Tracing the cellular origin of cancer

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Although many genes that lead to different types of cancer when mutated have been identified, the cells that initiate tumour formation following accumulation of these mutations have, until recently, remained elusive. This review explores how mouse genetic lineage-tracing experiments that allow the expression of oncogenes and/or the deletion of tumour suppressor genes in defined cell lineages have been instrumental in defining the cellular origin of different solid tumours in mouse models for various human cancers.

Cancer is usually viewed as an evolutionary process that results from the accumulation of somatic mutations in the progeny of a normal cell, leading to a selective growth advantage in the mutated cells and ultimately to uncontrolled proliferation[1,2]. The most frequent human cancers arise in epithelial tissues such as the skin, colon, breast, prostate or lung, and collectively lead to several million deaths per year[3].

Cancer research has in recent decades characterized the cellular and molecular events that enable the malignant transformation of cells harbouring oncogenic alterations. These events include: uncontrolled proliferation; evasion of tumour suppression; inhibition of cell death; creation of a particular microenvironment containing blood vessels, stromal and immune cells; and the acquisition of invasive and metastatic potential[4]. Furthermore, our knowledge of oncogenes and tumour suppressor genes has been enriched by the development of next-generation sequencing techniques, which have identified many genes that are mutated in different types of cancer[5]. Although this information is critical in understanding the genetic basis of cancer development and progression, the order of acquisition of these mutations and the cells in which they accumulate to allow cancer formation remain poorly understood.

The tissues from which most cancers arise are maintained by multipotent and/or unipotent stem cells and their progeny, the committed progenitors or transit amplifying cells that ultimately give rise to the non-dividing terminally differentiated cells that accomplish the various tissue functions[6]. Genetic lineage-tracing experiments in mice allowing the conditional expression of a reporter gene (β-galactosidase or a fluorescent protein) in a particular cell lineage and all its future progeny have greatly contributed to our understanding of the cellular hierarchy of normal tissues under physiological conditions[6]. By conditionally expressing oncogenes or deleting tumour suppressor genes through the targeted activation of Cre recombinase expression in different epithelial cell populations, including tissue-specific stem cells, it is now possible to determine with great precision the cellular origin of solid tumours in mice (Fig. 1).

In this Review, I discuss how lineage-tracing approaches have allowed the identification of the cellular origin of solid tumours in mouse models for the most frequent human cancers, including those of the skin, brain, gut, lung, prostate and breast.

Distinct cells of origin in skin basal and squamous cell carcinoma

The skin epidermis is composed of the interfollicular epidermis (IFE), which forms the skin barrier and the different epidermal appendages including the hair follicles, sebaceous glands and sweat glands[6,7]. Lineage-tracing experiments reveal that during homeostasis, the different compartments of the epidermis are self-sustained by their own resident stem cells (Fig. 2). Hair follicle bulge stem cells expressing the typical markers keratin 15 (K15), CD34 and Lgr5 (leucine-rich repeat-containing G-protein-coupled receptor 5) mediate hair follicle regeneration[8–12]; $Lgr6^+$ isthmus stem cells mediate turnover of the sebaceous gland cells and possibly of other epidermal lineages[13,14]; and IFE stem and progenitor cells expressing keratin 14 (K14) sustain IFE homeostasis and maintenance of the skin barrier[15–17]. Although under physiological conditions bulge stem cells do not contribute to the maintenance of the IFE (refs 10,18), almost all stem cell populations of the epidermis, including the hair follicle bulge[18,19], $Lgr6^+$ isthmus[13] and IFE (ref. 17) stem cells, can contribute to healing of the IFE after wounding. During morphogenesis, epidermal progenitors also give rise to sweat gland progenitors[20] and merkel cells[21,22], the neuroendocrine cells that mediate fine touch sensation.

The two most frequent human skin cancer subtypes are basal cell carcinoma (BCC), which resembles the hair follicle, and squamous cell carcinoma (SCC), which presents features of squamous differentiation called keratin pearls (which are reminiscent of the differentiation features that occur in the IFE)[23,24].

BCC is the most frequent tumour in humans, with more than a million new cases annually worldwide[25]. These slow-growing tumours, which in certain cases can be highly invasive and lead to morbidity, arise from mutations that constitutively activate the Hedgehog (HH) signalling

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pathway[26]. BCC was thought to derive from hair follicle stem cells due to its histological and biochemical similarities to hair follicles[23,24]. Constitutive activation of the HH pathway in the mouse epidermis by loss of function of the Patched tumour suppressor — achieved through overexpressing the Sonic hedgehog homolog (SHH) ligand or through activating mutations in the Smoothened receptor (such as the constitutively active SmoM2 mutant), or by activating mutations in the downstream transcription factors Gli1 and Gli2 — leads to the formation of tumours that closely resemble human BCC (refs 27–30).

The cellular origin of SmoM2-induced BCC in mice was identified by expressing a conditional allele of *SmoM2* (which is known to induce BCC-like tumours when expressed in the adult epidermis[31]) in different epidermal cell lineages[11]. Surprisingly, under physiological conditions, SmoM2 expression in hair follicle stem cells and their progeny induced hyperplastic and dysplastic lesions that did not progress to invasive BCC. Clonal analysis of SmoM2-targeted cells revealed that more than 90% of superficial BCCs arise from long-lived stem and/or progenitor cells residing in the IFE, whereas the remaining tumours arise from the upper infundibulum[11] (Fig. 2a). Transcriptional profiling of adult IFE cells following SmoM2 expression revealed that tumour-initiating cells are reprogrammed into an embryonic hair follicle progenitor-like-fate before progressing into invasive BCC (ref. 32). The gradual expression of hair follicle markers by oncogene-targeted IFE cells that progress to BCC demonstrated that follicular marker expression could be misleading in extrapolating the cellular origin of BCCs.

The cellular origin of Patched-induced BCC in the mouse epidermis remains unclear. The increased tumour formation following ultraviolet radiation (UV)-induced Patched loss of heterozygosity during the active stage of the hair follicle cycle implicated hair follicle stem cells in the origin of these tumours[33]. However, retinoic acid treatment of K6CRE–Patched1 (Ptch1)-floxed mice, which induces Cre expression and *Ptch1* deletion specifically in the IFE and infundibulum, results in BCC development — demonstrating that IFE cells can also initiate BCC upon Patched loss of function[34]. Consistent with this finding, *Ptch1* deletion in hairless paw epidermis also induced BCC formation[32]. Lineage tracing of bulge stem cells in K15CREPR (K15CRE fused to the progesterone receptor)–Rosa–LacZ mice heterozygous for *Ptch1* before administering ionizing radiation (which induced BCC formation through the loss of the remaining *Ptch1* allele) led to BCCs that were β-galactosidase-positive, suggesting that BCC arises from hair follicle bulge stem cells in this mouse model[35]. However, the apparent bulge stem cell origin of BCC in this study may also be explained by the leaky expression of K15CREPR in the absence of the Cre-activating drug[36], the K15CREPR activity in some cells outside the hair follicle lineage[9,36], or the early upregulation of K15 expression in IFE following HH activation[11]. Furthermore, the higher resistance of bulge stem cells to ionizing-radiation-induced cell death and their rapid repair of DNA damage through error-prone non-homologous end joining[37] may favour the loss of Patched heterozygosity more frequently in bulge stem cells than in IFE cells.

Another study confirmed that oncogenic SmoM2 expression in hair follicle bulge cells does not induce BCC formation during tissue homeostasis[38]. However, following wounding, SmoM2-expressing bulge stem cells that migrated to the IFE were able to initiate BCC formation at this location[38]. Likewise, whereas homozygous Patched loss of function in bulge stem cells can induce BCC initiation at low frequency, the number of BCCs was increased when these cells were stimulated to migrate to the IFE by wounding[39]. These experiments suggest that either the microenvironment of the IFE is crucial for BCC initiation, or that following migration into the IFE, bulge stem cells adopt an IFE-like fate[40] which may be required to initiate tumour formation following oncogenic HH signalling. Combined deletion of *Ptch1* and the tumour suppressor *p53* using K14CREER (K14CRE fused to the oestrogen receptor), which is expressed at a higher level in IFE cells (leading to their preferential targeting following Cre-mediated recombination[11]), induced BCC formation much more efficiently than *Ptch1* and *p53* deletion in bulge stem cells[35]. This reinforces the notion that the IFE is the most sensitive compartment to BCC initiation following *Patched* deletion. Overexpression of a constitutively active version of Gli2, which bypasses the normal transduction of HH oncogenic signals, leads to BCC formation in all epidermal lineages including the progeny of bulge stem cells[41]. This suggests that the mechanisms dictating the competence of epidermal cells to initiate BCC formation depend on their ability to transduce the constitutive HH signal.

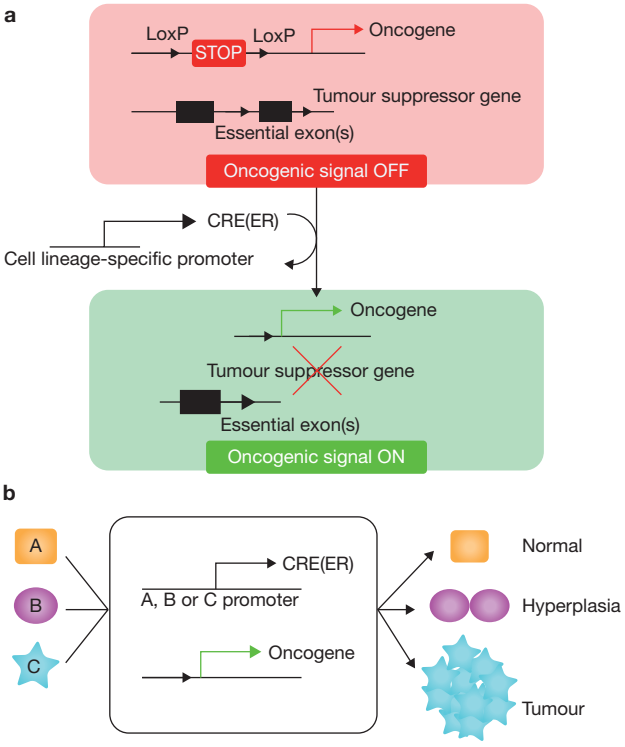
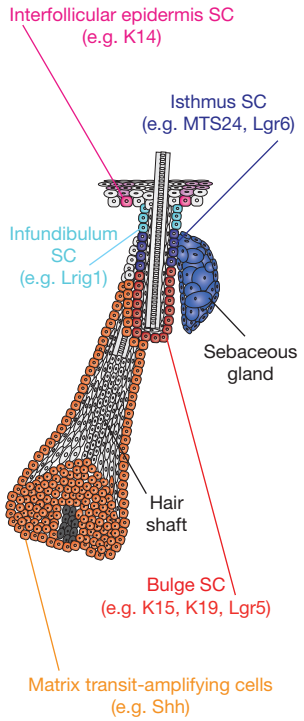


Figure 1 Genetic approach to define the cellular origin of epithelial cancers. (**a**) Mice are generated to co-express a conditional oncogene or tumour supressor gene and CRE recombinase, which expressed under the control of a promoter active specifically in one of the different cell lineages of a tissue. After administration of a drug that stimulates nuclear translocation of CRE (such as tamoxifen (TAM) in the case of CREER), or spontaneously in the case of a non-inducible CRE, the CRE recombinase is able to excise the stop cassette preceding the oncogene or essential exons in the tumour suppressor gene. This leads to the activation of the oncogenic program in one of the cell lineages of a tissue. (**b**) The oncogene is expressed specifically in only one cell lineage in any given tissue (A, B and C represent cell lines). In this example, oncogene expression will lead to tumour formation only in type C cells.

SCC is the second most frequent skin cancer, with more than 500,000 patients per year worldwide[42], and is also the predominant cancer in locations such as the oral cavity, head, neck and oesophagus[3]. The most



b

		Cells of origin				
	Mutation	IFE	Infundibulum	Isthmus	Bulge	Matrix
Basal cell carcinoma (SmoM2 β_4 integrin)	SmoM2 gain of function	Tumour ++	Tumour ++		Tumour ++ from the IFE following wounding	No tumour
	Patched loss of function	Tumour ++	Tumour ++		•Tumour (+) •Tumour ++ from the IFE following wounding	
	ΔN-Gli2 overexpression	Tumour ++	Tumour ++	Tumour ++	Tumour ++ (progenitors)	
Papilloma (K1 β_4 integrin)	DMBA-TPA induced papilloma				Tumour	
	$KRas^{G12D}$ induced papilloma	Tumour ++ (progenitors)			Tumour ++	No tumour
Squamous cell carcinoma (K14 β_4 integrin)	$KRas^{G12D}$/p53 cKO induced SCC				Tumour ++	No tumour

Figure 2 Multiple cells of origin in epithelial skin cancers. (**a**) Schematic representation of the different epidermal stem cells and their lineages. Typical molecular markers of each cell type are indicated. SC, stem cell. (**b**) Cells of origin in: basal cell carcinomas (top); mouse papilloma following DMBA/TPA treatment and oncogenic KRas expression (middle); and mouse squamous cell carcinoma following oncogenic KRas expression and *p53* deletion. The top image illustrates the IFE (interfollicular epidermis) origin of SmoM2-induced basal cell carcinoma in mouse-tail epidermis (adapted from Youssef, K. K. *et al.*[11]). The middle image illustrates a papilloma arising after $KRas^{G12D}$ expression in bulge stem cells (adapted from Lapouge, G. *et al.*[36]). The bottom image illustrates a squamous cell carcinoma arising following $KRas^{G12D}$ expression in bulge stem cells (adapted from Lapouge, G. *et al.*[36]).

extensively used mouse model for skin SCC is a multistage, chemically induced carcinogenesis approach[43,44], in which mice are treated first with the mutagen 9,10-dimethyl-1,2-benzanthracene (DMBA), and then with a drug that stimulates epidermal proliferation, such as 12-*O*-tetradecanoyl phorbol-13-acetate (TPA). During TPA treatment, benign tumours (papillomas) arise — probably owing to additional mutations — and some of these progress into invasive SCC. This model selects cells with activating mutations in the Ras pathway[24]. Although the most common papilloma mutations occur in the HRas gene[45], KRas mutations have also been reported in mouse and human skin SCCs (refs 46–48). SCCs often display signs of squamous differentiation, suggesting that they may originate from cells that naturally undergo this process, such as IFE cells[23]. TPA treatment even a year after the last DMBA administration can still stimulate papilloma formation, suggesting that the initial mutation arises in long-lived stem cells[49–51]. The decreased incidence, but not abrogation, of tumour formation when the IFE is removed by dermabrasion and repaired by hair follicle cells after DMBA treatment suggests that tumour-initiating cells may arise from the IFE as well as from hair follicle cells[52]. Oncogenic mutations have also been suggested to occur in differentiated cells of the epidermis[53]. For example, active MEK expression in suprabasal terminally differentiated cells enhances papilloma formation following wounding[54]. However, tumour initiation by differentiated cells has been shown to depend on a non-cellular autonomous mechanism involving inflammation[55]. Furthermore, transgenic mice expressing a mutated form of HRas in IFE suprabasal cells under the promoter of *K10*, a gene expressed in differentiated epidermal cells, develop papillomas at sites of wounding[56], suggesting that wounding may also stimulate skin squamous tumorigenesis. Transgenic mice overexpressing oncogenic HRas under the control of a truncated form of the K5 promoter, which is expressed in all epidermal progenitors during development but restricted to hair follicle lineages in adult mice, develop papillomas and SCC (ref. 57). These studies implicated different cell types, including hair follicle cells and differentiated IFE cells, in squamous skin tumour formation in oncogene-overexpressing transgenic mice and/or after wounding. However, the cells at the origin of squamous skin tumours following oncogene expression at a physiological level in adult epidermis remained unclear. Using a knock-in $KRas^{G12D}$ mutant[58], two independent groups demonstrated that bulge stem cells — but not their transient amplifying matrix cell progeny — can initiate papilloma formation on oncogenic KRas expression[36,59] (Fig. 2b). IFE progenitors were also able to form papillomas following $KRas^{G12D}$ expression[36]. Using K15CREPR–Rosa-LacZ lineage tracing, the majority of papillomas following DBMA and TPA treatment were shown to arise from K15-derived cells[60]. These studies show that the expression of differentiation markers can also be misleading in extrapolating the cellular origin of papillomas[36,59,60].

None of the papillomas arising from oncogenic $KRas^{G12D}$ expression alone progress into invasive SCC (refs 36,59). In contrast, combined

p53 deletion and oncogenic KRas expression in bulge stem cells initiate invasive and aggressive SCC (refs 36,59) (Fig. 2c). Only a small fraction of the oncogene-targeted cells form tumours, suggesting that other genetic and/or epigenetic hits are required to initiate tumorigenesis in this oncogenic context[36,59]. Although p53 loss of function has been associated with SCC initiation[36,59] and progression[61], a stabilizing p53 mutation (R172H) also increased tumour initiation and progression[62]. More studies are needed to better define the cells able to initiate SCC, and to identify the molecular mechanisms controlling the renewal and malignant progression of squamous skin tumours.

Medulloblastomas originate from different progenitors

Medullobastomas form in the cerebellum and are the most common brain tumours in childhood[63]. Medulloblastoma is a heterogeneous disease that can be classified into distinct molecular and histological subgroups that present different clinical prognoses[63]. Recent studies have demonstrated that the different medulloblastoma subtypes arise from different types of progenitors and from the activation of distinct oncogenic pathways[64–69].

30% of medulloblastoma cases represent a subtype marked by a HH expression signature, which presents a good-to-intermediate prognosis and arises from mutations that constitutively activate HH signalling, such as Smoothened gain of function, and Patched or Sufu loss of function[63]. Expression of SmoM2 or deletion of *Ptch1* in brain stem cells (using GFAP–Cre) or cerebral granule neuronal precursors (CGNPs) (using Olig2, Math1, Tlx3 or Gli1–Cre) induced a similar histological and molecular type of medulloblastoma[64,65], although with an accelerated appearance when HH was activated in stem cells[64]. Interestingly, the tumorigenic effect of constitutive HH activation is only manifested following the commitment of multipotent stem cells to granular neurons, as no tumours arise from the constitutive HH signalling in astrocytes, oligodendrocytes or non-granule neurons that derived from stem cells expressing GFAP (refs 64,65).

The second group (10% of cases) exhibits Wnt pathway activation, presents an excellent prognosis and arises from constitutive activation of β-catenin[66], although novel mutated genes were identified recently[70–72]. Analyses of the Wnt and HH signatures revealed their expression in distinct anatomical regions of the developing mouse brain[66], with HH genes preferentially expressed in the developing cerebellum, and Wnt genes concentrated in the lower rhombic lip of the brainstem. In line with this, the human HH and Wnt medulloblastoma subtypes are located in the cerebellum hemisphere and infiltrating the brain stem, respectively[66]. Conditional expression of a constitutively active form of β-catenin in CGNPs did not impair their proliferation and/or differentiation and did not induce medulloblastoma formation, whereas active β-catenin induced hyperproliferation when expressed in dorsal brain stem progenitors and resulted in medulloblastoma when *p53* was also deleted[66].

The third group (25% of cases) presents a Myc signature and has a poor prognosis[63]. Enforced c-Myc expression in p53-deficient CGNPs transplanted in the cerebellar cortex of imunodeficient mice gives rise to medulloblastomas that molecularly resemble the Myc subgroup[67,68], suggesting that *p53* deletion and c-Myc expression in CGNPs may induce a more profound dedifferentiation of these cells, leading to the loss of neuronal lineage markers[67,68]. These data also indicate that although both the HH and Myc groups arise from CGNPs, different

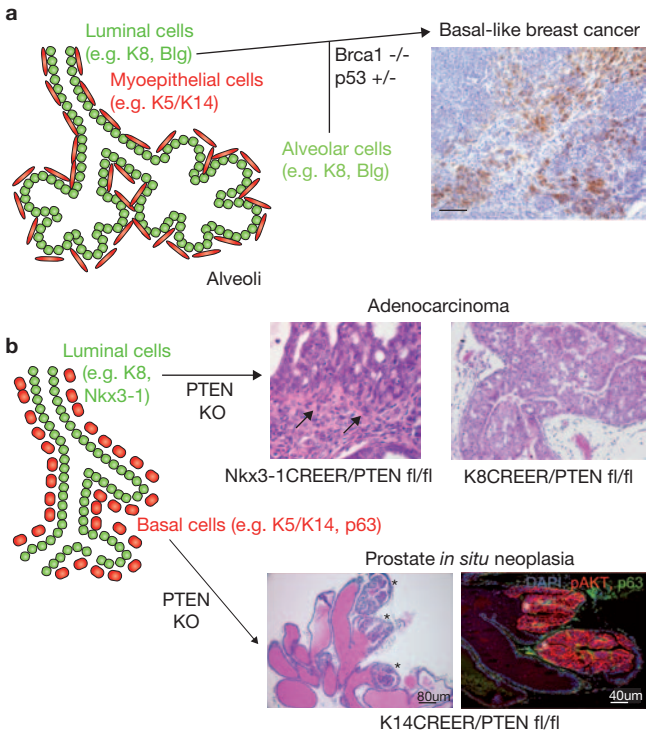


Figure 3 Cells of origin in basal-like breast and prostate cancers. (**a**) Schematic representation of the mammary gland and its lineages. Typical molecular markers of each cell type are indicated. Luminal progenitors induce basal-like breast cancer following *Brca1* and *p53* deletion (adapted from Molyneux, G. *et al.*[82] with permission from Elsevier). (**b**) Schematic representation of the prostate and its lineages. *PTEN* deletion (KO, knockout; fl, floxed) in Nkx3-1-expressing (adapted from Wang, X. *et al.*[93]) or K8-expressing luminal progenitors (adapted from Choi, N. *et al.*[95] with permission from Elsevier) induces prostate *in situ* neoplasia formation within 3 months, whereas *PTEN* deletion in K14$^+$ basal progenitors induces lesions that progress more slowly through a step involving luminal progenitor expansion.

oncogenic stimuli in these cells induce distinct medulloblastoma classes. Interestingly, overexpression of activated N-Myc in cerebellar and brain stem neuronal stem cells induces medulloblastoma, whereas the same oncogene expressed in forebrain stem cells leads to glioma[69].

The last medulloblastoma subtype presents a classic histology similar to the Wnt group, but has a worse prognosis[63]. Genomic analyses demonstrated that this subgroup harbours many recurrent genomic rearrangements and mutations in histone modifiers[70–72]. Future studies are needed to define the cellular origin of this medulloblastoma group.

Luminal origin of basal-like breast and prostate cancers

Breast cancer, the most frequent tumour type in women, is a heterogeneous disease that is classified into different immunohistological and molecular subclasses presenting distinct clinical prognoses[73]. One of the unresolved questions in breast cancer is whether the observed heterogeneity is related to the cellular origin of its distinct subtypes. Although histology and gene expression profiling are extremely informative for disease classification and prognosis, such analyses do not identify the mammary cell lineages responsible for tumour development following oncogenic mutations.

The mammary gland is composed of basal and luminal cells including the ductal and the alveolar cells[6,74]. A population of basal cells

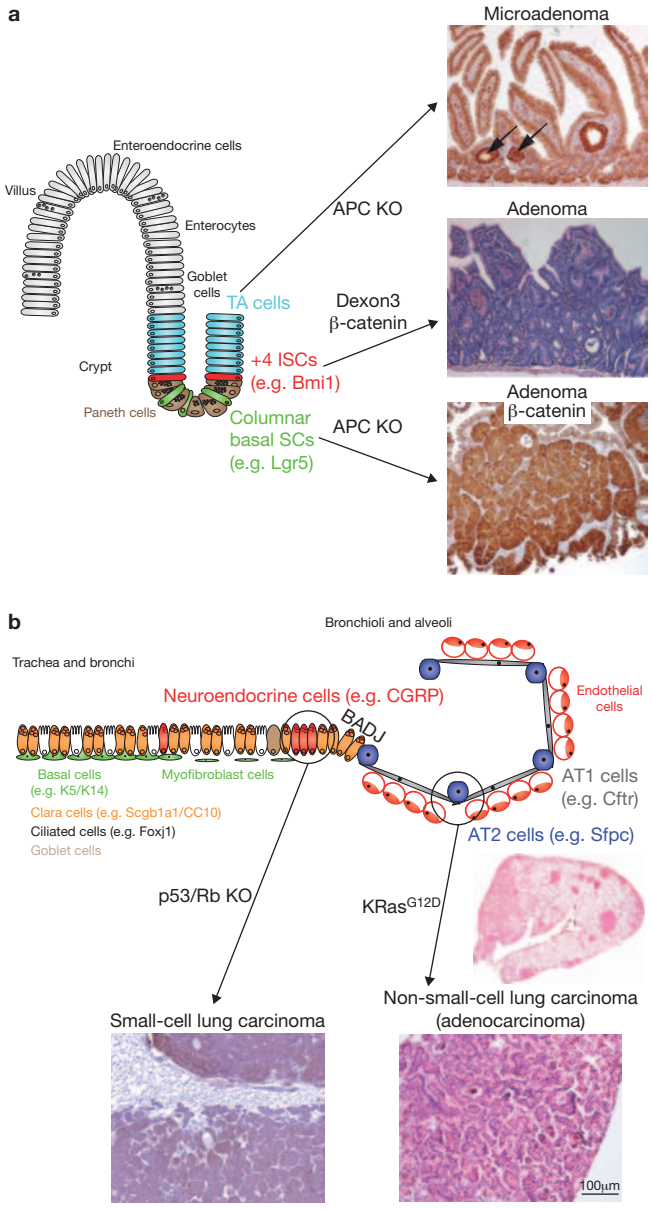


Figure 4 Cells of origin in intestinal and lung cancers. (**a**) Schematic representation of the intestinal stem cells, transit amplifying (TA) cells and the different intestinal lineages. Constitutive activation of Wnt/β-catenin signalling in intestinal basal columnar stem cells through *APC* deletion (adapted from Barker, N. *et al.*[112]) or in +4 ISCs (intestinal stem cells at position +4 from the crypt base) through stabilized β-catenin expression (adapted from Sangiorgi, E. *et al.*[103]) leads to intestinal adenoma within a month, whereas *APC* deletion in TA cells only induces microadenoma in the same time frame. (**b**) Schematic representation of different cell lineages in the lung. Neuroendocrine cells are the most sensitive cell lineage to initiate SCLC upon *p53* and *Rb* deletion (adapted from Sutherland, K. D. *et al.*[120] with permission from Elsevier). AT2 cells initiate adenocarcinoma upon $KRas^{G12D}$ expression (adapted from Xu, X. *et al.*[127] with permission from PNAS).

expressing α_6 and β_1 intergrin, CD24 and K14/K5 was shown to be highly enriched for mammary repopulation activity following transplantation into cleared mammary fat pads[75,76], suggesting that the mammary cell expansion during puberty, pregnancy and lactation are sustained by multipotent mammary stem cells[74]. Lineage-tracing experiments using a large panel of different inducible CREER proteins expressed in the basal (K5CREER, K14rtTA/TetOCRE and Lgr5CREER) and luminal (K8CREER and K18CREER) cells demonstrated that during embryonic development, multipotent $K14^+$ progenitors give rise to all the different epithelial lineages of the mammary tissue[77]. However, the epithelial expansion occurring during puberty and the multiple cycles of pregnancy, lactation and involution is sustained by unipotent basal and luminal stem cells rather than by multipotent stem cells[77]. Transplantation of basal cells (which are unipotent *in vivo*, according to lineage-tracing experiments) into the mammary fat pad induced the regeneration of functional mammary gland containing basal and luminal lineages, demonstrating that the transplantation experiments can expand the normally restricted differentiation potential of basal stem cells[77]. Recent studies, using Axin-2CREER and Lgr5CREER, two Wnt target genes, have demonstrated that these preferentially mark luminal progenitors during the early stage of mammary gland development and myoepithelial stem cells during puberty — probably reflecting a change in the localization of Wnt signalling during mammary gland development[78,79].

The heterogeneity of breast cancers has been proposed to relate to their cellular origin, with basal-like tumours originating from basal stem cells and luminal ones from luminal progenitors. Women with *Brca1* mutations preferentially develop basal-like breast cancer[80]. However, conditional deletion of *Brca1* and *p53* in the basal and luminal cells using K14CRE mice, which targets multipotent embryonic progenitors and all future adult mammary epithelium, induced mainly basal-like adenocarcinomas, but also other types of tumors such as adenomyoepithelioma[81]. To assess the cellular origin of Brca1/p53 basal-like breast cancer, the latency and the type of tumours arising in $Brca1^{fl/fl}$ $p53^{fl/+}$ mice were compared using two distinct CRE transgenes: the BlgCRE that is preferentially active in luminal progenitors[82] and the K14CRE that targets all mammary epithelium[77,81]. They found that *Brca1/p53* deletion using the BlgCRE induced basal-like breast cancers (Fig. 3a), demonstrating that luminal progenitors can initiate this type of breast cancer[82]. Consistent with this, it was found that mutant *Brca1* carriers present an expansion of the luminal progenitors that are more clonogenic *in vitro*,and that human basal-like breast tumours present a molecular signature that is more similar to the luminal progenitor signature than that of mature luminal cells and the myoepithelial lineage, including basal stem cells[83]. Moreover, luminal progenitors from *Brca1* mutation carriers express higher levels of basal markers, and several oncogenes expressed in luminal progenitors induced tumours with the same histology as unfractionated breast epithelium[84], supporting the luminal origin of Brca1-derived tumours.

The demonstration that luminal progenitors can be the cells at the origin of basal-like breast cancers[82,83] reinforces the notion that tumour differentiation cannot be used to deduce the cellular origin of a cancer[11,36]. Surprisingly, when *p53* and *Brca1* are deleted in the embryonic mammary gland using K14CRE, which induces *p53* and *Brca1* deletion in all mammary epithelial cells including basal and luminal cells, they only resulted in the formation of adenomyoepithelioma, in which *Brca1* deletion could not be formally demonstrated[82]. The absence of basal-like tumours in this model could be related either to the temporal or chimeric expression of the K14CRE used in this study leading to partial allele recombination, the genetic background of the mice or other mechanisms. New CREER mice, allowing more specific targeting of

basal and luminal stem cells at different stages of their ontogeny[77], will be instrumental in elucidating the cellular origin of the different breast cancers, including the very common ER$^+$ luminal subtype.

Increased susceptibility of luminal progenitors during prostate cancer initiation

Prostate cancer is the second leading cause of death in men[3,85]. Different genetic abnormalities have been linked to human prostate cancers including *Pten* and/or *p53* deletion, downregulation of Nkx3.1 and genetic translocations such as TMPRSS2-ERG (ref. 85). The prostate epithelium is composed of three different cell lineages including basal, luminal and neuroendocrine cells[85]. Different theories have been proposed to account for the maintenance of the prostate epithelium during homeostasis and following androgen-mediated prostate regeneration after castration[85]. Transplantation of murine and human basal, but not luminal, prostate cells can regenerate a normal prostate containing all epithelial lineages after transplantation into immunodeficient mice[86–92], suggesting that the prostate is maintained by multipotent basal progenitors. However, mouse lineage-tracing approaches suggest that androgen-induced prostate regeneration following castration is mediated by basal and luminal unipotent progenitors rather than multipotent stem cells[93–95], although luminal progenitors may also contain rare multipotent cells[93].

Evidence exists for the involvement of both basal and luminal lineages in prostate tumour initiation. Transplantation of basal cells harbouring oncogenic mutations can induce prostate cancer formation, suggesting that mouse and human prostate cancers may originate from basal cells[87,96,97]. However, conditional deletion of *pten* using mice expressing Cre under the control of probasin or PSA promoter (which are preferentially, but not exclusively, expressed by luminal cells) also induces prostate cancers[98–100], suggesting that luminal cells can initiate prostate cancer on *pten* deletion. Supporting the notion that luminal cells are competent to initiate prostate cancer, *pten* deletion in castration-resistant luminal cells expressing Nkx3.1 also induces prostate cancer formation[93] (Fig. 3b). Recently, Choi *et al.* compared the frequency and timing of prostate tumour initiation following *pten* deletion in basal and luminal progenitors[95]. Deletion of *pten* in luminal progenitors using K8CREER induces tissue hyperplasia and tumour formation in all mice within approximately 2 months. In contrast, deletion of *pten* in basal cells using K14CREER induces tissue hyperplasia only in a few mice and with a much greater latency, with some lesions progressing to early cancer 6-8 months after *pten* deletion. Conditional deletion of *p53* together with *pten* did not accelerate tumour initiation after recombination in basal cells (Fig. 3b), suggesting that basal cells are intrinsically more resistant to oncogenic transformation compared with luminal cells[95]. Interestingly, *pten* deletion increased luminal differentiation of basal cells, which always preceded tumour progression, suggesting that some basal cells may contribute to prostate cancer initiation, but that this must be preceded by a transition to a luminal fate[95].

Stem cell origin of intestinal tumours

The gut is subdivided into the small intestine and the colon, both presenting a similar proliferative unit called the crypt, but with distinct composition and relative abundance of differentiated cells[101]. Both the colon and the small intestine contain Lgr5$^+$ multipotent stem cells. These are located at the bottom of the crypts (positions 1–3 from the crypt base) and give rise to transit amplifying (located higher up along the crypt) and differentiated cells facing the lumen[102]. In the intestine, there is also another stem cell population expressing higher levels of Bmi1 (ref. 103), mTERT (ref. 104) or Hopx (ref. 105), and located around position 4. These cells seem to be in equilibrium with Lgr5$^+$ cells[105] and able to replace them upon lineage ablation[106]. It is still unclear whether these cells represent a truly distinct intestinal stem cell population or a subpopulation of Lgr5$^+$ stem cells[107,108].

Cancer of the digestive tract is very common in humans and occurs preferentially in the colon and the rectum, but surprisingly very rarely in the small intestine — despite its higher cellular turnover. Colorectal cancers arise from the accumulation of mutations in different oncogenes such as KRas and β-catenin, and/or tumour suppressor genes such as *p53* and *Apc* (adenomatous polyposis coli)[109]. Constitutive activation of the Wnt pathway through *Apc* deletion or stabilized β-catenin expression are among the most frequently found mutations in colorectal carcinoma[110,111] and represent the most common intestinal tumour mouse model, which results in the formation of benign tumours (adenoma) of the small intestine. Wnt activation in intestinal stem cells following *Apc* deletion using Lgr5- or prominin-CREER results in the formation of adenomas in a fraction of oncogene-targeted cells, whereas *Apc* deletion in transit amplifying (TA) cells and more differentiated intestinal cells only rarely induces the formation of slow growing benign intestinal tumours[112,113] (Fig. 4a). Similarly, activation of the Wnt/β-catenin pathway in Bmi1-expressing cells also induces adenoma formation[103] (Fig. 4a). Although these studies show that pathological Wnt/β-catenin activation in intestinal stem cells can lead to adenoma, further work is required to precisely determine the relative tumour-initiating potential of the different intestinal stem cells following Wnt or Ras activation, whether additional mutations expand the repertoire of intestinal tumour-initiating cells, and which cell lineages initiate colorectal cancer.

Distinct cellular origin of small- and non-small-cell lung carcinoma

The airways system is divided into the trachea, bronchi and bronchioles, which branch into and end in the alveoli, the site of gas exchange[114]. Different stem cell populations are responsible for the maintenance and repair of airway epithelia[6,114]. The trachea and bronchi are maintained and repaired by multipotent basal stem cells that can differentiate into all lineages including transit amplifying luminal progenitor and postmitotic basal and luminal cells[115]. However, in the brochioli, Clara cells, which are labelled by Scgb1a1/CC10-CREER, constitute a self-sustained population that contains long-term stem cells[116]. Rare neuroendocrine cells are also scattered in the trachea and the bronchioli[114]. The alveoli are composed by alveolar type I (AT1) and type II (AT2) cells, which are squamous cells covering the alveoli and secretory cells secreting surfactant protein C (SPC), respectively. However, the precise mechanisms maintaining and repairing alveoli are less well understood[6,114].

Lung cancers are divided into two main groups: the small-cell lung cancers (SCLCs), composed of cells expressing neuroendocrine markers; and the non-small-cell lung cancers (NSCLCs), which account for 80% of all lung cancers and can be further subdivided into adenocarcinoma, squamous cell, bronchioalveolar and large-cell carcinomas[117].

As SCLCs express neuroendocrine markers, they are thought to arise from neuroendocrine cells or neuroendocrine progenitors. Mutations of *p53* and the retinoblastoma protein (*Rb*) tumour suppressor genes

have been found in the majority of human SCLCs (ref. 118) and their inactivation in mice recapitulates the key features of human SCLC (ref. 119). CRE expression under the control of promoters targeting Clara, neuroendocrine and AT2 cells (CC10, CGRP and SPC, respectively) in mice harbouring conditional alleles of *p53* and *Rb1* demonstrated that neuroendocrine cells are the cell lineage most likely to initiate SCLC, with a subset of AT2 cells also giving rise to SCLC and Clara cells being apparently unable to initiate SCLC at all (ref. 120) (Fig. 4b). Another report using a similar strategy showed that deletion of *p53* and *Rb1* in Clara or AT2 cells does not lead to SCLC, and that SCLC probably arises from neuroendocrine cells[121]. These two studies are consistent with neuroendocrine cells being the most frequent cell lineage at the origin of SCLC, with apparent contradictory findings concerning the contribution of AT2 cells during tumour initiation likely to be explained by the strategies used to express the different CRE transgenes (adenovirus versus transgenic mice), the fidelity of the promoters used to express CRE into a particular cell lineage, the heterogeneity within the AT2 cell population, or the genetic background of the mice.

Oncogenic mutations of the KRas gene are found in 25–50% of human adenocarcinomas[122]. Introduction of constitutively active $KRas^{G12D}$ in mice using intranasal infection led to lung adenocarcinomas[123], with analysis of tumorigenic lesions indicating that KRas adenomas comprise a population of cells co-expressing Clara cell (Scgb1a1) and AT2 cell (SPC) markers[123]. In normal lungs, a population of cells with stem cell properties located at the bronchioalveolar junction (BASC) also expresses these two markers[124]. Stimulating proliferation of BASC with naphtalene treatment resulted in increased tumour formation[124], suggesting that BASCs could be the cells at the origin of adenocarcinoma. Constitutive expression of oncogenic KRas in cells expressing CC10 (CC10-CRE) or overexpression HRas in neuroendocrine cells also led to adenocarcinoma[125,126]. However, a recent study compared the oncogenic potential of $KRas^{G12D}$ in different airway cell populations using two different inducible CREER targeting AT2 and the majority of BASCs (Sftp2-CREER) or Clara cells and some AT2 cells (CC10-CREER). It was found that BASCs do indeed proliferate in response to oncogenic KRas and form hyperplasias that do not progress into malignant adenocarcinomas[127] (Fig. 4b). In contrast, $KRas^{G12D}$ expression using sfp-CREER in the AT2 cells leads to adenocarcinoma formation, whereas no lesions were found to originate from the BASCs despite the high frequency of genetic recombination in these cells. Although the expression of oncogenic KRas led to a histologically similar form of adenocarcinoma, some interesting differences (including different Sox2 expression) were observed between the AT2 cells targeted by the CC10 and sfpc-CREER (ref. 127). This study clearly demonstrates that adenocarcinomas arise preferentially from AT2 cells and strongly suggests that heterogeneity among these cells can lead to molecularly distinct adenocarcinoma subclasses[127].

Perspectives

The use of lineage-tracing approaches in mice to identify the cell at the origin of different cancers has uncovered several common features in distinct mouse models. In almost every model, oncogene expression in a given tissue led to the transformation of some cells but not others, which did not always correlate with the cell's proliferation potential or the ability of the oncogene to stimulate cell proliferation, suggesting that the epigenetic/transcriptional state of oncogene-targeted cells is important for tumour initiation. Understanding why some cells are sensitive to oncogene-induced tumorigenesis whereas others are resistant, and whether these mechanisms are conserved across different tissues, will be important for the development of strategies to prevent cancer initiation and possibly block tumour progression. A second important conclusion from these studies is that tumour differentiation is not necessarily indicative of their cellular origin. Some tumours, such as SCLCs, are indeed derived from neuroendocrine cells as expected from their differentiation, whereas others, such as some BCC or basal-like breast cancers, originate from cells that normally do not express the markers observed in the resulting tumours. It would be interesting to determine whether this change in gene expression and potentially cell fate following oncogene expression plays a causal role during tumour initiation and, if so, by which mechanisms. Future studies are also required to define the cellular origin of other cancer types, and the roles of putative oncogenes identified by cancer genome sequencing.

The identification of the cellular origins of cancer is also crucial in enhancing our understanding of the mechanisms regulating the different steps of tumour initiation and progression. Using inducible tumour mouse models, it is now possible to isolate tumour-initiating cells at different time points after the initial oncogenic event, to more precisely define the genetic, epigenetic, transcriptional and post-translational modifications associated with each step of tumour progression.

Lineage-tracing experiments have also been used to mark individual tumour cells and assess their respective contribution to tumour growth and relapse after therapy. Such studies demonstrated that benign skin and intestine tumours contain cancer stem cells that fuel tumour growth[128,129] and that glioblastoma, a brain tumour, contains a discrete population of cells responsible for relapse after therapy[130].

In summary, genetic lineage-tracing approaches are instrumental in uncovering the cell lineages at the origin of different cancers, and also provide powerful tools to better understand the modes of tumour growth and development of resistance to therapy.

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