in the suprabasal layers of the IFE, where it promotes keratinocyte differentiation and acts as a tumour suppressor[81–83]. Deletion of *Notch1* in mouse epidermis accelerates and increases skin tumorigenesis[81]. Mutations in *NOTCH1* and *NOTCH2* genes are found in around half of human CSCCs[52–54] and to a lesser extent in HNSCCs[59–64], ESCCs[55–58] and LSCCs[65–67] (FIG. 3).

Tumour protein p63 (*TP63*) and *SOX2* are frequently co-amplified in SCCs owing to their adjacent chromosomal localization (3q)[84] (FIG. 3). *TP63* is a member of the *TP53* gene family and is expressed in the basal compartment of the skin, oesophagus, lung airways and larynx during development and homeostasis[85,86]. In skin, TP63 is required for epidermal stratification during development and to maintain the proliferative potential of basal keratinocytes during homeostasis[87]. A reciprocal negative regulation of TP63 expression and NOTCH activity has been described in the skin, where TP63 expressed by basal cells represses NOTCH activity, controlling the switch between proliferation and differentiation[88]. SOX2 is a transcription factor that controls pluripotency in embryonic stem cells, where it acts together with octamer-binding protein 4 (OCT4; also known as POU5F1)[84]. In contrast, in LSCC and ESCC, SOX2 acts together with TP63 to regulate the expression of genes involved in squamous carcinogenesis[84].

SOX2 gene expression is upregulated in the majority of mouse and human CSCCs[89,90] (FIG. 3). Deletion of *Sox2* before chemical-induced carcinogenesis (DMBA followed by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) application) prevents CSCC formation, showing the essential role of SOX2 during CSCC initiation[89]. Moreover, SOX2-expressing CSCC cells

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showed higher clonogenic potential upon subcutaneous injection into immunodeficient mice. *Sox2* genetic lineage ablation, which allows the selective killing of *Sox2*-expressing cells in mice with CSCC, leads to tumour regression, consistent with the notion that SOX2 marks skin cancer stem cells[89]. Deletion of *Sox2* impairs tumour propagation and induces CSCC regression[89]. Altogether, these studies demonstrated that SOX2 is essential for skin tumour initiation and progression and that it marks and regulates the function of cutaneous cancer stem cells, suggesting the presence of a continuum between tumour initiation and progression in skin SCCs[89].

Epigenetic regulators

Mutations in chromatin-modifying enzymes leading to DNA and histone modifications have been described in numerous cancers[91]. Loss-of-function mutations in the histone 3 lysine 4 (H3K4) methyltransferases lysine methyltransferase 2C (*KMT2C*) and *KMT2D* have been described in the different types of SCCs[52,55,57–59,61–63,65,66]. In aggressive CSCC, *KMT2C* mutations are associated with poor outcome and increased bone invasion[52], suggesting that *KMT2C* functions as a tumour suppressor gene. Additionally, HNSCCs[59–64] and ESCCs[55–58] carry loss-of-function mutations for nuclear receptor-binding SET domain protein 1 (*NSD1*), an H3K36 methyltransferase. Individuals carrying inactivating mutations in *NSD1* often have overgrowth syndromes (Soto syndrome) and are more prone to cancer, including SCC[92]. Moreover, human HPV-negative HNSCC with *NSD1* inactivating mutations showed decreased expression of genes involved in epithelial differentiation, indicating that NSD1 may act as a tumour suppressor by promoting differentiation[93]. Histone demethylase (such as lysine demethylase 6A (*KDM6A*)) inactivating mutations or deletions have been reported in HNSCC[59–64], ESCC[55–58] and LSCC[65–67,94] (FIG. 3). However, the mechanisms by which mutations in these epigenetic regulators promote tumour initiation and progression remain poorly understood[95].

Cell of origin in SCCs

The activation of oncogenes or deletion of tumour suppressor genes in specific cell populations using Cre expressed under different promoters has allowed the identification of the cells of origin of the different SCCs. Before the development of lineage tracing techniques, it was often believed that the tumour cells and the cells of origin expressed the same markers and therefore that the cells of origin could be inferred by the marker expression in tumour cells. However, lineage tracing studies have demonstrated that the expression of differentiation markers by tumour cells is sometimes misleading when used to extrapolate or determine their cellular origin (that is, basal cell carcinoma originating from the IFE expresses markers of hair follicle)[31,96,97].

Cutaneous SCC

CSCC is one of the most common cancers in humans, accounting for over 700,000 new patients per year in the United States[98]. CSCCs rarely metastasize (5%), but metastasis is associated with a poor prognosis, with a patient survival of 10–20% over 10 years[2]. Sun exposure, chronic wounds and immunosuppression are the major risk factors of CSCC. CSCC also occurs in patients receiving BRAF inhibitors for the treatment of melanoma[99].

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Actinic keratosis (human) and papilloma (mouse) represent benign squamous lesions that progress into malignant CSCCs. The most extensively used mouse model for CSCC is a carcinogen-induced protocol consisting of a topical application of DMBA, a mutagen, followed by administration of TPA, which stimulates epidermal proliferation and inflammation[100]. TPA administration one year after the last DMBA application induced papilloma formation[101,102], suggesting that DMBA-induced mutations occur in long-term epidermal stem cells or that DMBA-induced mutations immortalize a fraction of progenitors and confer them with stem cell properties. DMBA treatment followed by repeated dermo-abrasion, which removes IFE cells, leads to papilloma and carcinoma formation, although it does occur with reduced frequency[103], suggesting that different epidermal lineages, including hair follicles and IFE, act as the cells of origin in CSCC. In addition, hair follicle stem cell lineage tracing demonstrates the direct contribution of hair follicle stem cells to DMBA–TPA-induced benign skin tumours[104,105].

DMBA–TPA-induced CSCC is almost invariably (>90%) associated with mutations in RAS family members, most frequently *Hras*, followed by *Kras* and *Rras2* (REFS[77,106–108]. Overexpression of *Hras* in differentiated epidermal cells using the *K10* promoter leads to the generation of papilloma only at sites of wounding or irritation[109], suggesting that either differentiated cells revert back to a stem-like state during wound healing or that wounding represents the second hit in K10+ cells already carrying and expressing the *Hras* mutant allele. In addition, *Hras* mutant allele expression in basal cells, using a truncated form of the *K5* promoter results in papilloma and CSCC formation in mice[110], indicating that CSCC originates from basal cells in the absence of wounding.

The classical transgenic approach described before, in which the *K5* or *K14* promoter is used to express an oncogene, leads to the constitutive expression (non-inducible) of the oncogene in all basal cells from the early stages of epidermal development to adulthood[110]. Therefore, this strategy cannot be used to determine from which parts of the epidermis tumours arise. To assess from which adult epidermal compartments CSCCs arise, different groups have used *Cre–ER* or *Cre–PR* transgenic mice to conditionally express oncogenic *Kras*^{G12D} in different epidermal compartments at physiological levels in mice (FIG. 4a). Papilloma was observed following activation of *Kras*^{G12D} expression in K19^{+} or K15^{+} hair follicle stem cells and their progeny (using *K19–Cre–ER* and *K15–Cre–PR* transgenic mice)[96,97]. By contrast, activation of the same oncogenes in the rapidly dividing hair follicle transit-amplifying cells using a Cre-inducible sonic hedgehog (*Shh*) promoter-driven system (*Shh–Cre–ER*) did not lead to benign or malignant tumour formation[96,97], suggesting that tumours arise from long-lived hair follicle progenitors and/or stem cells rather than transit-amplifying cells (FIG. 4a). Expression of *Kras*^{G12D} in K14^{+} basal cells or IVL^{+} progenitors using *K14–Cre–ER* or *Ivl–Cre–ER* transgenic mice leads to papilloma formation[96]. Combined *Kras*^{G12D} expression and *Trp53* deletion in mice are required to initiate malignant SCCs[96,97]. Interestingly, whereas *K14–Cre–ER*-targeted IFE basal cells can give rise to SCC upon *Kras*^{G12D} expression and *Trp53* deletion, *Ivl–Cre–ER* IFE progenitors cannot (C.B., unpublished observations). Altogether, these studies indicate that hair follicle and IFE stem cells represent the cells of origin of mouse CSCCs (FIG. 4a).

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Human CSCCs present a high degree of cellular heterogeneity, varying from well-differentiated to poorly differentiated tumours, which present a higher rate of recurrence and lower rate of cure after treatment[2]. To determine whether the cells of origin control tumour heterogeneity in CSCCs, intertumoural and intratumoural heterogeneity was assessed in SCCs arising from the same oncogenic hits in different cell lineages of the mouse epidermis[111]. Whereas conditional oncogenic *Kras*^{G12D} expression and *Trp53* deletion specifically in IFE basal cells, using *K14–Cre– ER*, lead to well-differentiated tumours in mice, activation of the same oncogenic hits in hair follicle lineages using *Lgr5–Cre–ER* leads to more invasive and less differentiated tumours with features of epithelial to mesenchymal transition (EMT) or purely mesenchymal-like tumours resembling spindle cell carcinoma[111] (FIG. 4). These data demonstrate that oncogene-targeted hair follicle cells are primed to undergo EMT during tumorigenesis. Cancer cells derived from hair follicle lineages that underwent EMT have much higher clonogenic and metastatic potential, suggesting that the cells of origin in CSCC influence tumour stemness, local invasion and lung metastasis. The transcriptional and chromatin landscape of the cells of origin and the presence of different populations of tumour cells revealed that the different epidermal stem cells are epigenetically primed to undergo different differentiation programmes upon oncogenic transformation, giving rise to tumours with different degrees of squamous differentiation and EMT[111] (FIG. 4b). *Trp63* overexpression restricts EMT in oncogene-targeted hair follicle cells, leading to the formation of well-differentiated SCC, and this finding indicates that *Trp63* is a key regulator of epithelial fate. Altogether, this study demonstrates the role of the cell lineages (hair follicle cells versus IFE cells) at the origin of CSCC in regulating EMT and identifies gene regulatory networks that promote squamous differentiation and EMT in primary skin SCCs[111].

Head and neck SCC

HNSCCs are the sixth most common cancer worldwide[112] and are associated with high mortality — approximately 50% of patients die of the disease. Smoking and alcohol consumption are the major risk factors in developing countries, and infection with HPV is a risk factor for HNSCC among non-smokers[4]. HPV serotype 16 is associated with increased risk of developing oropharyngeal SCC[113] and cervical cancer[114]. Fanconi anaemia, a rare autosomal recessive disorder characterized by a high degree of genomic instability, predisposes to HNSCC[115].

Oral leukoplakia is described to be the precursor lesion of HNSCC in humans[116]. Several chemical HNSCC carcinogen models have been developed in rodents, including DMBA-based and 4-nitroquinoline 1-oxide (4-NQO)-based methods, which recapitulate some features of human oral SCC (OSCC), including the progression of pre-malignant lesions to differentiated SCCs[117]. A combination of *K14–Cre–ER* lineage tracing with 4-NQO treatment in mice leads to the generation of papilloma and OSCC, providing evidence that basal cells represent the cells of origin in OSCC[118].

RAS and *TP53* genes are mutated in HNSCC[59–64] (FIG. 3). The Cre-inducible expression of tumorigenic *Kras*^{G12D} driven by the *K14* or *K15* promoter in basal cells of the oral mucosa in *K14–Cre–ER* and *K5–Cre–ER* transgenic mice consistently leads to the generation of

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papillomas in mouse oral mucosa and to hyperplasia of the tongue[119,120]. Combined *Kras*^{G12D} activation and *Trp53* deletion in mouse oral basal cells using the *K14–Cre–ER* system resulted in the generation of tongue SCC[121] (FIG. 5a).

Mutations in the transforming growth factor-β (TGFβ) and PTEN–AKT signalling pathways are frequently found in HNSCC[59]. Loss of function of SMAD family member 4 (*Smad4*) in mouse oral basal cells, using the Cre-inducible promoters *K14–Cre–PR* and *K5–Cre–PR*, leads to the generation of tumours ranging from moderately differentiated to poorly differentiated SCC with RAS activation and genomic instability[122]. Deletion of *Pten* or TGF-beta receptor type-1 (*Tgfbr1*) alone in basal cells using the Cre-inducible *K14–Cre–ER* promoter resulted in hyperproliferation and very few instances of HNSCC, whereas the combined deletion of *Pten* and *Tgfbr1* in the same cells using *K14–Cre–ER* led to the formation of HNSCC[123] (FIG. 5a). Altogether, these reports demonstrate that HNSCC can originate from oral basal cells.

Oesophageal SCC

Oesophageal cancer is the eighth most common cancer and the sixth leading cause of cancer death worldwide[112]. Oesophageal cancer includes two major histological subtypes: ESCC and oesophageal adenocarcinoma[5]. ESCC accounts for the majority (around 90%) of the cases of oesophageal carcinomas and is particularly common in certain regions of Asia. ESCC predominates in the upper and mid-oesophagus and is associated with smoking and alcohol exposure in Western countries. Adenocarcinoma generally occurs in the lower oesophagus near the gastric junction and is associated with gastric reflux and obesity[5].

Several studies using the Epstein–Barr virus *ED-L2* promoter, which is expressed in both basal and suprabasal compartments of the oraloesophageal squamous epithelia, identified the combination of genetic alterations that lead to ESCC development in mice[124–127] (FIG. 5b). *TP53* loss of function and *CCND1* amplification represent the most frequent genetic alterations in ESCC[58]. Overexpression of *Ccnd1* in mouse oesophageal squamous epithelia using the *ED-L2* promoter leads to dysplasia, the precursor lesion of ESCC[124]. The combined overexpression of *Ccnd1* and deletion of *Trp53* driven by the *ED-L2* promoter resulted in invasive oral-oesophageal cancer, leading to lymph node metastasis in 25% of mice with ESCC[125] (FIG. 5b).

Inflammation was suggested to play an important role in ESCC carcinogenesis by fostering a microenvironment favourable for tumour initiation. Kr pel-like factor 4 (*Klf4*) overexpression in the oesophageal epithelia using the *ED-L2* promoter leads to the production of proinflammatory cytokines (tumour necrosis factor (TNF), CXC-chemokine ligand 5 (CXCL5), granulocyte colony-stimulating factor (G-CSF) and interleukin-1α (IL-1α)) by the oesophageal basal cells, resulting in the recruitment of inflammatory cells and SCC formation in mice[126]. Deletion of catenin δ1 (*Ctnnd1*) in the tongue, oesophagus and forestomach using *ED-L2–Cre* mice led to immune cell infiltration, increased proliferation of basal cell, defects in differentiation and generation of ESCC[127].

Lineage tracing experiments in a chemical-induced mouse model of oesophageal cancer, consisting of diethylnitrosamine (DEN) and sorafenib application, revealed that high-grade

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dysplasias resulted from a small bias towards symmetrically renewing division over asymmetrical division or symmetrical differentiation in oesophageal progenitors[128]. In invasive murine SCCs, which were induced by DEN treatment followed by *Kras*^{G12D} expression in oesophageal cells of *Ah–Cre–ER* mice and following sorafenib application, the tumour expansion was driven by a larger bias towards self-renewing division in basal tumour cells[128], suggesting that unbalanced cell fate is associated with ESCC development.

Genes controlling squamous cell differentiation, such as *NOTCH* or *SOX2*, are frequently altered in human ESCC[55–58] (FIG. 3). Inactivation of the NOTCH signalling pathway in the oesophageal epithelium (using a dominant negative mutant of mastermind-like transcriptional coactivator 1 (*Maml1*) expressed in oesophageal cells of *Ah–Cre–ER* mice) in mice resulted in the replacement of wild-type epithelium by mutant cells, but this was not sufficient to promote dysplasia or ESCC formation, suggesting that NOTCH inhibition is not sufficient to initiate ESCC carcinogenesis[129]. *SOX2* is amplified in human LSCCs and ESCCs[130]. *Sox2* overexpression in murine oesophageal K5+ basal cells and their progeny (using *K5–Cre–ER*) leads to the expansion of the basal compartment, defects in squamous differentiation and hyperplasia[131]. When these *Sox2*-overexpressing cells are infected with lentivirus expressing constitutively activated signal transducer and activator of transcription 3 (STAT3), they give rise to SCC upon transplantation into immunodeficient mice. However, differentiated suprabasal cells co-expressing *Sox2* and *Stat3* did not lead to SCC upon transplantation. These findings demonstrate that activated STAT3 and SOX2 overexpression in murine oesophageal basal progenitors, but not differentiated suprabasal cells, leads to ESCC formation, indicating that oesophageal progenitors and/or stem cells represent the cells of origin in ESCC131 (FIG. 5b). Moreover, this study suggested that SOX2 overexpression represents a tumour-initiating event in ESCC and that cooperation with inflammation-mediated STAT3 activation is required for SOX2-driven ESCC tumorigenesis. Altogether, these reports suggest that ESCC originates from oesophageal progenitors and/or stem cells and that cell fate imbalance in the cells of origin is an important feature of tumour initiation.

Lung SCC

Lung cancer is one of the leading causes of cancer-related mortality, resulting in an estimated 1.4 million deaths per year worldwide[132]. Non-small-cell lung carcinoma and small-cell lung carcinoma are the two most frequent lung cancers. Non-small-cell lung carcinomas account for more than 85% of lung cancer cases and are classified into lung adenocarcinomas (50%), LSCCs (30–40%) and large cell carcinomas. Adenocarcinomas and SCCs present distinct molecular abnormalities[65,133,134] and are thought to arise from distinct cells of origin[135]. Adenocarcinomas and large cell carcinomas usually arise peripherally (from the small bronchi, bronchioles or alveoli), and LSCCs usually arise proximally (from the main bronchi)[3]. Smoking and chronic inflammation are the major risk factors of LSCCs[135].

The combination of *Kras*^{G12D} expression and serine/threonine kinase 11 (*Lkb1*; also known as *Stk11*) deletion in the mouse lung epithelium using inoculation of adenovirus-derived Cre intranasally leads to the generation of a large spectrum of lung tumour types including

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LSCC[136]. Combined *Pten* and *Lkb1* deletion in lung epithelia leads to the formation of highly penetrant, well-differentiated, rarely metastatic LSCCs, which appeared in both proximal and distal murine lungs, suggesting that different cell lineages are at the origin of LSCC[137]. *SOX2* is one of the most frequently amplified genes (23%) and is highly expressed in human LSCCs[130]. SOX2 is expressed physiologically during the development of the airway system and in the adult trachea[138]. Conditional deletion of *Sox2* in the developing airway epithelium using NK2 homeobox 5 (*Nkx2-5*)*–Cre* mice resulted in mouse perinatal death due to defects in the airway system and lung development[138], including an excess of mucus-producing cells and a decrease of basal, ciliated and secretory cells[138]. Deletion of *Sox2* in adult lung epithelial cells using the ubiquitously expressed cyto-megalovirus-derived *Cre–ER* promoter leads to a decrease of their renewal potential and repair capacity during tissue injuries[138]. These data demonstrate the essential role of SOX2 during airway system development and maintenance in adults. Overexpression of *Sox2* combined with *Lkb1* deletion in murine lung cells, using intranasal inhalation of a lentivirus engineered to overexpress *Sox2* under the ubiquitous beta-actin (*Actb*) promoter and a lentivirus expressing Cre under the ubiquitous phosphoglycerate kinase (Pgk) promoter (*Pgk–Cre*), leads to the generation of LSCC[139] (FIG. 6). By contrast, lentivirus expression of *Sox2* in combination with *Trp53* deletion or *Trp53* and *Rb1* double deletion in mouse lung epithelium, using *Pgk*–Cre lentivirus to delete *Trp53* and*Rb1*, leads to the generation of adenocarcinomas[139].

The previous studies define the combination of mutations required for LSCC development but do not define the lung lineages from which LSCCs arise. Overexpression of *Sox2* using *Scgb1a1–Cre–ER* in mice, which targets secretory cells and rare AT2 cells located in the bronchioalveolar duct junction that display stem cell properties[140], leads to the metaplastic transformation of pseudostratified epithelium of the bronchioles and alveoli into columnar cells expressing TP63. Some of these lesions progress into adenocarcinoma-like tumours but express squamous markers[141]. Oncogenic *Kras*^{G12D} expression in secretory cells and rare AT2 cells using *Scgb1a1–Cre–ER* leads to the formation of alveolar adenocarcinomas, whereas the expression of *Kras*^{G12D} combined with NOTCH inhibition by expression of the dominant negative *Maml1* mutant in the same cells resulted in *Sox2* expression and alveolar hyperplastic lesions that expressed squamous markers[142], suggesting that NOTCH and SOX2 drive squamous differentiation in *Kras*-induced lung tumours. To gain insight into the cell of origin of LSCCs, the tumour suppressor genes (that is, *Pten* and *Cdkn2a* and/or *Cdkn2b*) or putative amplified oncogenes (that is, *Fgfr1* and *Sox2*) in LSCCs in different lung cell types were deleted or overexpressed[143] (FIG. 6). Deletion of *Pten* and *Cdkn2a and/* or *Cdkn2b* in mouse lung basal cells using intratracheal administration of adenovirus-expressing Cre under the *K5* promoter (adeno-*K5–Cre*) or adeno-*K14–Cre* resulted in low-penetrance tumours of mixed histology. Deletion of *Pten* and *Cdkn2a and/*or *Cdkn2b* in combination with *Fgfr1* overexpression in basal cells using intratracheal administration of adeno-*K5–Cre* or adeno-*K14–Cre* leads to heterogeneous tumours with sporadic squamous differentiation. By contrast, overexpression of *Sox2* and deletion of *Pten* and *Cdkn2a and/*or *Cdkn2b* in basal cells resulted in the generation of multiple proximal LSCCs ranging from moderately differentiated to well-differentiated SCCs, providing evidence that basal cells can represent the cells of origin in LSCCs and that *Sox2* overexpression promotes squamous

differentiation. Interestingly, *Sox2* overexpression in the context of *Pten* and *Cdkn2a and/*or *Cdkn2b* deletion in secretory and AT2 cells (using intratracheal administration of adeno-*Scgb1a1–Cre* and adeno-*Spcc–Cre*, respectively) leads to the formation of distal LSCCs, demonstrating that bronchus, bronchiole and alveolus cells can also act as the cells of origin of LSCC[143]. This study nicely illustrates that basal, secretory and AT2 cells represent different possible cells of origin for LSCCs in mice[143]. Moreover, it demonstrates that *Sox2* overexpression is a key promoter of squamous cell differentiation in LSCCs, as previously reported in CSCCs[89].

Limitations of the SCC mouse models

Although some progress has been made in the characterization of the cell of origin of SCCs using murine models, many questions still need to be resolved. The cellular hierarchy present in some tissues from which SCC arises (cervical, oral cavity and head and neck tissues) is poorly characterized. The distinction between stem cells, progenitors and differentiated cells within the basal layer of these epithelia remains complicated. The generation of new specific Cre mice allowing targeting of these different populations will be essential to refine the respective contribution of stem cells versus progenitors in cancer initiation. The mechanisms that confer the competence or resistance of a given cell population to SCC initiation should be further studied to design new strategies to prevent and treat SCCs.

Moreover, caution is needed when extrapolating the results obtained in mice to the human setting, especially where histological differences exist between the two species. This is the case of the oesophageal epithelium, which is keratinized in mice but non-keratinized in humans. It has been suggested that keratinization in mouse oesophagus protects it from injuries and ESCC development. Additionally, the transition from squamous epithelia (oesophagus) to columnar epithelia (gastric cardia) occurs at the gastro-oesophageal junction (between the oesophagus and stomach) in humans, whereas in mice, it occurs within the stomach. Another important difference is observed in the lung; basal cells and cartilage rings extend from the trachea to the bronchioles in humans, whereas in mice, they are limited to the trachea and bronchi (FIG. 2).

Conclusions and future directions

Comparisons across different SCCs illustrate that SCCs are characterized by very similar mutation landscapes, including alterations in the *TP53*, *SOX2*, *TP63*, *CDNK2A* (*P16-INK4A*), *NOTCH1*, *KMT2D*, *PIK3CA* and *PTEN* genes. Comparative genomic studies have reported that LSCCs and ESCCs share more commonly mutated genes with other types of SCCs from other body locations than with adenocarcinomas originating from the same tissue[56,94,134]. The type and number of oncogenic mutations that occur in cells of origin determine SCC invasiveness, differentiation and EMT features. In mouse models, more than one mutation in an oncogene or a tumour suppressor gene is usually required for the formation of invasive SCCs. *TP53* deletion is the most frequent tumour suppressor gene mutation in human and mouse SCCs, and *Trp53* deletion is usually sufficient to convert benign tumours into malignant SCCs when another oncogene (*Ras* or *Ccnd1*) is expressed in mouse models of SCC[96,97,125]. In addition to these key oncogenic mutations, cell fate

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determinants, such as SOX2 or TP63 overexpression, are required to promote the squamous fate of SCCs[143].

The different studies that investigate the cells of origin in SCCs highlight another common feature — the cells of origin arise from mutations in proliferative basal cells that are characterized by their ability to self-renew and to generate terminally differentiated cells. No clear demonstration that oncogenic mutations can induce the reversion of non-proliferative epithelial cells to a stem cell-like state and consequently drive SCCs initiation has been provided. Very often, despite their rapid turnover, transit-amplifying progeny seem unable to initiate malignant SCCs, although in some cases, committed progenitors are able to initiate benign squamous tumours. It will be interesting to study whether additional oncogenic mutations in more committed progenitors can lead to SCC formation, as described in basal cell carcinoma or intestinal tumours[144,27]. However, when mouse tumours arise upon experimental introduction of oncogenic mutations in stem cells, it remains unclear whether the cancer originates directly from the stem cell or from the more committed progeny.

Next-generation sequencing techniques allowed the identification of new drivers in the different types of SCC. New SCC mouse models could be generated to better model the diversity and tumour heterogeneity found in SCCs from different tissues and to better understand how the order and combination of different genetic alterations modify the cell at the origin of cancer and tumour progression. Moreover, a better understanding of the transcriptional and chromatin landscape of the different types of SCCs will uncover common and tissue-specific determinants that regulate tumour initiation and squamous differentiation programmes across different SCCs. In addition, it would be interesting to assess whether the cells of origin in SCCs can be inferred from the DNA methylation patterns of cancer cells, as suggested in CSCC[145]. Moreover, understanding how the cell of origin controls the generation and properties of cancer stem cells would be essential to designing new strategies to prevent tumour progression and metastasis and to decrease resistance to therapy.

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Box 1

Cellular origin of cancer: stem cells versus committed cells

Stem cell theory

Stem cells typically persist for a longer duration in the tissue, where they self-renew and give rise to progenitors and differentiated cells. It has been suggested that the longer a cell persists in a tissue, the higher the likelihood that this cell will accumulate the necessary mutations required to become tumorigenic. For this reason, stem cells are commonly considered the cells of origin of cancer[146]. Recent studies have suggested that the rate of stem cell turnover of a given human tissue is correlated with the probability of this tissue to develop cancer[147–149].

Committed cell contribution

It has been suggested that the plasticity observed in epithelial tissues during tissue repair plays a role during tumour initiation. Extrinsic cues and/or oncogenic mutations can confer the ability to induce tumour development to already committed cells. In the intestine, activation of the WNT signalling pathway through the expression of a constitutively active form of catenin beta 1 (*Ctnnb1*) together with nuclear factor-κB (NF-κB) activation (through deletion of NF-κB inhibitor alpha (*Nfkbia*)) or simultaneous expression of constitutively active forms of *Ctnnb1* and *Kras*^{G12D} in committed epithelial intestinal cells, using the X-box-binding protein 1 (XBP1) promoter fused with a Cre recombinase and oestrogen receptor (*Xbp1–Cre–ER*), led to the dedifferentiation of previously committed cells into stem-like cells and to tumour development[144]. In the skin interfollicular epidermis, progenitors targeted by involucrin (*Ivl*)*–Cre–ER* mice require two hits (overexpression of a constitutively activated form of smoothened, frizzled class receptor (*SmoM2*) and *Trp53* deletion) to induce basal cell carcinoma, whereas stem cells require only *SmoM2* expression for tumour development[27].

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Glossary

Squamous cell carcinomas

(SCCs). Cancers that present with squamous differentiation, which is visible by the presence of keratin materials.

Lineage tracing

A method involving experiments that allow the labelling of a cell or a group of cells and assess the fate of these labelled cells and their progeny overtime.

Stem cells

Cells that are at the top of the cellular hierarchy and are characterized by long-term self-renewing capacity and give rise to progenitors, transit-amplifying cells and differentiated cells.

Progenitors

Cells that can self-renew and give rise to terminally differentiated cells. Depending on the proportion of asymmetric renewal and symmetric differentiation upon division, progenitors can live long term or short term.

Stratified squamous epithelium

Epithelium composed of a layer of basal proliferative cells and several suprabasal layers of differentiated cells that express keratins and progressively flatten near the surface, eventually presenting as enucleated cells that are shed from the surface. These amorphous keratinized ghost cells are known as squames. The inner surface of the body is lined with non-keratinized stratified squamous epithelium, which is characterized by superficial cells that are flattened and nucleated.

Keratin pearls

Keratin-derived amorphous materials arising from the differentiation of tumour cells.

Transit-amplifying cells

Cells that divide a finite number of times and then terminally differentiate.

Clonal analysis

The study of the fate, renewal and long-term maintenance of single isolated cells over time.

Secretory cells

Cells found throughout the body that secrete components to affect cell; secretory cells of the airway system (also known as Clara cells) produce mucins and antimicrobial peptides.

Ciliated cells

Cells that contain tiny hair-like structures on their surface, found in the airway system of mammals and the fallopian tube of female mammals; ciliated cells of the airway system

propel debris and dirty mucus out of the respiratory tract through the movement of their cilia.

Type 1 cells

(AT1 cells). Cells of the alveolar epithelium that allow gas exchange.

Type 2 cells

(AT2 cells). Cells of the alveolar epithelium that produce surfactant, which helps the alveolar structure to stay open and thus allows gas exchange.

Lineage ablation

The selective killing of a cell lineage, which is usually performed by inducing expression of a toxin or a toxin receptor in a cell of interest and then administering that toxin.

Dedifferentiation

A process that occurs when committed or differentiated cells revert to a less committed state.

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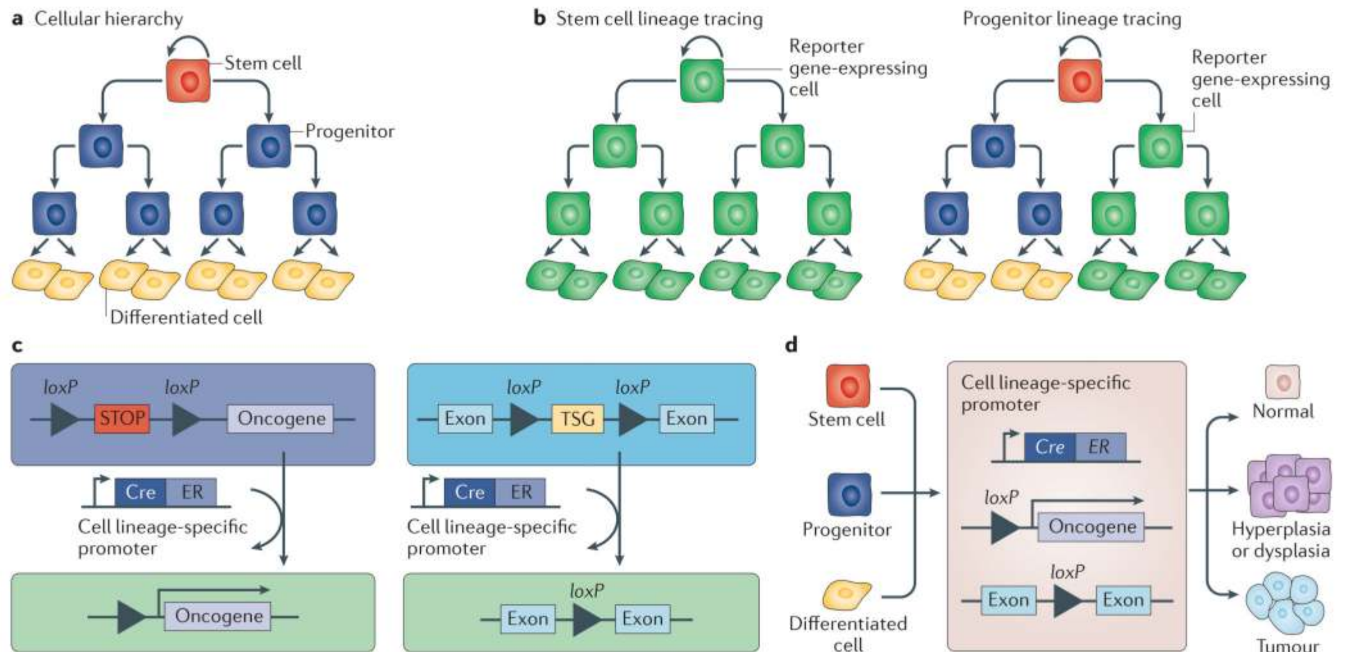

Fig 1. Lineage tracing and the cells of origin of cancer.

Many cancers arise from epithelial tissues, which are maintained by stem cells and their progeny. Stem cells are at the top of the cellular hierarchy and have the ability to self-renew and generate progenitors, which can self-renew and give rise to progenitors and terminally differentiated cells (part **a**). Tamoxifen-induced activation of the Cre recombinase under a cell lineage-specific promoter, for example, a stem cell-specific or progenitor-specific promoter, leads to the elimination of the STOP cassette between *loxP* sites, resulting in the expression of the reporter gene (part **b**), the expression of the oncogene or the deletion of the tumour suppressor gene (TSG) between *loxP* sites (part **c**) in the respective cell population and its progeny (parts **b** and **c**). If a progenitor-specific promoter is used, the reporter gene is expressed in progenitors and their progeny and in differentiated cells but not in the stem cell population. A progenitor usually has a limited lifetime, and thus, the reporter expression may be lost over time (part **b**, right panel). Conditional activation of oncogenes or deletion of TSGs has allowed the identification of the cells of origin in different mouse tumour models (part **d**). ER, oestrogen receptor.

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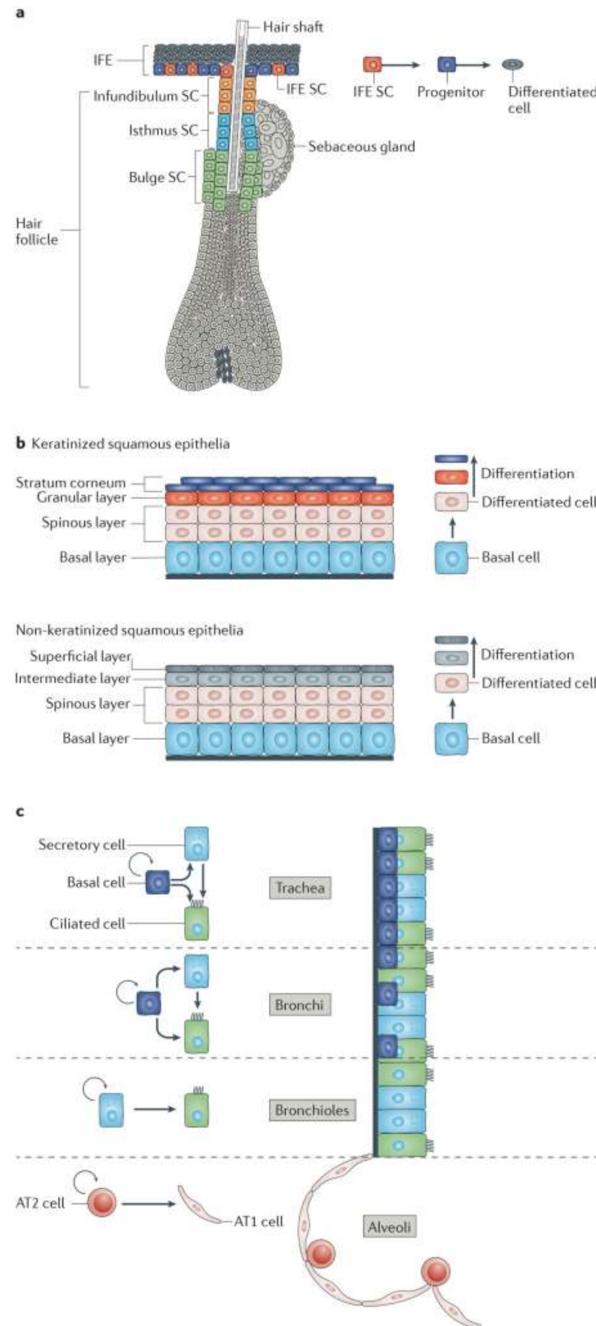

Fig 2. Architecture and cellular hierarchy present in the tissues from which SCC arise.

a | The different skin compartments are maintained by their own resident stem cells (SCs). The epidermis is composed of the interfollicular epidermis (IFE), hair follicles and sebaceous glands. The different anatomical regions that form the hair follicle, namely, bulge, infundibulum, isthmus and sebaceous glands, have their own pool of SCs. The hair follicle SCs are slow-cycling cells, residing below the sebaceous gland, in the permanent region of hair follicles. During physiological conditions, hair follicle SCs sustain the cyclic production of the hair, giving rise to transit-amplifying progenitors that rapidly divide and differentiate

into the different concentric hair follicles lineages. **b |** The squamous epithelia of the skin, oral cavity, head and neck and oesophagus are composed of a layer of basal proliferative cells and several suprabasal layers of differentiated cells that progressively flatten before being lost. In keratinized squamous epithelium (for example, oesophageal epithelium), the differentiated cells are enucleated and shed from the surface; these amorphous keratinized ghost cells are called squames. The inner surface of the body is lined with non-keratinized stratified squamous epithelium (for example, oral epithelium), which is characterized by superficial cells that are flattened and nucleated. **c |** The different lung compartments are maintained by their own pool of SCs during homeostasis. Multipotent basal SCs maintain the mouse trachea and bronchi and give rise to secretory and ciliated cells. In the bronchioles, secretory cells represent a bipotent population of SCs that self-renew and give rise to ciliated cells. In the alveoli, bipotent type 2 (AT2) SCs self-renew and give rise to type 1 (AT1) and AT2 cells.

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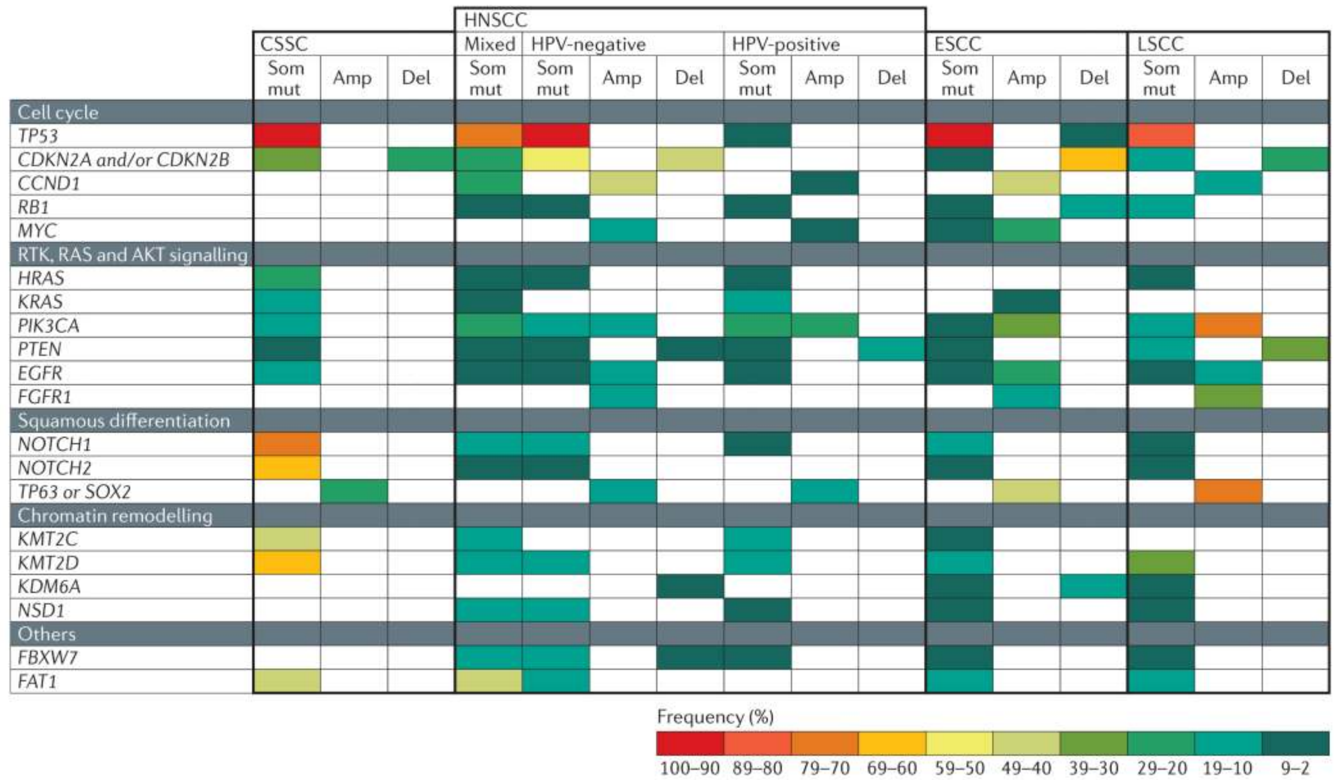

Fig 3. Common genetic alterations found in the different types of SCC.

Squamous cell carcinomas (SCCs) from different body locations (cutaneous SCC (CSCC)[52–54], oesophageal SCC (ESCC)[55–58], head and neck SCC (HNSCC)[59–64] and lung SCC (LSCC)[65–67]) have somatic mutations (Som mut), amplifications (Amp) and deletions (Del) in genes controlling the cell cycle, the receptor tyrosine kinase (RTK), RAS and AKT signalling pathways, squamous differentiation and chromatin remodelling. The colour code represents the frequency of a given alteration among patients with each disease subtype. The white fields indicate that Del, Amp or Som mut have not been described for a given gene and SCC type. *CCND1*, cyclin D1; *CDKN2*, cyclin-dependent kinase inhibitor 2; *EGFR*, epidermal growth factor receptor; *FAT1*, FAT atypical cadherin 1; *FBXW7*, F-box and WD repeat domain-containing 7; *FGFR1*, fibroblast growth factor receptor 1; HPV, human papilloma virus; *KDM6A*, lysine demethylase 6A; *KMT2C*, lysine methyltransferase 2C; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit-α; *NSD1*, nuclear receptor-binding SET domain protein 1.

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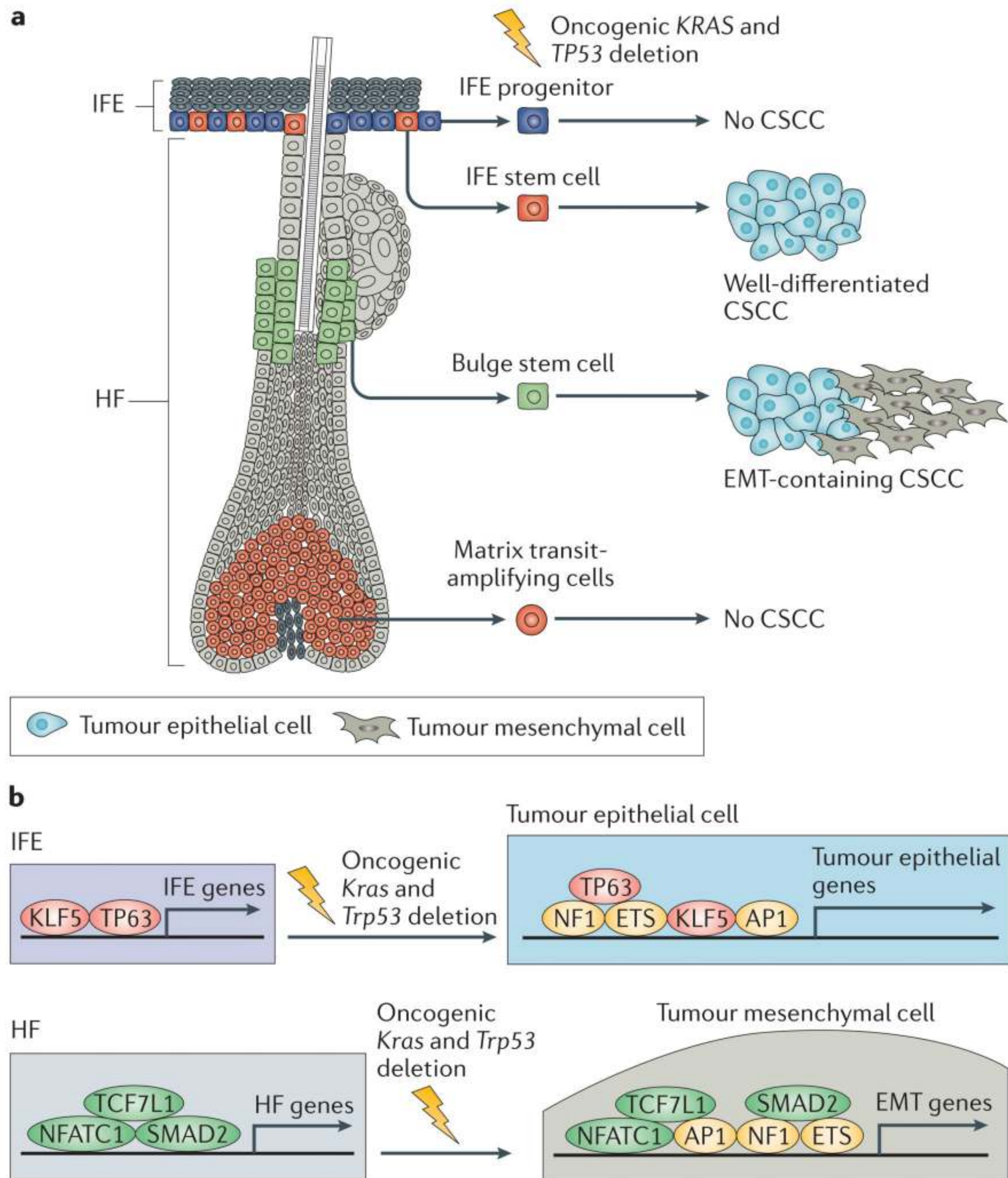

Fig 4. The cells at the origin of CSCC.

a | Activation of oncogenic *Kras* and deletion of *Trp53* in interfollicular epidermis (IFE) and hair follicle (HF) stem cells lead to cutaneous squamous cell carcinoma (CSCC) formation, whereas these gene alterations in IFE progenitors or matrix transit-amplifying cells do not lead to CSCC formation. Oncogenic activation of *Kras* and deletion of *Trp53* in IFE stem cells lead to the generation of well-differentiated CSCCs, whereas activation of the same oncogenic hits in HF stem cells leads to the generation of CSCCs with epithelial to mesenchymal transition (EMT) features. **b |** The transcriptional and epigenetic landscape of

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the cell of origin influences tumour differentiation. Upon oncogenic *Kras* expression and *Trp53* deletion, a core of transcription factors (including members of the adaptor protein 1 (AP1), E26 transformation-specific (ETS) and nuclear factor 1 (NF1) families) promote tumour gene expression independently of the cell of origin. In addition to this core of transcription factors, lineage-specific transcription factors controlled by the cells of origin of CSCCs influence the specific differentiation of the tumours. Tumour protein 63 (TP63) and Krüppel-like factor 5 (KLF5) promote the expression of IFE genes and the development of well-differentiated squamous cell carcinomas (SCCs), whereas SMAD family member 2 (SMAD2), nuclear factor of activated T cell, cytoplasmic 1 (NFATC1) and transcription factor 7-like 1 (TCF7L1) promote the expression of HF genes and the development of SCCs in which EMT occurs.

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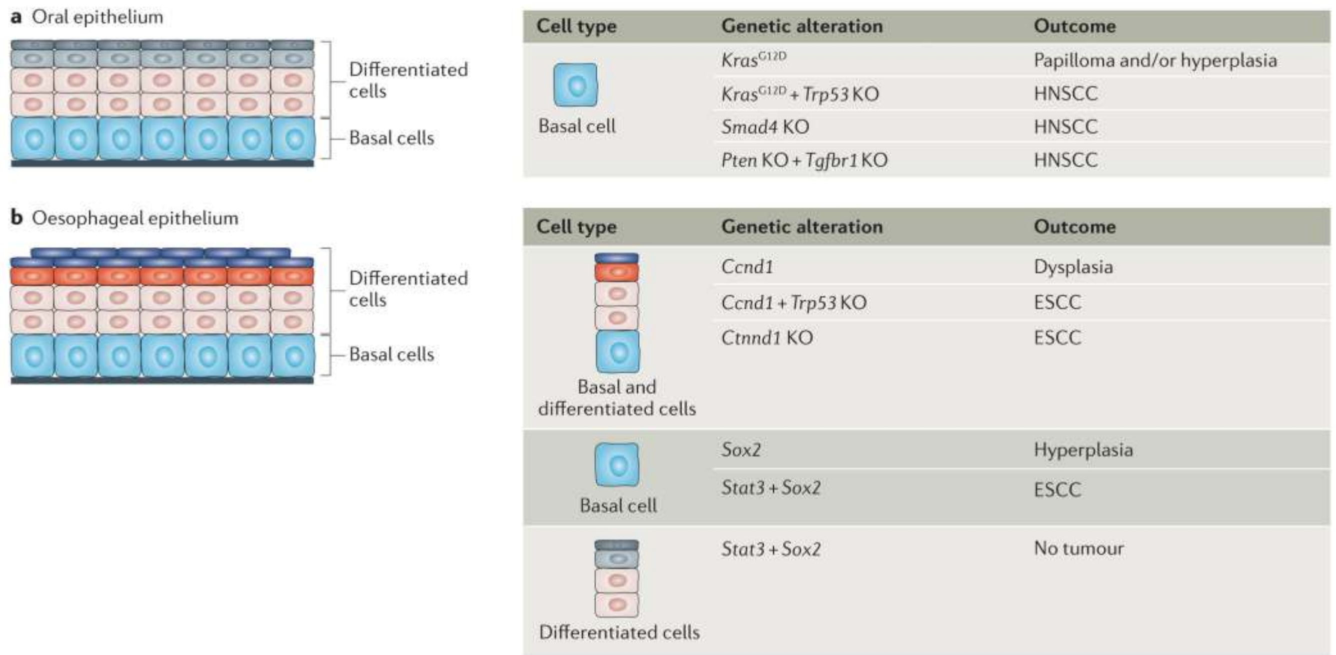

Fig 5. The cells of origin in HNSCC and ESCC.

a | The cells at the origin of head and neck squamous cell carcinoma (HNSCC) are shown. Basal cells of the oral epithelia can give rise to hyperplasia and/or papilloma formation upon oncogenic *Kras* activation. HNSCC can result from the activation of *Kras*, the combination of activation of *Kras* and deletion of *Trp53*, SMAD family member 4 (*Smad4*) deletion or double deletion of *Pten* and transforming growth factorbeta receptor type-1 (*Tgfbr1*) in basal cells. **b |** The cells at the origin of oesophageal squamous cell carcinoma (ESCC) are shown. Activation of cyclin D1 (*Ccnd1*) in combination with *Trp53* deletion and deletion of catenin delta-1 (*Ctnnd1*) in oesophageal epithelial cells leads to ESCC formation. Expression of signal transducer and activator of transcription 3 (*Stat3*) and SRY-box 2 (*Sox2*) in basal cells but not in suprabasal cells promotes ESCC. KO, knockout.

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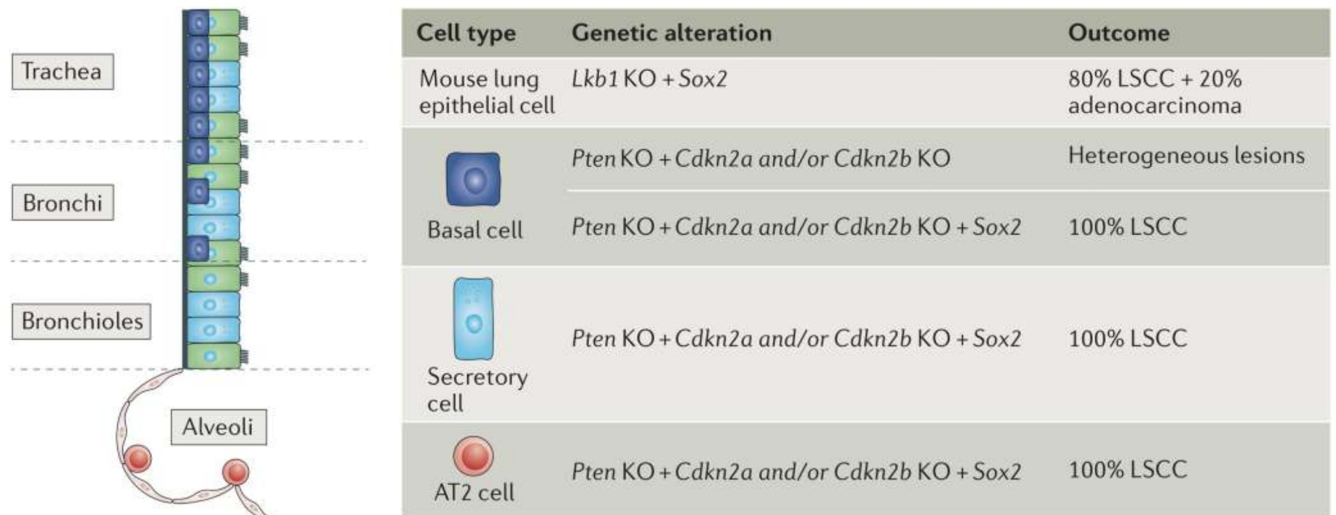

Cell type	Genetic alteration	Outcome
Mouse lung epithelial cell	*Lkb1* KO + *Sox2*	80% LSCC + 20% adenocarcinoma
Basal cell	*Pten* KO + *Cdkn2a and/or Cdkn2b* KO	Heterogeneous lesions
Basal cell	*Pten* KO + *Cdkn2a and/or Cdkn2b* KO + *Sox2*	100% LSCC
Secretory cell	*Pten* KO + *Cdkn2a and/or Cdkn2b* KO + *Sox2*	100% LSCC
AT2 cell	*Pten* KO + *Cdkn2a and/or Cdkn2b* KO + *Sox2*	100% LSCC

Fig 6. SOX2 promotes LSCC differentiation irrespective of the cell of origin.

Transcription factor SRY-box2 (SOX2) promotes squamous cell fate in lung tumours regardless of the cell of origin. *Sox2* overexpression and serine/threonine kinase 11 (*Lkb1* deletion in cells of the lung epithelium lead to lung squamous cell carcinoma (LSCC) and adenocarcinoma. In the absence of *Sox2* overexpression, *Pten* and cyclin-dependent kinase inhibitor 2A (*Cdkn2a*) and/or *Cdkn2b* deletion in basal cells leads to heterogeneous lesions including adenocarcinoma and LSCCs. *Sox2* overexpression and *Pten* and *Cdkn2a* and/or *Cdkn2b* deletion in basal, secretory or type 2 (AT2) cells lead to the formation of LSCCs. KO, knockout.