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Towards a holistic and mechanistic understanding of tumourigenesis via genetically engineered mouse models

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Abstract

Mouse models have been an invaluable tool to systematically study tumour progression upon expression of an oncogene or knockdown of tumour suppressors in an immune-proficient microenvironment. Today, tractable genetically engineered mouse models (GEMMs) of human disease permit the regulation of cancer inducing genes at a given time-point in a tissue specific manner and can be combined with cell type specific marking approaches to follow, isolate and study cells during disease. Organoid cultures of primary cells taken directly from these mice are capable of preserving the original architecture and signalling events within the tumour, allowing in-depth mechanistic analysis. Here we present an overview of combined approaches, involving GEMMs that expand on our knowledge obtained from patient material and contribute to our in-depth understanding of human cancer.

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Introduction

Despite major advances in diagnostics and treatment options, cancer remains one of the leading causes of morbidity and mortality worldwide. Moreover, the number of new cases is expected to rise by 70% in the next 2 decades [1], emphasizing the need to intensify research efforts on the causes and mechanisms of carcinogenesis.

Recent advances in the genomic analysis of human cancers, including single cell sequencing approaches, has led to a much better understanding of tumour evolution and heterogeneity, has aided better classification of cancer subtypes [2] and-in conjunction with sophisticated histological analysis [3]- also helped to shed light on the role of the tumour microenvironment. However, large sample numbers have to be obtained to analyse vaguely defined human tumour subtypes, confounding lifestyle factors have to be considered and ethical hurdles to be overcome. Further, a mechanistic analysis of tumour progression and therapy response is hard to achieve with independent patient samples, since they reflect only a snap shot of these dynamic processes.

To this end, mouse models have proved to be an invaluable resource to systematically and reproducibly analyse mechanisms in tumourigenesis [4,5]. Specifically genetically engineered mouse models permit us to delineate the cell of origin in lineage tracing approaches and to study as well as visualize the outcome of drug treatment. They also serve as a tool to understand late tumour stages by giving access to minimal residual disease following therapy and homing metastatic cells, both cellular substrates that largely remain elusive in patient samples (see Table 1).

Tumour initiation

Despite our increased understanding of tumour progression, the initiating and driving cancer cells remain largely uncharacterized, as does their evolution via accumulation of mutations. It is imperative to understand contextual evolution of tumours to develop efficient therapeutics for the different tumour subpopulations, including tumour re-initiating cells (so called cancer stem cells).

Lineage tracing approaches in mouse models are used to elucidate the mechanisms of tumour initiation and progression into pre-neoplastic disease and involve marking a single cell with a label that is transferred to all its progeny and retained stably over time [6,7]. For this, the Cre-loxP system adapted from bacteriophage P1 is widely used. In short, Cre recombinase is expressed under the control of a tissue/cell type-specific promoter and will excise a loxP-STOP-loxP ("floxed" STOP) sequence to activate expression of a reporter gene. Temporal control of Cre activity can be achieved by inducible recombination systems like Cre-ER and Cre-PR fusion proteins. These systems have been carefully developed over the years, both in terms of preventing "leakiness" of Cre-induction [8] and development of robust reporter genes as well as multi-label approaches [9].

Table 1
Examples of GEMMs used for studying human tumourigenesis.

Cancer stages	GEMMs employed for a mechanistic analysis
Tumour initiation	Lineage tracing models [10–13,15] used in conjunction with next generation sequencing [12,14].
Tumour progression	Models used in conjunction with non-invasive imaging methods [22–27,29] , intra-vital microscopy(IVM) [35], 3D organoid technology [55,58,59], next generation sequencing [67,68] . Also, recently developed stochastic models [19,21].
Drug response and minimal residual disease	Tetracycline regulated mouse models[65,66] in conjunction with 3D organoid technology [67,68], and models used in conjunction with intra-vital microscopy(IVM) [31,73]
Metastatic cascade	Models used in conjunction with intra-vital microscopy (IVM) [71] and 3D organoid technology [57].

Such tracing models combined with tractable mouse models of human cancer permit us to follow nascent tumours and to isolate marked cells at different steps of tumour progression for subsequent in-depth analysis.

A number of such studies have identified the cell of origin in cancer tissues and also demonstrated that only a small fraction of the oncogene-targeted cells are able to contribute to tumour formation, suggesting that other genetic and/or epigenetic hits are required to initiate tumourigenesis. The same oncogenic pathways activated in different organ progenitors resulted in different histological cancers [10,11], suggesting that certain tissue specific cell types are biased towards neoplastic transformation, for instance in the case of expansion of the luminal progenitor lineage in pancreatic cancer [12]. The dependence of certain tumour cell lineages towards the driving oncogenic signal is of potentially enormous therapeutic value, as found in the case of β-catenin inhibition for patients with basal-derived prostrate cancer [11].

Neoplastic transformation, as shown by linage tracing experiments, can result in re-programming of tumour initiating cells (TICs) to a different cell fate within the normal lineage hierarchy of the organ. For example, during skin BCC (basal cell carcinoma) progression, initiating differentiated cells were shown to be re-programmed to an embryonic hair follicle like progenitor state [13]. Conversely, oncogene expression and effect in brain stem cells is only manifested following the commitment of multipotent stem cells to granular neurons, while no tumours arose from other cell lineages [14]. In the mammary epithelium, luminal progenitors were shown to initiate basal-like breast cancers, proving that cell type markers might be misleading in determining the cell of origin of differentiated tumours [15].

Lastly, lineage tracing studies can also be used to study the role of the microenvironment during tumour initiation and showed a location dependent permissive tumour environment in skin cancer. Wong and colleagues [16] discovered that bulge stem cells remain benign upon oncogene induction, but when recruited to the inter follicular epidermis during wounding they initiate BCC.

Overall, the ability to unravel the cell lineages at the origin of different cancers, has led to a better understanding of the kinetics and cell type specific expansions during the growth of heterogeneous tumours and currently extends towards understanding of the development of therapy resistance [17].

Tumour progression

Conditional GEMMs are engineered to allow normal developmental processes in mice, enabling genomic manipulation that leads to *de novo* tumour formation in the adult tissue. These conditional mouse models allow tissue specific gene regulation either via CreER-mediated gene recombination upon tamoxifen administration or through the use of tetracycline inducible transgenes that permit reversible control over target-gene expression [18]. However, contrary to the paradigm that human cancers develop from a single mutated cell, the oncogenic event usually occurs simultaneously in all the cells of the organ in these GEMMs. To model a stochastic transformation, sporadic expression of oncogenes can be induced via inhalation of engineered adenoviruses/lentiviruses and was described for conditional models of non-small cell lung cancer (NSCLC) [19]. Similarly, viral delivery of a CRISPR/Cas9 based system [20] for *in vivo* engineering of the oncogenic EML4-Alk fusion protein [21] has proven the potential of such genome editing techniques to rapidly advance stochastic cancer modelling in mice.

The combination of these specialized mouse models with methods to visualize tumour progression in the animals yields valuable information; fluorescent light imaging (FLI), bioluminescent imaging (BLI), magnetic resonance imaging (MRI) and positron emission tomography (PET), among other whole body imaging technologies have been used to study early tumourigenesis events [22], to monitor tumour growth [23] and localization, to evaluate biodistribution and uptake of drugs [24], to test target efficacy [25] as well as to study homing of specific cell types [26] and reveal localisation and interactions of immune cells [27–29]. These techniques allow for non-invasive in-vivo imaging of mice but lack the resolution to observe single cell behaviour. Imaging of live animals at microscopic