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Beyond genetics: driving cancer with the tumour microenvironment behind the wheel

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Abstract

Cancer has long been viewed as a genetic disease of cumulative mutations. This notion is fuelled by studies showing that ageing tissues are often riddled with clones of complex oncogenic backgrounds coexisting in seeming harmony with their normal tissue counterparts. Equally puzzling, however, is how cancer cells harbouring high mutational burden contribute to normal, tumour-free mice when allowed to develop within the confines of healthy embryos. Conversely, recent evidence suggests that adult tissue cells expressing only one or a few oncogenes can, in some contexts, generate tumours exhibiting many of the features of a malignant, invasive cancer. These disparate observations are difficult to reconcile without invoking environmental cues triggering epigenetic changes that can either dampen or drive malignant transformation. In this Review, we focus on how certain oncogenes can launch a two-way dialogue of miscommunication between a stem cell and its environment that can rewire downstream events non-genetically and skew the morphogenetic course of the tissue. We review the cells and molecules of and the physical forces acting in the resulting tumour microenvironments that can profoundly affect the behaviours of transformed cells. Finally, we discuss possible explanations for the remarkable diversity in the relative importance of mutational burden versus tumour microenvironment and its clinical relevance.

Introduction

For over a century, cancer has been described as a genetic disease[1]. Most human cancers contain 2–8 driver mutations[2], and much work has focused on the order in which mutations arise[2–7]. For instance, in colorectal cancer, progression often correlates with a sequential

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addition of mutations in *APC*, RAS, SMAD and *TP53* (ref. 8). These studies have led to the view that the temporal accumulation of somatic mutations up to a threshold of the oncogenic load underlies both initiation of and progression to a metastatic state.

Research in mice has now revealed that even cells that harbour only a single or a few oncogenic mutations can progress to malignancy[9–11] (Table 1). Similarly, in humans, paediatric cancers have far fewer mutations than adult cancers but are just as aggressive[2,12], suggesting that a small number of mutations in combination with epigenetic changes can trigger the development and progression of tumours. Further evidence is provided by studies showing that metastatic spread to secondary sites does not always correlate with a higher mutational burden[7], and that even non-transformed epithelial cells can spontaneously disseminate and survive in distant organs[13]. The organotropism exhibited by certain cancers also supports the notion that the environment dictates the formation of successful metastases[14] and that non-genetic avenues often control the acquisition of metastatic traits.

Hints surfaced long ago that cancer is not simply a matter of genetics. In the 1970s, pioneering studies by Mintz and colleagues demonstrated that malignant teratocarcinoma cells could contribute to healthy tissue morphogenesis in chimeric mice[15,16]. Soon thereafter, it was shown that the skin of chickens infected with Rous sarcoma virus or of mice transgenic for transforming growth factor-α (TGFα) exhibited no overt phenotype until they were wounded, after which tumours developed along a wound site[17–20]. As secreted factors, TGFα and TGFβ can exhibit paracrine transforming functions without genetic alterations[21,22]. Conversely, the cumulative effect of harmful sun rays in ageing human skin manifests in numerous clones of genetically altered epidermal cells, which carry oncogenic mutations, but do not necessarily progress to cancer[23,24]. Together, these studies demonstrate that whether a cell afflicted by an oncogenic mutation will initiate a tumour or behave normally is context-dependent, invoking additional factors, such as the tissue microenvironment, in the decision-making process. Included in this equation are high-risk factors for cancers, including obesity, diet, chronic inflammatory conditions, pollution, hormonal changes, injury and a host of other aberrations that affect the fitness of the organism and in turn the microenvironment of a premalignant tissue stem cell.

In the past decade, researchers have focused on investigating the spatial-temporal changes in various components of the tumour microenvironment (TME)[25–27] and in the changes of the transformed cells as they progress to malignant, invasive and metastatic cancers. Such studies have led to a number of newfound cancer drivers, including several genetic alterations, such as oncogenic histone mutations[28], and many non-genetic alterations, such as histone and DNA modifications and altered transcriptional regulation on the tumour side[29], and the extracellular matrix (ECM), tissue architecture, and physical forces on the TME side[30], all of which can have a profound impact on malignancy. Additionally, judging from their normal tissue counterparts, the TMEs of most cancers are complex, comprised of a diverse array of cells and cellular networks that include both adaptive and innate immune cells, fibroblasts, blood vessels, lymphatics, neurons and adipocytes. Cancer stem cells (CSCs)[9,31,32] — the cells that self-renew, maintain and propagate tumour growth —

act as a cellular switchboard that integrates the various incoming signals from the TME and transmits back new signals that together shape cancer prognosis[9,10].

Given these complexities, it is not surprising that TMEs are tailored to suit the diversity of cancer behaviours[33–35]. In a number of cases, for instance, invasion is driven by active TGFβ emanating from pro-tumorigenic immune cells such as monocyte-derived macrophages, which associate with the perivascular niche and elicit marked transcriptional changes in nearby CSCs and cancer-activated fibroblast (CAF) neighbours[32,36–38]. By contrast, lung adenocarcinomas present a relatively constant tumour-immune signature maintained throughout progression and invasion to lymph nodes and secondary sites[39], emphasizing that whether the immune system inhibits or promotes invasion is dependent on context.

In addition to the local environment, dietary effects can affect tumour formation and progression. For example, although individuals with obesity are at higher risk of developing tumours, they are, paradoxically, better responders to cancer treatment and have an improved prognosis[40,41]. In this case, the chronic inflammatory state that is caused by overweight and obesity may support tumorigenesis but deter recalcitrant lesions upon therapy[42]. Vasculature and neural innervation provide additional avenues for long-range signalling; see below.

Together, studies on tumour initiation and metastasis have revealed myriad non-genetic factors that are critical, and often major, drivers in the formation and progression of malignancies. Here, we review recent advances in understanding how cells that undergo a single oncogenic mutation can acquire many features of malignancy simply through epigenetic rewiring driven by interactions with their environment. By exploring the various ways in which non-genetic factors drive tumour progression, these studies begin to shed light on why mutational burden can sometimes be perplexingly low in cancers. Finally, we place microenvironmental and epigenetic drivers in the context of their potential as therapeutic targets for cancer.

Cell-extrinsic drivers of malignancy

The TME encompasses immune cells, CAFs, blood and lymphatic vasculature and nerves, as well as extracellular components such as the ECM, basement membrane, growth factors and metabolites, all of which are known to influence tumorigenesis and tumour progression[25]. Epithelial cells that harbour a single oncogenic mutation have made it possible to disentangle the multifarious impacts of mutational burden and unravel the environmental factors that influence early tumour-initiating CSCs and their roles in tumorigenesis and progression[43].

Competition between a transformed cell and its healthy neighbours—Cell competition within the mammalian epithelium is a natural physiological process that occurs during tissue morphogenesis[44]. First described in *Drosophila* wing development, cell competition is orchestrated by a mechanism whereby fit cells recognize and actively kill a less-fit neighbour, after which it is expelled from the tissue[45]. Often misunderstood as simply proliferative advantages, phenomena resembling classical cell competition have been described for mammalian tissues. Thus, by using live imaging, researchers have observed

out-competition of transformed cells by healthy neighbours within both hair follicles[46] and pancreatic ductal and acinar regions[47]. Although mechanistic insight was lacking, such studies have highlighted the need for transformed cells to outcompete their healthy neighbours in order to avoid cell death and extrusion, and to allow tumours to form.

Cellular competition in tumorigenesis in human cancers has recently been examined in mammary organoids in which a transformed cell only proliferates efficiently when surrounded by transformed neighbours[48]. Hence, in order for clones of transformed cells to emerge, they must generate sufficient paracrine signals to block competition by their healthy neighbours. Indeed, in intestinal crypts, transformed enterocytes harbouring mutations in *KRAS* or *PI3K* secrete bone morphogenic protein ligands into neighbouring healthy crypts, curbing normal stem cell activity and gaining a competitive advantage[49].

Together, these studies provide compelling evidence that from the early stages of tumorigenesis and without the accumulation of genetic mutations, transformed epithelial cells can (and must) alter their surrounding epithelium to successfully progress to malignancy (Fig. 1). It is both interesting and notable in this regard that there are also cases in which particular mutations, for example, *TP53* and *NOTCH1*, can accumulate aphenotypically in the skin and oesophagus over extended periods of time without progressing to malignancy or being eliminated by their neighbours[23,50], indicating that niche remodelling is required for tumorigenesis in addition to oncogenic mutations. Overall, clonal competition probably both contributes to and fuels intra-tumoural heterogeneity to promote tumour evolution.

Inflammation as an accelerator of tumorigenesis—Inflammation is a crucial driver of cancer initiation and progression across tissues[51]. One of the first links between inflammation and cancer was drawn from classical skin chemical carcinogenesis studies, in which mice treated with the potent mutagen 7,12-dimethylbenz(a) anthracene (DMBA) that frequently induces *Hras* mutations and papillomas were subsequently treated with the inflammation-stimulus 12-*O*-tetradecanoylphorbol-13-acetate (TPA) to accelerate progression to malignant squamous cell carcinomas (SCCs)[52,53]. Similarly, exposure to the pancreatitis-inducing agent caerulein predisposes mice harbouring a single oncogenic $Kras^{G12D}$ mutation in pancreatic ductal cells to progress to ductal adenocarcinoma[10]. Although the field is still nascent, the extent to which inflammation is enhancing versus essential in driving cancer seems to be predicated on the natural activity of the stem cells of the tissue. Epidermal turnover is constant and thus oncogenic RAS can drive a malignant cutaneous SCC state on its own[9], whereas pancreatic turnover is infrequent, requiring preconditioning with inflammation to prime RAS cells to a pancreatic ductal adenocarcinoma state[10].

Recent studies confirm that the links between inflammation and cancer are far-reaching. Thus, intestinal tumorigenesis is accelerated when the guts of mice heterozygous for a mutation in the adenomatosis polyposis coli (*Apc*) gene (Apc^{min} mice) are treated with the inflammation-inducing agent dextran sodium sulfate (DSS)[54]. Analogously, mice carrying mutations in either epidermal growth factor (*Egfr*) or *Kras* show accelerated formation of lung adenocarcinomas when exacerbated by air pollutants[55]. Together, despite the long-

term assumption that additional spontaneous genetic events must occur to progress to malignancy[47], these examples infer that exposure to inflammatory stimuli that occur either before or after the initial genetic insult can replace this need.

Like *HRAS*^{G12V} SCC cells, *KRAS*^{G12D}-expressing pancreatic ductal adenocarcinoma cells secrete interleukin-33 (IL-33), a powerful immunomodulatory cytokine. However, in contrast to skin carcinoma, where the cytokine acts on macrophages to enhance their pro-tumorigenic features, including release of active TGFβ[38], IL-33 release from pancreatic tumours seems to affect regulatory T (T_{reg}) cells[29]. Although tumour context may fine-tune the crosstalk, the outcome of this signal transmitted by the tumour cell and received by immune cell neighbours appears to be cancer progression.

Another interesting twist to the relationship between inflammation and cancer comes from the remarkable discovery, first made in skin[56] but now extended to many tissue stem cells[57], that exposure to an inflammatory stimulus imparts to the stem cells an epigenetic inflammatory memory of the experience that can last long after the inflammation subsides and pathology has returned to normal. Embedded within enhancers of a cohort of key inflammatory genes, these ‘memory domains’ keep their genes in an open state, poised for rapid, hyper-sensitive re-activation upon a stressful encounter[58]. Inflammatory memory can accelerate a wound response in injured epithelial tissues[56] and skew haematopoietic progenitors to favour the myeloid lineage[59,60]. This field is still unfolding, but such attributes are likely to underlie the link between inflammation and cancer, at least in part.

Despite the ability of inflammation to accelerate tumorigenesis in oncogenic RAS-expressing cells, expression of oncogenic *HRAS*^{G12V} on its own can in some cases trigger a cascade of dysregulated signals within the TME that, over time, progress to tumours with features of invasive cancer[9,32,61]. In mouse skin, once CSCs secrete IL-33, the altered local immune milieu elevates the release of active TGFβ[38], causing the CSCs to generate angiogenic factors and provoke a massive influx of blood vessels[8]. In this way, the CSCs truly become the architects of their tumorigenic niche without the need for high mutational burden or inflammation to set a course towards cancer, even though they undoubtedly benefit by such inputs (Fig. 1).

Stromal fibroblasts as drivers of tumorigenesis—CAFs are TME cells with an intra-tumoural heterogeneity phenotype of subtypes and subspecializations, which function critically in ECM deposition and remodelling, and also in communicating extensively with cancer cells and infiltrating leukocytes[62]. One of the earliest examples illustrating the effect of the stroma on tumorigenesis came from the work of Moses, who observed that when a Cre driver expressed by stromal fibroblasts was used to conditionally ablate the essential TGFβ co-receptor gene *Tgfbr2*, mice developed spontaneous epithelial forestomach tumours[63,64]. Soon thereafter, studies showed that CAFs, but not normal fibroblasts, are sufficient to drive tumorigenesis in the prostate with transformed epithelial cells, lessening the need for high mutational burden in the cancer[65].

The powerful effect that CAFs can have on epithelial tumorigenesis has also been demonstrated with genetically normal fibroblasts. CAFs strongly respond to TGFβ and

Yes-associated protein (YAP) signalling, which induces cell-type-specific changes in ECM production and immunosuppressive capabilities[66–68]. In *Apc*^{min} mice, genetically normal fibroblasts can contribute to intestinal tumorigenesis by activating the myeloid differentiation primary response gene 88 protein–Toll-like receptor-4 (MYD88–TLR4)-mediated inflammatory pathways, once thought to be exclusive to innate immune cells[69]. That the microbiota that infiltrate these early *Apc*^{min} lesions are involved in triggering the innate immune response in CAFs seems likely, but confirmation must await future investigation.

Finally, through their ability to weave a dense matrix web surrounding some tumours, for example pancreatic cancers, CAFs have the ability to alter the metabolic and hypoxic milieu of the TME and thus the CSC niche[70].

Mechanical forces as part of the TME—Although many interactions between the stroma and transformed epithelial cells are influenced by secreted cytokines and growth factors, others can be triggered by acute, rapid changes in cell–cell and cell–ECM interactions, or by aberrant mechanical forces resulting from oncogenic mutations. Indeed CAFs secrete type I collagen and are responsible for most of the stromal ECM[71].

The ability of tumour cells to invade depends upon the stiffness of their surroundings. In young, healthy tissues, stromal ECM is often stiff, impeding tumorigenesis and often requiring ECM-degrading enzymes to facilitate invasion[72]. As tissues age, stromal ECM becomes weakened, particularly in sun-exposed or mechanically stressed regions, probably contributing to the marked age-related rise in tumorigenesis[73,74]. The stiffness of the basement membrane also contributes to tumour progression. In basal cell carcinomas, for instance, basement membrane proteins are secreted at a fast pace, inducing the formation of a softer, viscoelastic basement membrane that can contain lesions and keep tumours benign[75]. Of additional note, resistance to Hedgehog inhibitors is determined by basal cell carcinoma tumour architecture, with cells in close contact with the basement membrane constituting the resistant population[76]. By contrast, skin epithelium harbouring *HRAS*^{G12V} mutations form cutaneous SCCs, which have thinner and stiffer basement membranes that rupture more easily in the face of the strong mechanical forces emanating from the stiff keratin pearls that typify these cancers[75]. These examples illustrate how fundamental changes in the biology established early by a particular oncogene can have profound non-genetic effects on later steps in tumour progression.

The importance of local mechanical forces as non-genetic factors in tumour progression has also been investigated in studies using a higher mutational burden. For example, in an array of mouse and human cancer cell lines grown in different patterns and hydrogel stiffness, geometric cues alone were shown to enhance CSC features in clonal tumouroids, including proliferation, migration, invasion and survival[77]. This principle has been documented in vivo in a model of pancreatic ductal adenocarcinoma driven by *Kras*^{G12D} and loss of *Trp53*, in which it was demonstrated that the geometry of an incipient lesion can predict the aggressiveness of the tumour[78]. Thus, the diameter of the duct where the pancreatic tumours arise determines whether the lesion will grow into the lumen (endophytically) or

fold externally (exophytically). Exophytic lesions interact closely with the TME[78], so they also recruit pro-tumorigenic CAFs, facilitating tumour progression.

Another example of how the mechanical landscape of the extracellular space affects tumorigenicity and invasiveness comes from studies on human hepatocellular carcinoma lines, where stiffening of the ECM induces their secretion of exosomes, which in turn leads to NOTCH activation and growth enhancement upon xenografting[79]. Extracellular fluid viscosity can also reprogram breast cancer cells in vitro into disseminating phenotypes that favour metastasis, affecting the actin cytoskeleton, sodium and calcium transport and contractility[80].

In another study, researchers used a magnetic device applied to mouse intestines to mimic the mechanical pressures of tumour growth. The result was phosphorylation of β-catenin on Tyr654, which destabilized E-cadherin in adherens junctions, releasing β-catenin to translocate into the nucleus and activate WNT signalling[81]. The mechanical force transcriptional regulator YAP is also sequestered in the cytoplasm by adherens junctions, in this case through α-catenin, providing another mechanical activation link in this tumour-promoting pathway[82]. These more recent examples shed further light on the long-standing knowledge that inactivating mutations in *CDH1*, encoding E-cadherin, are associated with human cancers[83], and reveal non-genetic ways to alter adherens junctions, cancer cell dissemination and mechanical properties of tumours.

In short, it is clear that context matters when it comes to the tumour cells, their TME and internal mechanical forces within the TME. Additionally, although mechanical forces emanating from the external environment can affect tumour architecture and behaviour, mechanical forces arising from within the cancer itself are equally and in some cases more important in shaping the properties of the growing cancer[30].

Angiogenesis, lymphangiogenesis and neurogenesis—Other well known impacts of the TME on cancer are angiogenesis and lymphangiogenesis[84,85], both of which are triggered by signals from CSCs. As exemplified by genetic ablation of the gene encoding vascular endothelial growth factor A (VEGFA) in a mouse skin tumour model, VEGFA is a potent inducer of tumour-associated angiogenesis[86]. Of note, *Vegfa* can be induced by a variety of factors featured in the early tumorigenic microenvironment, including the integrated stress response, hypoxia and TGFβ signalling[8,87]. The ability of the vasculature to shuttle nutrients, hormones and immune cells to tumours makes it an essential lifeline for CSCs, which reside at the tumour–stromal interface. Vasculature can also create intra-tumoural heterogeneity, driving differential transcriptomics in neighbouring CSCs and contributing to their therapeutic resistance[88].

In skin SCCs, the vasculature rises precipitously when TGFβ signalling causes CSCs to upregulate expression of VEGFA and other angiogenic factors[9]. TGFβ target genes also include those encoding epithelial–mesenchymal transition proteins as well as the leptin receptor (LEPR)[9]. For tumours like SCCs that are not in direct contact with fatty tissue (which is the source of leptin production), the vasculature becomes all the more important as the circulation must then provide leptin to trigger LEPR signalling. Activation of

this pathway in turn elevates the PI3K–AKT–mTOR pathway and maintains the energy balance as tumour growth makes nutrients scarcer[9]. Because LEPR signalling can also boost cholesterol biosynthesis, required for HRAS farnesylation, this pathway may also function in enhancing RAS activity[89,90].

Less is known about how lymphatic capillaries function in tumorigenesis, although metastasis often begins by cancer cell migration to lymph nodes. Although the field is still unfolding, recent studies suggest that melanoma cells may use lymphatic vasculature as a preferred means of minimizing death from ferroptosis[91]. Through their ability to traffic immune cells, regulate fluid pressure and provide key stimulatory growth factors, lymphatics function critically in regulating normal tissue stem cell behaviour[92–94]. Given that these features profoundly affect cancer properties, and that jamming forces due to tumour growth may in turn affect lymphatics, it should be interesting to interrogate the role of lymphatics on CSCs.

Recent studies have begun to explore the effect of nerves in cancer progression. To date, their effects appear context-dependent, without a consistent tumour-promoting or -suppressing role[95]. However, in the single-oncogene *Apc*^{min} model of colorectal cancer, vagotomy significantly reduced tumour number[96], suggesting that visceral sensory neurons may function as non-genetic drivers of tumorigenesis and progression. Neurons can, in addition, mount an antitumour response in the immune compartment. For example, in melanoma, nociceptors can induce CD8$^+$ T cell exhaustion through the secretion of the immunomodulatory neuropeptide calcitonin gene-related peptide (CGRP)[97] (Fig. 1).

Together, these discoveries exemplify the power of long-range as well as short-range environmental inputs in orchestrating a tumour-promoting response even when the associated CSCs have a low mutagenic burden (Fig. 1). Given that the various pathways activated in CSCs from these inputs are frequently mutated in metastatic cancers[98], it is tempting to speculate that much of the increased mutational burden in cancer may reflect a locking into place of oncogenic pathways already established by the communication between the TME and CSCs.

Cell-intrinsic non-genetic drivers

Communication between the TME and tumour cells shapes the cancer epigenome, which encompasses posttranslational modifications in DNA, histones and other chromatin-associated proteins. These alterations can leave long-lasting marks that affect both transcription[99] and translation[100] of CSCs. Although they often elicit similar outcomes on cellular behaviours, epigenetic modifications differ from genetic mutations in that they are potentially reversible, making this an interesting field for developing anticancer therapies in the future[101].

TME-induced transcription factor dysregulation—Pro-tumorigenic signalling triggered by the TME often leads to transcription factor dysregulation in the transformed cell. The MAPK pathway is activated by tyrosine kinase receptors such as EGFR, which in turn activate RAS and/or RAF, altering the expression of transcription factors such as JUN, FOS, ELK, ETS, MYC, MSK[102] and even β-catenin, the lymphoid enhancer-binding

factor (LEF1, also known as TCF1) cofactor of WNT signalling[103]. Although genes along this pathway are frequently mutated in cancer, the pathway can be hyperactivated by the TME[2,104]. In oncogenic RAS-driven pancreatic cancer, for instance, sterol *O*-acyltransferase 1 (SOAT1), which enhances RAS membrane localization and function, is hyperactivated[105]. *Soat1* is also among the transcriptional changes activated in skin SCCs exposed to elevated TGFβ signalling from the angiogenic-rich TME[9] (Fig. 2).

The effect of chromatin dynamics—As tumorigenesis ensues, the intricate interplay of external factors and downstream transduction signalling pathways can profoundly alter the chromatin organization of CSCs. Recent high-throughput technical advances that enable the mapping of accessible chromatin regions, binding of transcription factors, chromatin modifications and tertiary chromatin dynamics begin to reveal how non-genetic changes in chromatin contribute to tumour initiation and evolution, as exemplified by studies on mouse lung adenocarcinoma[106]. Chromatin dysregulation during carcinogenesis leads to plastic states that contribute to cancer transformation, heterogeneity and evolution, as demonstrated in colorectal cancer[107]. In fact, even just sustained activation of pioneer factor SOX9 is sufficient to launch epidermal stem cells onto a molecular journey reminiscent of that of basal cell carcinoma[108]. The dynamic crosstalk between the emerging tumour and its microenvironment creates selective pressures that trigger transcription factors not usually active in the tissue. One such example is the Runt-related transcription factor (RUNX), which often elicits epigenomic alterations in chromatin remodelling involved in tumour progression and metastasis[106] (Fig. 2).

The metabolic state of the TME can also affect cancer epigenomics. In this regard, it is notable that the activities of many DNA and histone demethylases rely upon the cofactor α-ketoglutarate and hence are affected by the α-ketoglutarate-to-succinate ratio in the TME[109,110]. For instance, when the ratio is low and demethylases are inhibited, histone 3 lysine 27 trimethylation (H3K27me3) can accumulate, particularly when the methylase, enhancer of zeste homologue 2 (EZH2), is overly active, as it often is in cancer. EZH2 is generally high in stem cells, including CSCs, where it can inhibit differentiation and promote proliferation[111,112]. Although *EZH2*-activating mutations or gene amplifications have been identified in some cancers[113], EZH2 can also be elevated by the TME, as illustrated by studies showing that EZH2 levels are elevated by the bone microenvironment, and this is sufficient to induce metastasis of mammary tumours[114].

Although the precise underlying pathways await future investigation in most cases, these examples highlight the importance of TME-driven chromatin remodelling in tumour initiation, broadening our understanding of how a single oncogenic mutation can trigger a cascade of molecular dominos between the stem cell and its TME that contributes to successful cell transformation (Fig. 2). An additional layer in this process is the role of changes in tertiary chromatin topology in cancer. Emerging evidence has revealed that epigenetic increases in DNA methylation that occur in some cancers can mask CCCTC binding factor (CTCF) binding sites, reshaping the three-dimensional topology of chromatin, which can bring potent enhancers into contact with the promoters of tumorigenic genes[115]. The field is still in its infancy, and as yet, the impact of cancer-causing shifts in chromatin topologies resulting from TME-mediated alterations in metabolism and/or

signalling pathways has not been explored. If such effects are identified, this could suggest a new mode of regulation for future therapeutics.

Post-transcriptional control of cancer progression—Downstream of chromatin architecture and transcription, mechanisms regulating transcript stability (for example microRNAs (miRNAs)) and translation are also considered epigenetic modulators. In cancer, external cues from the TME can induce pathways that hijack these mechanisms to promote transformation, tumorigenesis and progression.

miRNAs.: Numerous miRNAs have been associated with cancer, and their dysregulation can be mediated by mutations[116]. An intriguing example of the power of miRNAs in altering the course of cancer is their ability to suppress an mRNA encoding a specific variant of apolipoprotein E that naturally occurs in the population but, when missing, increases the susceptibility to metastatic melanoma[117]. In this case, the molecular seed of metastasis is rooted in the germline in what was previously thought to be simply a homologue of a small gene family of no obvious consequence to cancer.

The cancer cell environment also plays a critical part in regulating the expression of these post-transcriptional regulators. Hypoxia and EGFR signalling — the latter being hyperactivated by mutations but also by growth factor secretion from cells of the TME[118] — modulate phosphorylation of argonaute 2 (AGO2) protein, which regulates miRNA maturation[119]. For example, in *Kras*^{G12D}-driven pancreatic cancer models, *Ago2* deletion does not prevent the formation of benign pancreatic intraepithelial neoplasia lesions, but in contrast to their *Ago2* wild-type counterparts, these lesions do not progress to pancreatic ductal adenocarcinoma unless p53 is lost[120]. These examples highlight the importance of the miRNA machinery as a tumour-driving alternative to classical oncogenic mutations (Fig. 2).

Translation.: Cancer cells must devise strategies that enable their survival under stressful conditions while simultaneously damping antitumour immune responses. Hostile TMEs, such as nutrient deprivation, oxygen restriction, high metabolic demand and oxidative stress, all provoke a cellular state of endoplasmic reticulum (ER) stress[121]. By activating ER stress sensors and adaptive stress responses, cancer cells can withstand environmental assaults, enhancing their metastatic and drug-resistant capacities[122]. At the heart of these responses is the translational machinery. By damping global translation while permitting translation of a few key stress-responsive mRNAs, cancer cells can better cope with the stresses of their environment[123]. Two key targets are involved. Phosphorylation of the eukaryotic translation initiation factor 4B (eIF4B) by the RAS–MAPK and PI3K–mTOR signalling cascades restricts cap-dependent translation[124]. Phosphorylation of eIF2α is mediated by any one of four stress kinases, one of which is regulated by amino acid deprivation, and another by DNA damage, events integrally linked to cancer progression[125] (Fig. 2). Upon eIF2α phosphorylation, the translational machinery is redirected towards unconventional initiation sites, some of which appear in oncogenic 5′ untranslated regions, allowing oncogenic transcripts to be translated in the face of global translational suppression[100].

These studies are just a few that exemplify how cancer cells can hijack the post-transcriptional machinery to drive tumorigenesis and progression without the need for a cumulative mutational burden (Fig. 2).

Clinical considerations of TME-driven tumours

The confounding effects of the complex communication circuitry between CSCs and their TME come into play in cancer therapeutics. This is especially clear from recent developments in immunotherapy[126–129]. For instance, although signals from the TME can increase the ability of CSCs to resist immunotherapy with cytotoxic T cells[31], the immunotherapy itself can promote tumour stemness by stimulating the cytotoxic T cells to secrete interferons, which in turn activate pro-metastatic transcripts in the CSCs[130]. These data underscore the importance of elucidating not only the effect of therapeutics on the CSCs, but also their effect on the TME.

Given the heterogeneity in the TME and its dynamic adaptations to anticancer therapeutics, targeting the TME is challenging. Maintaining a healthy diet, regular exercise and a low level of stress have been shown to improve therapeutic responses. Perhaps the most striking example of this is the effectiveness of anti-PI3K cancer drugs when patients are placed on a diet that keeps insulin levels down and maintains low blood sugar levels[131]. In another twist, a number of human cancers show elevated levels of LEPR, which, when activated by circulating leptin, can trigger the PI3K pathway, often mutated in advanced metastatic squamous cell carcinomas with high mutational burden[9,132]. Of note, obesity can boost plasma leptin levels by more than two-fold, and when the density of blood vessels rises during cancer progression, leptin levels in the TME rise further[9]. Should mechanical forces exerted on lymphatic vasculature also rise, this could elevate the effective stromal leptin levels even further by reducing drainage.

The links between obesity and cancer extend beyond leptin. A number of tissue changes in lipids and other factors such as lipokines and systemic inflammation occur in obesity and can further fuel the cancer path[133–135]. It has long been known that diet can shape the immunity of our bodies[136,137]. Recently, a high-fat, high-carbohydrate Western diet administered to mice has been shown to trigger inflammation in the bone marrow, which can skew the fate options of haematopoietic progenitors from lymphoid to myeloid[59]. Given that these two lineages may be predetermined by distinct niches within the bone marrow, it will be interesting to determine whether the skewing of fates is rooted in an inflammatory stimulus on a myeloid niche or whether it arises from true fate switching.

Overall, the consequences of a Western world diet and its ability to provoke an inflammatory response are likely to be broad-reaching and deleterious, not only because of the well established link between inflammation and cancer, but also because tissue stem cells harbour long-lasting epigenetic memories of their inflammatory experiences. This becomes all the more disconcerting when considering that in mice, an inflammatory experience during pregnancy can be passed on to offspring[138]. Finally, epigenetic memories of stem cell experiences go beyond inflammation to include memories of migration and stem cell plasticity, which are transiently activated in wound repair but constitutive in cancer[138,139]. The ability of stem cells, and presumably other long-lived cells, to harbour memories of

their past experiences is likely to profoundly affect susceptibility to many cancers in mice and in humans.

A perplexing question in the field of epigenetic memory is how it can be propagated for such a long time. Although attention to date has centred on histone modifications, effects on DNA methylation are likely to exert a longer effect, as they can be propagated independent of proliferation. In this regard, it is interesting that, as noted above, DNA methylation can be influenced by diet, hormones, stress, drugs or exposure to environmental chemicals, leading to a myriad of non-genetic ways in which the same phenotype can be achieved and propagated in the absence of genetic mutations[140].

In fact, we now know of a number of cases in which key cancer-promoting tumour-suppressor genes can be regulated by epigenetic means rather than mutations, reaching outcomes similar to those generated from gene mutations. One case melding DNA methylation and tumour suppression is in sporadic breast tumours, where CpG methylation-mediated repression of the breast cancer susceptibility gene *BRCA1* confers a 'BRCAness' tumour phenotype, akin to that commonly observed in *BRCA1* mutation carriers[141]. Additionally, the SRC pathway has been identified as an essential modulator of p53 activity in oestrogen-receptor-positive breast cancers that harbour a wild-type *TP53* gene[142]. The interaction between SRC and p53 suggests that SRC may phosphorylate the p53 inhibitor MDM2 to restrain p53 tumour-suppressor function[143]. Patients in these scenarios may exhibit clinical phenotypes analogous to those with genetic mutations. However, the screening and treatment of these individuals remain uncharted territory at present.

A holistic view of host factors affecting cancer epigenetics is also important. Apart from the granular dissection of the TME components, factors that affect whole-body fitness, including not only diet, but also stress, sleep and hormonal changes, can also affect tumour progression and response to therapy. Thus, for instance, emotional distress has been shown to correlate with poor response to immune checkpoint blockade in patients with melanoma[144]. Additionally, the circadian rhythm of mice and humans affects the metastatic potential of breast cancer, with most metastatic seeding occurring during sleep[145]. And finally, the enhanced cancer risk of postmenopausal women placed on hormone replacement therapy has been recognized for decades[146].

Although the systematic targeting of all host factors that affect tumorigenesis is clinically untenable, these recent discoveries hold promise for the development of new approaches to manage cancers.

Conclusions and perspectives

This Review focused on non-genetic drivers of tumour biology. Many of these drivers were uncovered long ago, but were relatively ignored during the initial decades of the genome revolution and the emergence of cancer genetics. Only when it became increasingly clear that mutational burden does not account for many features of cancers did cancer geneticists begin to turn their focus to non-genetic variations for explanations. The arrival of sophisticated molecular tools and high-throughput technologies, in combination with animal

models in which mutational burden was kept low and constant, have greatly advanced our knowledge of the TME and its importance to cancer progression.

Over the past decade, new data have pointed to the view that for most cancers, once a stem cell receives an initial oncogenic mutation, non-genetic drivers are probably responsible for many contributing factors that drive tumour initiation and progression. Indeed, as surprising as the finding that malignant, invasive features of mouse tumorigenesis can arise following activation of a single oncogene are the human cancer studies showing that genetic diversity is a relatively early event in tumour progression and that there are very few genetic alterations that accompany the metastatic transition[147]. Together, these recent studies emphasize the inadequacy of considering cancer solely as a genetic disease. By contrast, they exemplify how, upon acquiring an oncogene, a tissue stem cell embarks upon a temporal path of miscommunication with its microenvironment that results in the activation of new signalling cascades, accompanied by chromatin and post-transcriptional alterations.

The molecular details of these complex communication circuits are still unfolding, but the extent to which exacerbating factors contribute to cancer is likely to be attributable at least in part to the regenerative characteristics of its natural tissue. Thus, for tissues like the epidermis and intestine, which self-renew nearly constantly, inflammation may not be as essential as it is for the pancreas and sweat glands, where normal turnover rates are low and stem cells must undergo an additional mobilization step in the quest for tissue proliferation. Although many questions are still unaddressed, the emerging body of evidence holds promise for the development of diverse new therapies against cancer. In particular, the contribution of non-genetic factors in driving (and sometimes being required for) tumour progression is critical in the development of novel therapies, because most patients with cancer die from relapse and metastatic disease[31,32]. The ability of stem cells to accumulate long-lasting epigenetic memories of their encounters with stressful situations and heighten their reaction upon recall has further implications for non-genetic drivers of plasticity and cancer[56,58,139,148], as does the ability of DNA methylation to have long-lasting effects on the rewiring of three-dimensional chromatin topology[97]. As we have highlighted in this Review, the field is beginning to shift its view of mutational burden to one that may, in many cases, be irreversibly locking into place epigenetic pathways that would otherwise be activated through maladaptive crosstalk between tumour-initiating CSCs and their surrounding TMEs.

The previous decades have concentrated on genomic sequencing of cancers, and the next decade is likely to be focused on understanding cellular communication networks through spatial high-throughput ligand–receptor analyses of cancers[149,150]. Advanced computational tools now make it possible to incorporate epigenetic perspectives[29] and advances in human organoid cultures and mouse genetics are beginning to provide the functional means of interrogating the physiological importance of these findings. Advances have also been made in the study of tumour-architecture-driven mechanical forces that modify the cancer epigenome. Although this nascent field has been studied mostly in vitro, the recent development of new in vivo techniques of visualizing tumours in three dimensions through tissue clearing[151] and tissue-intrinsic forces through atomic force microscopy[75,152] paves the way to studying the interaction between mechanics and tumour biology.

In closing, the process of carcinogenesis and tumour progression can be viewed as a dynamic balance between genetic and non-genetic factors that is tailor-made to suit the particular cancer cell of origin and its ever-changing microenvironment during tumour progression (Fig. 3). Placing a cancer along this spectrum captures more accurately the complexity of cancer cell behaviour and should facilitate future investigations that reveal new vulnerabilities to be harnessed for cancer therapeutics.

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Glossary

Axonogenic factors
Soluble molecules that induce axon elongation

Caerulein
A ten-amino-acid peptide that induces pancreatic secretion and acute pancreatitis

Cancer stem cell
(CSC). A subset of tumour-initiating progenitors that mimic some stem cell behaviours and obtain increased resistance to chemotherapy and immunotherapy

Cre driver
Cell-type-specific recombinase

Dextran sodium sulfate
(DSS). Sulphated polysaccharide, toxic to colonic epithelial cells and used to induce colitis

Epigenetic rewiring
Environment-induced changes in chromatin accessibility that affect the biology of cells

Extracellular matrix
(ECM). Network of fibrous macromolecules between cells

Organotropism
Preferential invasion of specific distant organs by metastatic cells

T cell exhaustion
Acquired state of T cell dysfunction

Tissue architecture
Three-dimensional spatial organization of biological tissues

Transformed cell

A cell afflicted by an oncogenic genetic alteration, resulting in the development of a neoplastic phenotype

Translational machinery
Macromolecules (proteins and ribosomal RNA) involved in protein biosynthesis

Vagotomy
Surgical cauterization of vagus nerve

References

1. Varmus HE, Weiss RA, Friis RR, Levinson W. & Bishop JM Detection of avian tumor virus-specific nucleotide sequences in avian cell DNAs (reassociation kinetics-RNA tumor viruses-gas antigen-Rous sarcoma virus, chick cells). Proc. Natl Acad. Sci. USA 69, 20–24 (1972). [PubMed: 4333039] First evidence of an oncogene.
2. Vogelstein B. et al. Cancer genome landscapes. Science 339, 1546–1558 (2013). [PubMed: 23539594]
3. Gerlinger M. et al. Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing. Nat. Genet. 46, 225–233 (2014). [PubMed: 24487277]
4. McGranahan N. et al. Clonal status of actionable driver events and the timing of mutational processes in cancer evolution. Sci. Transl. Med. 7, 283ra254 (2015).
5. Hodis E. et al. Stepwise-edited, human melanoma models reveal mutations' effect on tumor and microenvironment. Science 376, eabi8175 (2022).
6. Drost J. et al. Sequential cancer mutations in cultured human intestinal stem cells. Nature 521, 43–47 (2015). [PubMed: 25924068]
7. Tauriello DVF et al. TGFβ drives immune evasion in genetically reconstituted colon cancer metastasis. Nature 554, 538–543 (2018). [PubMed: 29443964]
8. Fearon ER & Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 61, 759–767 (1990). [PubMed: 2188735] A study suggesting that sequential accumulation of oncogenes drives colorectal cancer initiation, progression and metastasis.
9. Yuan S. et al. Ras drives malignancy through stem cell crosstalk with the microenvironment. Nature 612, 555–563 (2022). [PubMed: 36450983] Deciphers miscommunication between an oncogenic HRAS skin stem cell and its microenvironment that generates a TME and CSC state resembling malignant, invasive SCC.
10. Alonso-Curbelo D. et al. A gene-environment-induced epigenetic program initiates tumorigenesis. Nature 590, 642–648 (2021). [PubMed: 33536616] Demonstrates how inflammation induces chromatin remodelling to promote pancreatic ductal adenocarcinoma initiation.
11. Hart JR et al. The butterfly effect in cancer: a single base mutation can remodel the cell. Proc. Natl Acad. Sci. USA 112, 1131–1136 (2015). [PubMed: 25583473]
12. Sweet-Cordero EA & Biegel JA The genomic landscape of pediatric cancers: implications for diagnosis and treatment. Science 363, 1170–1175 (2019). [PubMed: 30872516]
13. Rahrmann EP et al. The NALCN channel regulates metastasis and nonmalignant cell dissemination. Nat. Genet. 54, 1827–1838 (2022). [PubMed: 36175792]
14. Jin X. et al. A metastasis map of human cancer cell lines. Nature 588, 331–336 (2020). [PubMed: 33299191]
15. Mintz B. Teratocarcinoma cells as vehicles for introducing mutant genes into mice. Differentiation 13, 25–27 (1979). [PubMed: 288720] Early evidence that cancer cells introduced into mouse blastocysts can result in normal embryogenesis and contribute to healthy tissues in adult chimeric mice.
16. Mintz B. & Illmensee K. Normal genetically mosaic mice produced from malignant teratocarcinoma cells. Proc. Natl Acad. Sci. USA 72, 3585–3589 (1975). [PubMed: 1059147]
17. Coussens LM & Werb Z. Inflammation and cancer. Nature 420, 860–867 (2002). [PubMed: 12490959]

18. Ge Y. et al. Stem cell lineage infidelity drives wound repair and cancer. Cell 169, 636–650.e614 (2017). [PubMed: 28434617]
19. Dolberg DS, Hollingsworth R, Hertle M. & Bissell MJ Wounding and its role in RSV-mediated tumor formation. Science 230, 676–678 (1985). [PubMed: 2996144] Early evidence that wounding cooperates with oncogenes to induce tumorigenesis.
20. Vassar R. & Fuchs E. Transgenic mice provide new insights into the role of TGF-α during epidermal development and differentiation. Genes Dev. 5, 714–727 (1991). [PubMed: 1709129]
21. Moses HL, Roberts AB & Derynck R. The discovery and early days of TGF-β: a historical perspective. Cold Spring Harb. Persp. Biol. 8, a021865 (2016).
22. Sieweke MH, Thompson NL, Sporn MB & Bissell MJ Mediation of wound-related Rous sarcoma virus tumorigenesis by TGF-β. Science 248, 1656–1660 (1990). [PubMed: 2163544]
23. Martincorena I. et al. Tumor evolution. High burden and pervasive positive selection of somatic mutations in normal human skin. Science 348, 880–886 (2015). [PubMed: 25999502]
24. Jonason AS et al. Frequent clones of p53-mutated keratinocytes in normal human skin. Proc. Natl Acad. Sci. USA 93, 14025–14029 (1996). [PubMed: 8943054]
25. de Visser KE & Joyce JA The evolving tumor microenvironment: from cancer initiation to metastatic outgrowth. Cancer Cell 41, 374–403 (2023). [PubMed: 36917948]
26. Walsh LA & Quail DF Decoding the tumor microenvironment with spatial technologies. Nat. Immunol. 24, 1982–1993 (2023). [PubMed: 38012408]
27. Stahl PL et al. Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. Science 353, 78–82 (2016). [PubMed: 27365449]
28. Nacev BA et al. The expanding landscape of 'oncohistone' mutations in human cancers. Nature 567, 473–478 (2019). [PubMed: 30894748]
29. Burdziak C. et al. Epigenetic plasticity cooperates with cell-cell interactions to direct pancreatic tumorigenesis. Science 380, eadd5327 (2023). Confirms that inflammatory cues drive chromatin remodelling and pancreatic cancer progression.
30. Almagro J, Messal HA, Elosegui-Artola A, van Rheenen J. & Behrens A. Tissue architecture in tumor initiation and progression. Trends Cancer 8, 494–505 (2022). [PubMed: 35300951]
31. Miao Y. et al. Adaptive immune resistance emerges from tumor-initiating stem cells. Cell 177, 1172–1186.e1114 (2019). [PubMed: 31031009]
32. Oshimori N, Oristian D. & Fuchs E. TGF-β promotes heterogeneity and drug resistance in squamous cell carcinoma. Cell 160, 963–976 (2015). [PubMed: 25723170] Discovers that TGFβ from the TME drives skin carcinoma progression and resistance to chemotherapeutics.
33. Nader GPF et al. Compromised nuclear envelope integrity drives TREX1-dependent DNA damage and tumor cell invasion. Cell 184, 5230–5246.e5222 (2021). [PubMed: 34551315]
34. Szczerba BM et al. Neutrophils escort circulating tumour cells to enable cell cycle progression. Nature 566, 553–557 (2019). [PubMed: 30728496]
35. Gkountela S. et al. Circulating tumor cell clustering shapes DNA methylation to enable metastasis seeding. Cell 176, 98–112.e114 (2019). [PubMed: 30633912]
36. Ghajar CM et al. The perivascular niche regulates breast tumour dormancy. Nat. Cell Biol. 15, 807–817 (2013). [PubMed: 23728425]
37. Batlle E. & Massague J. Transforming growth factor-β signaling in immunity and cancer. Immunity 50, 924–940 (2019). [PubMed: 30995507]
38. Taniguchi S. et al. Tumor-initiating cells establish an IL-33-TGF-β niche signaling loop to promote cancer progression. Science 369, eaay1813 (2020). Reveals a crosstalk between IL-33 expressed by SCC CSCs and myeloid cells that in response elevate TGFβ to sustain the CSC niche and drives progression.
39. Lavin Y. et al. Innate immune landscape in early lung adenocarcinoma by paired single-cell analyses. Cell 169, 750–765 e717 (2017). [PubMed: 28475900]
40. Protani M, Coory M. & Martin JH Effect of obesity on survival of women with breast cancer: systematic review and meta-analysis. Breast Cancer Res. Treat. 123, 627–635 (2010). [PubMed: 20571870]

41. Tsang NM et al. Overweight and obesity predict better overall survival rates in cancer patients with distant metastases. Cancer Med. 5, 665–675 (2016). [PubMed: 26811258]
42. Multhoff G, Molls M. & Radons J. Chronic inflammation in cancer development. Front. Immunol. 2, 98 (2011). [PubMed: 22566887]
43. Hu B. et al. Multifocal epithelial tumors and field cancerization from loss of mesenchymal CSL signaling. Cell 149, 1207–1220 (2012). [PubMed: 22682244]
44. Ellis SJ et al. Distinct modes of cell competition shape mammalian tissue morphogenesis. Nature 569, 497–502 (2019). [PubMed: 31092920]
45. Moreno E, Basler K. & Morata G. Cells compete for decapentaplegic survival factor to prevent apoptosis in *Drosophila* wing development. Nature 416, 755–759 (2002). [PubMed: 11961558]
46. Brown S. et al. Correction of aberrant growth preserves tissue homeostasis. Nature 548, 334–337 (2017). [PubMed: 28783732]
47. Hill W. et al. EPHA2-dependent outcompetition of KRASG12D mutant cells by wild-type neighbors in the adult pancreas. Curr. Biol. 31, 2550–2560.e2555 (2021). [PubMed: 33891893]
48. Alladin A. et al. Tracking cells in epithelial acini by light sheet microscopy reveals proximity effects in breast cancer initiation. eLife 9, e54066 (2020). [PubMed: 32690136]
49. Yum MK et al. Tracing oncogene-driven remodelling of the intestinal stem cell niche. Nature 594, 442–447 (2021). [PubMed: 34079126]
50. Martincorena I. et al. Somatic mutant clones colonize the human esophagus with age. Science 362, 911–917 (2018). [PubMed: 30337457]
51. Greten FR & Grivennikov SI Inflammation and cancer: triggers, mechanisms, and consequences. Immunity 51, 27–41 (2019). [PubMed: 31315034]
52. Paul D. & Hecker E. On the biochemical mechanism of tumorigenesis in mouse skin. II. Early effects on the biosynthesis of nucleic acids induced by initiating doses of DMBA and by promoting doses of phorbol-12.13-diester TPA. Z. Krebsforsch. 73, 149–163 (1969). [PubMed: 4245380]
53. Balmain A, Ramsden M, Bowden GT & Smith J. Activation of the mouse cellular Harvey-*ras* gene in chemically induced benign skin papillomas. Nature 307, 658–660 (1984). [PubMed: 6694757]
54. Tanaka T. et al. Dextran sodium sulfate strongly promotes colorectal carcinogenesis in Apc(Min/+) mice: inflammatory stimuli by dextran sodium sulfate results in development of multiple colonic neoplasms. Int. J. Cancer 118, 25–34 (2006). [PubMed: 16049979]
55. Hill W. et al. Lung adenocarcinoma promotion by air pollutants. Nature 616, 159–167 (2023). [PubMed: 37020004] Deciphers the impact of air pollutants in the recruitment of pro-tumorigenic myeloid cells in lung cancer.
56. Naik S. et al. Inflammatory memory sensitizes skin epithelial stem cells to tissue damage. Nature 550, 475–480 (2017). [PubMed: 29045388] Seminal discovery that epithelial stem cells can acquire, retain and recall long-term memories of an inflammatory experience with profound implications for chronic inflammation and cancer.
57. Del Poggetto E. et al. Epithelial memory of inflammation limits tissue damage while promoting pancreatic tumorigenesis. Science 373, eabj0486 (2021). Identification of pro-tumorigenic inflammatory memory in pancreatic epithelial cells.
58. Larsen SB et al. Establishment, maintenance, and recall of inflammatory memory. Cell Stem Cell 28, 1758–1774.e1758 (2021). [PubMed: 34320411]
59. Christ A. et al. Western diet triggers NLRP3-dependent innate immune reprogramming. Cell 172, 162–175.e114 (2018). [PubMed: 29328911]
60. Mitroulis I. et al. Modulation of myelopoiesis progenitors is an integral component of trained immunity. Cell 172, 147–161.e112 (2018). [PubMed: 29328910]
61. Chen X. et al. Endogenous expression of $Hras^{G12V}$ induces developmental defects and neoplasms with copy number imbalances of the oncogene. Proc. Natl Acad. Sci. USA 106, 7979–7984 (2009). [PubMed: 19416908]
62. Sahai E. et al. A framework for advancing our understanding of cancer-associated fibroblasts. Nat. Rev. Cancer 20, 174–186 (2020). [PubMed: 31980749]

63. Bhowmick NA et al. TGF-β signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. Science 303, 848–851 (2004). [PubMed: 14764882]
64. Hockel M. & Vaupel P. Biological consequences of tumor hypoxia. Semin. Oncol. 28, 36–41 (2001).
65. Olumi AF et al. Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. Cancer Res. 59, 5002–5011 (1999). [PubMed: 10519415]
66. Costa A. et al. Fibroblast heterogeneity and immunosuppressive environment in human breast cancer. Cancer Cell 33, 463–479 e410 (2018). [PubMed: 29455927]
67. Roulis M. et al. Paracrine orchestration of intestinal tumorigenesis by a mesenchymal niche. Nature 580, 524–529 (2020). [PubMed: 32322056]
68. Su H. et al. Collagenolysis-dependent DDR1 signalling dictates pancreatic cancer outcome. Nature 610, 366–372 (2022). [PubMed: 36198801]
69. Koliaraki V. et al. Innate sensing through mesenchymal TLR4/MyD88 signals promotes spontaneous intestinal tumorigenesis. Cell Rep. 26, 536–545.e534 (2019). [PubMed: 30650348]
70. Chang CH et al. Metabolic competition in the tumor microenvironment is a driver of cancer progression. Cell 162, 1229–1241 (2015). [PubMed: 26321679]
71. Kay EJ et al. Cancer-associated fibroblasts require proline synthesis by PYCR1 for the deposition of pro-tumorigenic extracellular matrix. Nat. Metab. 4, 693–710 (2022). [PubMed: 35760868]
72. Kessenbrock K, Plaks V. & Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. Cell 141, 52–67 (2010). [PubMed: 20371345]
73. Fane M. & Weeraratna AT How the ageing microenvironment influences tumour progression. Nat. Rev. Cancer 20, 89–106 (2020). [PubMed: 31836838]
74. McCabe MC et al. Alterations in extracellular matrix composition during aging and photoaging of the skin. Matrix Biol. 8, 100041 (2020).
75. Fiore VF et al. Mechanics of a multilayer epithelium instruct tumour architecture and function. Nature 585, 433–439 (2020). [PubMed: 32879493]
76. Eberl M. et al. Tumor architecture and notch signaling modulate drug response in basal cell carcinoma. Cancer Cell 33, 229–243 e224 (2018). [PubMed: 29395868]
77. Lee J, Abdeen AA, Wycislo KL, Fan TM & Kilian KA Interfacial geometry dictates cancer cell tumorigenicity. Nat. Mater. 15, 856–862 (2016). [PubMed: 27043781]
78. Messal HA et al. Tissue curvature and apicobasal mechanical tension imbalance instruct cancer morphogenesis. Nature 566, 126–130 (2019). [PubMed: 30700911]
79. Wu B. et al. Stiff matrix induces exosome secretion to promote tumour growth. Nat. Cell Biol. 25, 415–424 (2023). [PubMed: 36797475]
80. Bera K. et al. Extracellular fluid viscosity enhances cell migration and cancer dissemination. Nature 611, 365–373 (2022). [PubMed: 36323783]
81. Fernandez-Sanchez ME et al. Mechanical induction of the tumorigenic β-catenin pathway by tumour growth pressure. Nature 523, 92–95 (2015). [PubMed: 25970250]
82. Schlegelmilch K. et al. Yap1 acts downstream of α-catenin to control epidermal proliferation. Cell 144, 782–795 (2011). [PubMed: 21376238]
83. Guilford P. et al. E-cadherin germline mutations in familial gastric cancer. Nature 392, 402–405 (1998). [PubMed: 9537325]
84. Folkman J. Tumor angiogenesis: therapeutic implications. N. Engl. J. Med. 285, 1182–1186 (1971). [PubMed: 4938153]
85. Folkman J, Merler E, Abernathy C. & Williams G. Isolation of a tumor factor responsible for angiogenesis. J. Exp. Med. 133, 275–288 (1971). [PubMed: 4332371]
86. Beck B. et al. A vascular niche and a VEGF-Nrp1 loop regulate the initiation and stemness of skin tumours. Nature 478, 399–403 (2011). [PubMed: 22012397]
87. Verginadis II et al. A stromal integrated stress response activates perivascular cancer-associated fibroblasts to drive angiogenesis and tumour progression. Nat. Cell Biol. 24, 940–953 (2022). [PubMed: 35654839]
88. Oshimori N, Guo Y. & Taniguchi S. An emerging role for cellular crosstalk in the cancer stem cell niche. J. Pathol. 254, 384–394 (2021). [PubMed: 33634866]

89. Gadbut AP et al. Induction of the cholesterol metabolic pathway regulates the farnesylation of RAS in embryonic chick heart cells: a new role for ras in regulating the expression of muscarinic receptors and G proteins. EMBO J. 16, 7250–7260 (1997). [PubMed: 9405354]

90. Kosztaczky B. et al. Leptin stimulates endogenous cholesterol synthesis in human monocytes: new role of an old player in atherosclerotic plaque formation. Leptin-induced increase in cholesterol synthesis. Int. J. Biochem. Cell Biol. 39, 1637–1645 (2007). [PubMed: 17560160]

91. Ubellacker JM et al. Lymph protects metastasizing melanoma cells from ferroptosis. Nature 585, 113–118 (2020). [PubMed: 32814895]

92. Gur-Cohen S. et al. Stem cell-driven lymphatic remodeling coordinates tissue regeneration. Science 366, 1218–1225 (2019). [PubMed: 31672914]

93. Goto N. et al. Lymphatics and fibroblasts support intestinal stem cells in homeostasis and injury. Cell Stem Cell 29, 1246–1261 e1246 (2022). [PubMed: 35931033]

94. Palikuqi B. et al. Lymphangiocrine signals are required for proper intestinal repair after cytotoxic injury. Cell Stem Cell 29, 1262–1272 e1265 (2022). [PubMed: 35931034]

95. Zahalka AH & Frenette PS Nerves in cancer. Nat. Rev. Cancer 20, 143–157 (2020). [PubMed: 31974491]

96. Liu V. et al. Extrinsic intestinal denervation modulates tumor development in the small intestine of Apc(Min/+) mice. J. Exp. Clin. Cancer Res. 34, 39 (2015). [PubMed: 25925839]

97. Balood M. et al. Nociceptor neurons affect cancer immunosurveillance. Nature 611, 405–412 (2022). [PubMed: 36323780]

98. Weinstein JN et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nat. Genet. 45, 1113–1120 (2013). [PubMed: 24071849]

99. Insco ML et al. Oncogenic CDK13 mutations impede nuclear RNA surveillance. Science 380, eabn7625 (2023).

100. Sendoel A. et al. Translation from unconventional 5’ start sites drives tumour initiation. Nature 541, 494–499 (2017). [PubMed: 28077873]

101. Mohammad HP, Barbash O. & Creasy CL Targeting epigenetic modifications in cancer therapy: erasing the roadmap to cancer. Nat. Med. 25, 403–418 (2019). [PubMed: 30842676]

102. Dhillon AS, Hagan S, Rath O. & Kolch W. MAP kinase signalling pathways in cancer. Oncogene 26, 3279–3290 (2007). [PubMed: 17496922]

103. Jackstadt R, Hodder MC & Sansom OJ WNT and β-catenin in cancer: genes and therapy. Annu. Rev. Cancer Biol. 4, 177–196 (2020).

104. Bradner JE, Hnisz D. & Young RA Transcriptional addiction in cancer. Cell 168, 629–643 (2017). [PubMed: 28187285]

105. Oni TE et al. SOAT1 promotes mevalonate pathway dependency in pancreatic cancer. J. Exp. Med. 217, 20192389 (2020). Identifies SOAT1 as a key regulator of tumour lipid metabolism and RAS oncogenesis in the absence of *TP53* mutations.

106. LaFave LM et al. Epigenomic state transitions characterize tumor progression in mouse lung adenocarcinoma. Cancer Cell 38, 212–228 e213 (2020). [PubMed: 32707078] Discovers epigenomic programmes in lung adenocarcinoma associated with ECM remodelling and metastasis.

107. Heide T. et al. The co-evolution of the genome and epigenome in colorectal cancer. Nature 611, 733–743 (2022). [PubMed: 36289335]

108. Yang Y. et al. The pioneer factor SOX9 competes for epigenetic factors to switch stem cell fates. Nat. Cell Biol. 25, 1185–1195 (2023). [PubMed: 37488435]

109. Carey BW, Finley LW, Cross JR, Allis CD & Thompson CB Intracellular α-ketoglutarate maintains the pluripotency of embryonic stem cells. Nature 518, 413–416 (2015). [PubMed: 25487152]

110. Xiao M. et al. Inhibition of α-KG-dependent histone and DNA demethylases by fumarate and succinate that are accumulated in mutations of FH and SDH tumor suppressors. Genes. Dev. 26, 1326–1338 (2012). [PubMed: 22677546]

111. Ezhkova E. et al. Ezh2 orchestrates gene expression for the stepwise differentiation of tissue-specific stem cells. Cell 136, 1122–1135 (2009). [PubMed: 19303854]

112. Chang CJ et al. EZH2 promotes expansion of breast tumor initiating cells through activation of RAF1–β-catenin signaling. Cancer Cell 19, 86–100 (2011). [PubMed: 21215703]
113. McCabe MT et al. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. Nature 492, 108–112 (2012). [PubMed: 23051747]
114. Zhang W. et al. The bone microenvironment invigorates metastatic seeds for further dissemination. Cell 184, 2471–2486 e2420 (2021). [PubMed: 33878291]
115. Flavahan WA et al. Altered chromosomal topology drives oncogenic programs in SDH-deficient GISTs. Nature 575, 229–233 (2019). [PubMed: 31666694]
116. Galka-Marciniak P, Urbanek-Trzeciak MO, Nawrocka PM & Kozlowski P. A pan-cancer atlas of somatic mutations in miRNA biogenesis genes. Nucleic Acids Res. 49, 601–620 (2021). [PubMed: 33406242]
117. Ostendorf BN et al. Common germline variants of the human APOE gene modulate melanoma progression and survival. Nat. Med. 26, 1048–1053 (2020). [PubMed: 32451497]
118. Zeng XY et al. M2-like tumor-associated macrophages-secreted EGF promotes epithelial ovarian cancer metastasis via activating EGFR–ERK signaling and suppressing lncRNA LIMT expression. Cancer Biol. Ther. 20, 956–966 (2019). [PubMed: 31062668]
119. Shen J. et al. EGFR modulates microRNA maturation in response to hypoxia through phosphorylation of AGO2. Nature 497, 383–387 (2013). [PubMed: 23636329]
120. Shankar S. et al. An essential role for Argonaute 2 in EGFR–KRAS signaling in pancreatic cancer development. Nat. Commun. 11, 2817 (2020). [PubMed: 32499547]
121. Riha R, Gupta-Saraf P, Bhanja P, Badkul S. & Saha S. Stressed out — therapeutic implications of er stress related cancer research. Oncomedicine 2, 156–167 (2017). [PubMed: 29445586]
122. Senft D. & Ronai ZE Adaptive stress responses during tumor metastasis and dormancy. Trends Cancer 2, 429–442 (2016).
123. Zucal C. et al. EIF2A-dependent translational arrest protects leukemia cells from the energetic stress induced by NAMPT inhibition. BMC Cancer 15, 855 (2015). [PubMed: 26542945]
124. Liu L. et al. Hypoxia-induced energy stress regulates mRNA translation and cell growth. Mol. Cell 21, 521–531 (2006). [PubMed: 16483933]
125. Cordova RA et al. GCN2 eIF2 kinase promotes prostate cancer by maintaining amino acid homeostasis. eLife 11, e81083 (2022). [PubMed: 36107759]
126. Leach DR, Krummel MF & Allison JP Enhancement of antitumor immunity by CTLA-4 blockade. Science 271, 1734–1736 (1996). [PubMed: 8596936]
127. Gross G, Waks T. & Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. Proc. Natl Acad. Sci. USA 86, 10024–10028 (1989). [PubMed: 2513569]
128. Iwai Y. et al. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc. Natl Acad. Sci. USA 99, 12293–12297 (2002). [PubMed: 12218188]
129. Ng KW et al. Antibodies against endogenous retroviruses promote lung cancer immunotherapy. Nature 616, 563–573 (2023). [PubMed: 37046094]
130. Beziaud L. et al. IFNγ-induced stem-like state of cancer cells as a driver of metastatic progression following immunotherapy. Cell Stem Cell 30, 818–831 e816 (2023). [PubMed: 37267916]
131. Hopkins BD et al. Suppression of insulin feedback enhances the efficacy of PI3K inhibitors. Nature 560, 499–503 (2018). [PubMed: 30051890] Identifies systemic insulin as a key driver of antitumour therapy resistance.
132. Lin D. et al. WISER survivor trial: combined effect of exercise and weight loss interventions on adiponectin and leptin levels in breast cancer survivors with overweight or obesity. Nutrients 15, 3453 (2023). [PubMed: 37571390]
133. Chung KM et al. Endocrine-exocrine signaling drives obesity-associated pancreatic ductal adenocarcinoma. Cell 181, 832–847 e818 (2020). [PubMed: 32304665] Discovers systemic consequences of obesity as a trigger of islet cholecystokinin endocrine secretion and stimulation of cancer development.

134. Aznar Benitah S. Metastatic-initiating cells and lipid metabolism. Cell Stress. 1, 110–114 (2017). [PubMed: 31225441]
135. Pascual G. et al. Targeting metastasis-initiating cells through the fatty acid receptor CD36. Nature 541, 41–45 (2017). [PubMed: 27974793]
136. Collins N. & Belkaid Y. Control of immunity via nutritional interventions. Immunity 55, 210–223 (2022). [PubMed: 35139351]
137. Parigi SM, Eldh M, Larssen P, Gabrielsson S. & Villablanca EJ Breast milk and solid food shaping intestinal immunity. Front. Immunol. 6, 415 (2015). [PubMed: 26347740]
138. Naik S. & Fuchs E. Inflammatory memory and tissue adaptation in sickness and in health. Nature 607, 249–255 (2022). [PubMed: 35831602]
139. Levra Levron C. et al. Tissue memory relies on stem cell priming in distal undamaged areas. Nat. Cell Biol. 25, 740–753 (2023). [PubMed: 37081165]
140. Plunk EC & Richards SM Epigenetic modifications due to environment, ageing, nutrition, and endocrine disrupting chemicals and their effects on the endocrine system. Int. J. Endocrinol. 2020, 9251980 (2020). [PubMed: 32774366]
141. Lips EH et al. Triple-negative breast cancer: BRCAness and concordance of clinical features with BRCA1-mutation carriers. Br. J. Cancer 108, 2172–2177 (2013). [PubMed: 23558900]
142. Abdullah C, Korkaya H, Iizuka S. & Courtneidge SA SRC increases MYC mRNA expression in estrogen receptor-positive breast cancer via mRNA stabilization and inhibition of p53 function. Mol. Cell Biol. 38, e00463–e00517 (2018). [PubMed: 29263157]
143. Batuello CN, Hauck PM, Gendron JM, Lehman JA & Mayo LD Src phosphorylation converts Mdm2 from a ubiquitinating to a neddylating E3 ligase. Proc. Natl Acad. Sci. USA 112, 1749–1754 (2015). [PubMed: 25624478]
144. Fraterman I. et al. Association between pretreatment emotional distress and neoadjuvant immune checkpoint blockade response in melanoma. Nat. Med. 29, 3090–3099 (2023). [PubMed: 37957378]
145. Diamantopoulou Z. et al. The metastatic spread of breast cancer accelerates during sleep. Nature 607, 156–162 (2022). [PubMed: 35732738]
146. Million Women Study Collaborators. Breast cancer and hormone-replacement therapy in the Million Women Study. Lancet 362, 419–427 (2003). [PubMed: 12927427]
147. Massague J. & Ganesh K. Metastasis-initiating cells and ecosystems. Cancer Discov. 11, 971–994 (2021). [PubMed: 33811127]
148. Gonzales KAU et al. Stem cells expand potency and alter tissue fitness by accumulating diverse epigenetic memories. Science 374, eabh2444 (2021). Discovery that epigenetic memories of diverse experiences — including inflammation, migration and plasticity — can accumulate within the stem cells of tissues, with profound implications for cancer and metastasis.
149. Yu Q, Jiang M. & Wu L. Spatial transcriptomics technology in cancer research. Front. Oncol. 12, 1019111 (2022).
150. Tran M. et al. A robust experimental and computational analysis framework at multiple resolutions, modalities and coverages. Front. Immunol. 13, 911873 (2022).
151. Almagro J, Messal HA, Zaw Thin M, van Rheenen J. & Behrens A. Tissue clearing to examine tumour complexity in three dimensions. Nat. Rev. Cancer 21, 718–730 (2021). [PubMed: 34331034]
152. Calo A. et al. Spatial mapping of the collagen distribution in human and mouse tissues by force volume atomic force microscopy. Sci. Rep. 10, 15664 (2020). [PubMed: 32973235]
153. Moser AR, Pitot HC & Dove WF A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science 247, 322–324 (1990). [PubMed: 2296722]
154. Pollard P. et al. The *Apc1322T* mouse develops severe polyposis associated with submaximal nuclear β-catenin expression. Gastroenterology 136, 2204–2213 (2009). [PubMed: 19248780]
155. Smits R. et al. Apc1638N: a mouse model for familial adenomatous polyposis-associated desmoid tumors and cutaneous cysts. Gastroenterology 114, 275–283 (1998). [PubMed: 9453487]
156. Oshima M. et al. Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 87, 803–809 (1996). [PubMed: 8945508]

157. Sasai H, Masaki M. & Wakitani K. Suppression of polypogenesis in a new mouse strain with a truncated $Apc^{\Delta 474}$ by a novel COX-2 inhibitor, JTE-522. Carcinogenesis 21, 953–958 (2000). [PubMed: 10783317]
158. Di Cristofano A, Pesce B, Cordon-Cardo C. & Pandolfi PP Pten is essential for embryonic development and tumour suppression. Nat. Genet. 19, 348–355 (1998). [PubMed: 9697695]
159. Saal LH et al. Recurrent gross mutations of the *PTEN* tumor suppressor gene in breast cancers with deficient DSB repair. Nat. Genet. 40, 102–107 (2008). [PubMed: 18066063]
160. Boelens MC et al. PTEN loss in E-cadherin-deficient mouse mammary epithelial cells rescues apoptosis and results in development of classical invasive lobular carcinoma. Cell Rep. 16, 2087–2101 (2016). [PubMed: 27524621]
161. Narlik-Grassow M. et al. Conditional transgenic expression of PIM1 kinase in prostate induces inflammation-dependent neoplasia. PLoS One 8, e60277 (2013). [PubMed: 23565217]
162. Malanga D. et al. AKT1E(1)(7)K is oncogenic in mouse lung and cooperates with chemical carcinogens in inducing lung cancer. PLoS One 11, e0147334 (2016).
163. Politi K. et al. Lung adenocarcinomas induced in mice by mutant EGF receptors found in human lung cancers respond to a tyrosine kinase inhibitor or to down-regulation of the receptors. Genes. Dev. 20, 1496–1510 (2006). [PubMed: 16705038]
164. Johnson L. et al. Somatic activation of the *K-ras* oncogene causes early onset lung cancer in mice. Nature 410, 1111–1116 (2001). [PubMed: 11323676]
165. Guerra C. et al. Tumor induction by an endogenous *K-ras* oncogene is highly dependent on cellular context. Cancer Cell 4, 111–120 (2003). [PubMed: 12957286]
166. Drosten M, Guerra C. & Barbacid M. Genetically engineered mouse models of K-Ras-driven lung and pancreatic tumors: validation of therapeutic targets. Cold Spring Harb. Perspect. Med. 8, a031542 (2018).
167. Chin L. et al. Essential role for oncogenic Ras in tumour maintenance. Nature 400, 468–472 (1999). [PubMed: 10440378]
168. Adams JM et al. The *c-myc* oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. Nature 318, 533–538 (1985). [PubMed: 3906410]
169. Stewart M. et al. Conditional expression and oncogenicity of *c-myc* linked to a CD2 gene dominant control region. Int. J. Cancer 53, 1023–1030 (1993). [PubMed: 8473043]
170. Stewart TA, Pattengale PK & Leder P. Spontaneous mammary adenocarcinomas in transgenic mice that carry and express *MTV/myc* fusion genes. Cell 38, 627–637 (1984). [PubMed: 6488314]
171. Lewis BC, Klimstra DS & Varmus HE The *c-myc* and *PyMT* oncogenes induce different tumor types in a somatic mouse model for pancreatic cancer. Genes. Dev. 17, 3127–3138 (2003). [PubMed: 14681205]
172. Weiss WA, Aldape K, Mohapatra G, Feuerstein BG & Bishop JM Targeted expression of MYCN causes neuroblastoma in transgenic mice. EMBO J. 16, 2985–2995 (1997). [PubMed: 9214616]
173. Ehrhardt A. et al. Development of pulmonary bronchiolo-alveolar adenocarcinomas in transgenic mice overexpressing murine c-myc and epidermal growth factor in alveolar type II pneumocytes. Br. J. Cancer 84, 813–818 (2001). [PubMed: 11259097]
174. Adams JR et al. Cooperation between Pik3ca and p53 mutations in mouse mammary tumor formation. Cancer Res. 71, 2706–2717 (2011). [PubMed: 21324922]

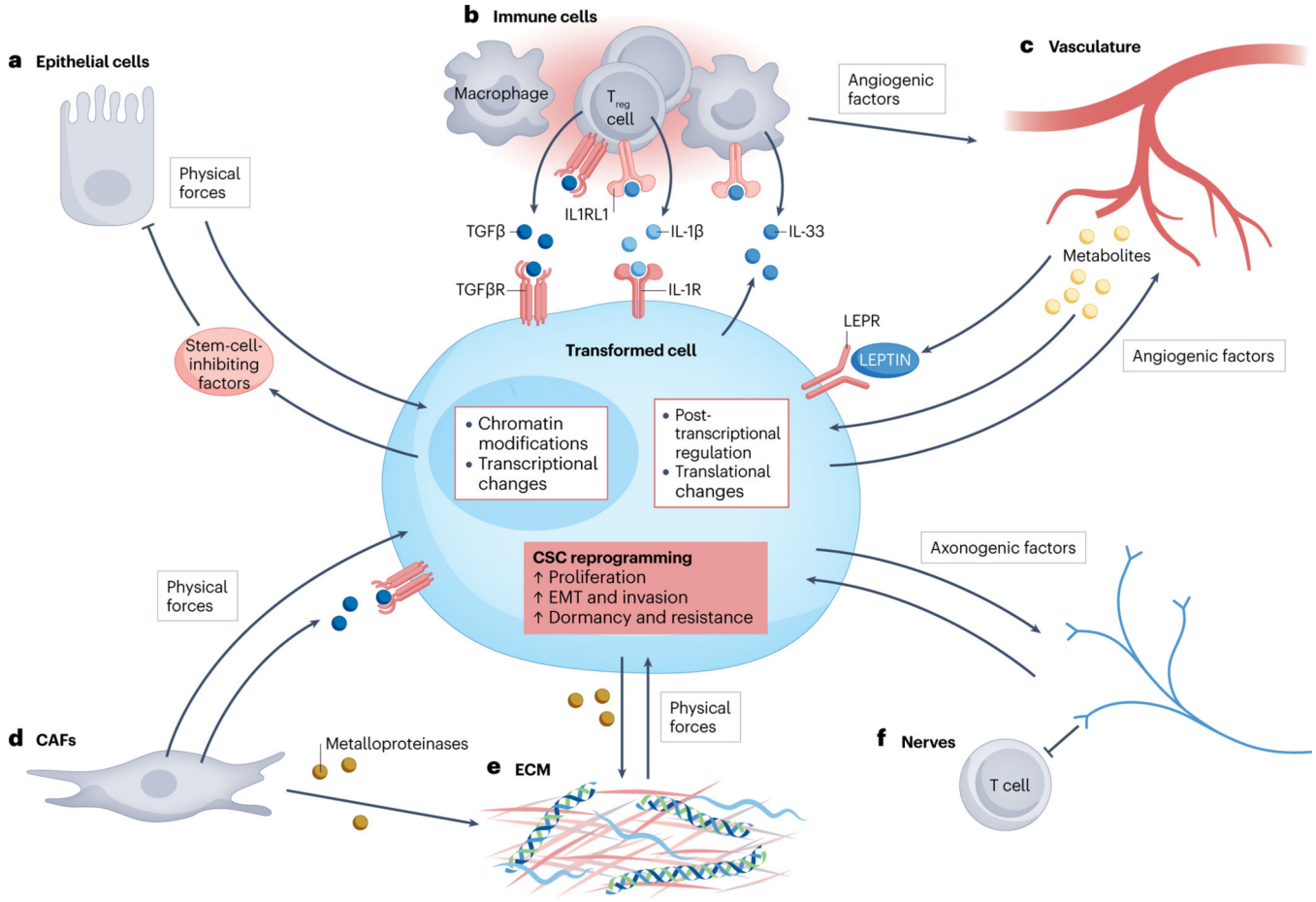

Fig. 1 |. Crosstalk between transformed cells and the microenvironment induces the cancer stem cell state.

Components of the tumour microenvironment crosstalk with the transformed epithelial cell, driving its epigenetic reprogramming towards a cancer stem cell (CSC) state. **a**, Non-transformed epithelial cells compete with transformed cells, for example, by extrusion physical forces. In colorectal cancer initiation, the release of bone morphogenic proteins from transformed cells inhibits the stem cell function of healthy epithelium. **b**, The release of immunomodulatory cytokines such as interleukin-33 (IL-33) from transformed cells stimulates immune cells, including macrophages and regulatory T cells (T_{reg} cells) to produce cytokines such as transforming growth factor-β (TGFβ) and the interleukin IL-1β, which bind to their respective receptors and reprogram transformed cells towards malignancy. **c**, TGFβ stimulates the release of angiogenic factors that drive vessel formation to increase the supply of nutrients. This also increases tissue concentration of the peptide hormone leptin, which cooperates with oncogenic RAS to drive malignancy. Notably, expression of the leptin receptor (LEPR) in transformed cells is stimulated by TGFβ. **d**, Cancer-activated fibroblasts (CAFs) also secrete cytokines that support CSC formation. **e**, In addition, CAFs as well as transformed cells themselves contribute to the remodelling of the extracellular matrix (ECM), for example through the release of metalloproteinases or by exerting physical forces. ECM remodelling affects tissue stiffness, basement membrane integrity and invasion, which collectively drive malignant transformation and progression.

f, Finally, nerves have been shown to contribute to tumorigenesis. Transformed cells secrete axonogenic factors and de novo innervation facilitates tumour growth, for example, by promoting $CD8^+$ T cell exhaustion. EMT, epithelial–mesenchymal transition; TGFβR, TGFβ receptor; IL1RL1, IL-1 receptor-like 1 (receptor for IL-33, also known as ST2).

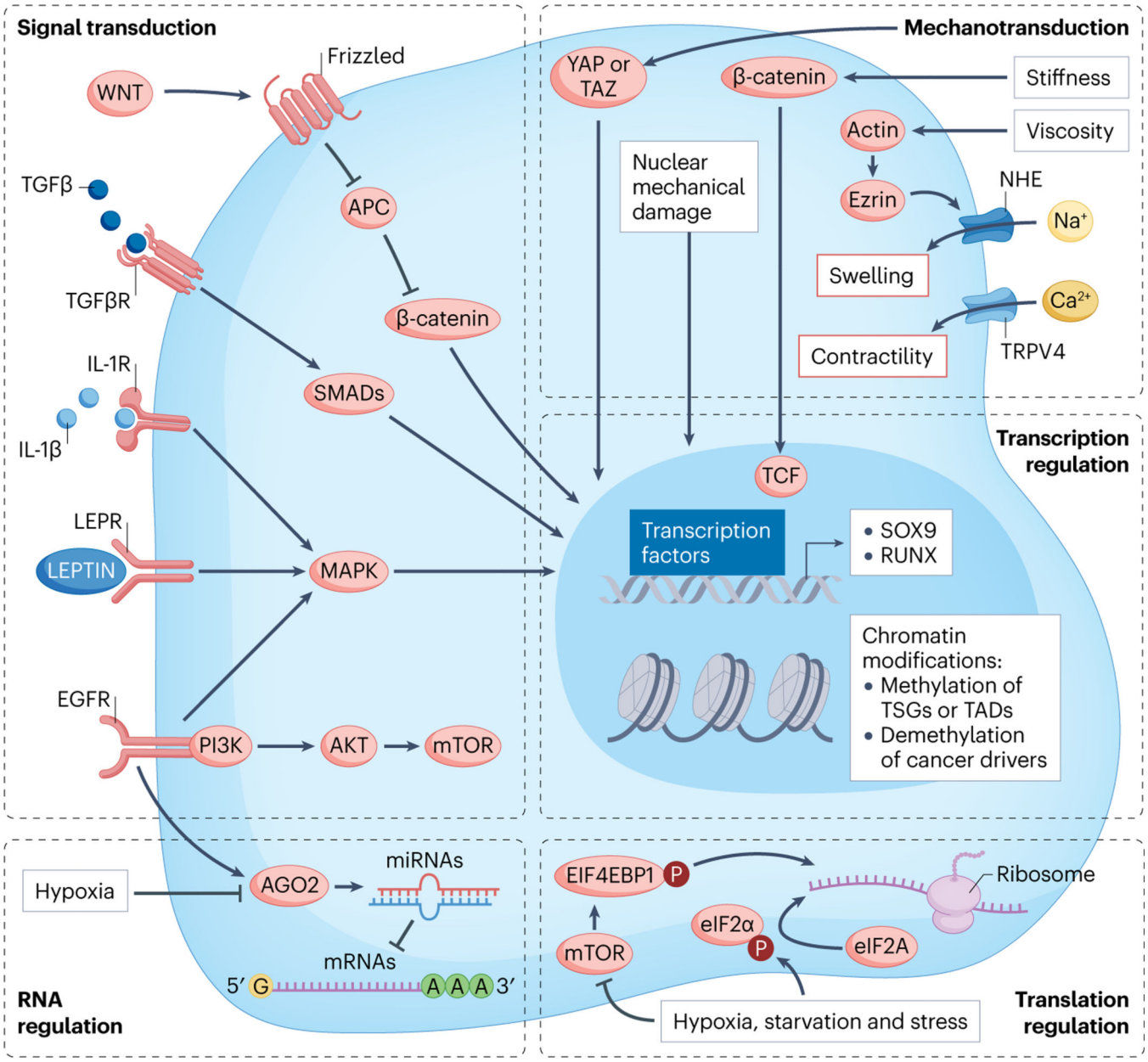

Fig. 2 |. Epigenetic changes drive tumorigenesis and progression.

Pathways involved in the epigenetic reprogramming of the cancer stem cell. Numerous external factors — including receptor–ligand interactions, physical forces, hypoxia or nutrient deprivation, and other stresses — influence malignant progression. Activation of cell surface receptors — such as frizzled, transforming growth factor-β (TGFβ) receptor (TGFβR) or the interleukin IL-1β receptor IL-1R, the leptin receptor LEPR or the epidermal growth factor (EGF) receptor EGFR — signal through β-catenin, SMAD family members or MAPK signalling. These pathways regulate transcription through the modulation of transcription factors and chromatin accessibility by modulating the methylation pattern of tumour-suppressor genes (TSGs), topology-associated domains (TADs) or cancer-driver genes. Together, these processes lead to altered transcriptional output, mediated in part

by upregulation of the transcriptional regulators SRY (sex-determining region Y)-box 9 (SOX9) or Runt-related transcription factor (RUNX). Mechanical forces are also sensed and transduced to alter transcriptional regulation through Yes-associated protein (YAP) or transcriptional coactivator with PDZ-binding motif (TAZ) signalling. Increases in extracellular viscosity are transduced through actin–ezrin, leading to increased sodium influx by Na^+/H^+ exchanger (NHE) and cell swelling. The increased sodium uptake through NHE activates calcium channel transient receptor potential cation channel subfamily V member 4 (TRPV4), calcium intake, cell contractility and invasive behaviour. Stresses encountered in the tumour microenvironment can affect tumorigenesis and progression. Hypoxia-mediated effects on argonaut 2 (AGO2) inhibits microRNA (miRNA) processing. Hypoxia, starvation or other environmental stresses affect translation through inhibition of mTOR signalling, resulting in phosphorylation of the eukaryotic translation initiation factor 4E (eIF4E) binding protein-1 (EIF4EBP1) and through phosphorylation of eIF2α, allowing eIF2A-dependent translation initiation. APC, adenomatous polyposis coli; TCF, T cell factor.

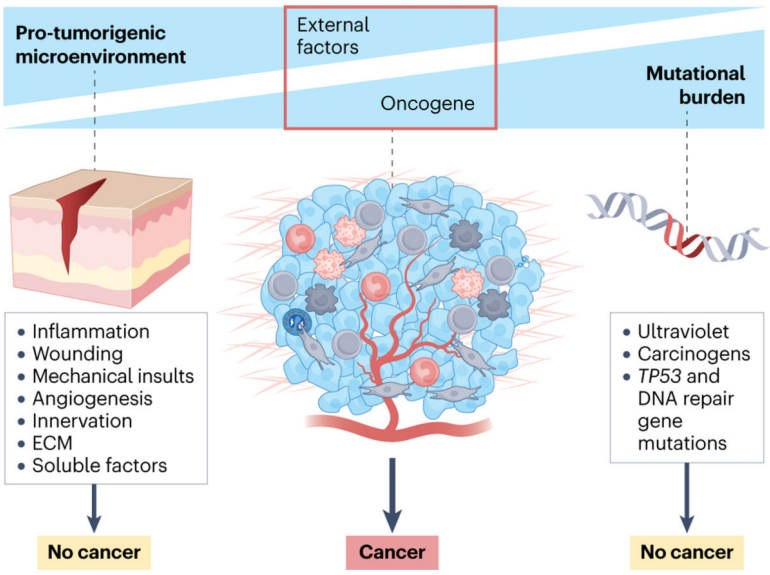

Fig. 3 |. The balance between mutations and the tumour microenvironment drives tumorigenesis and progression.

Neither external insults (left) nor mutagenesis (right) are enough to initiate a malignancy. Carcinomas are caused by a combination of external factors driving reprogramming of the epithelial cell in combination with a genetic oncogenic mutation. ECM, extracellular matrix.

Table 1 |

Mouse models of cancer driven by a single mutation

Gene	Mutation	Mouse model	Originating cancer type	Promoting stimulus	Molecular or cellular tumour microenvironment drivers	Downstream signalling[a]	Refs.
Tumour-suppressor genes							
Apc	min/+	L2549STOP whole-body mutants	Intestinal neoplasia or adenoma[153]	None/DSS	Innate immune cells	**WNT**	154
	1638N						155
	Δ716						156
	Δ474						157
	1322T						154
Pten	Knockout; hypomorphic allele	Haploinsufficiency (heterozygous deletion) in whole body	Adenocarcinomas	None/unknown	Innate immune cells	**PI3K–AKT–mTOR**	158
			Basal-like breast cancers	Inflammation, loss of E-cadherin			159,160
			Prostate cancer	Inflammation			161
Oncogenes							
Akt1	E17K	Inducible through Cre under TTF1 promoter	Lung hyperplasia	None	Unknown	**PI3K**	162
Egfr	L858R	Doxycycline-inducible expression in type II pneumocytes	Lung adenocarcinoma	None, air pollutants	IL-1β, macrophages	**MAPK–AKT–JAK**	55
	ΔL747–S752			None	Unknown		163
Kras	G12D	Whole-body spontaneous recombination and expression of G12D	Lung adenocarcinoma	None/unknown	Innate immune cells, T_{reg} cells	**ERK–MAPK,** PI3K–AKT–mTOR	164
	G12V	Cre-dependent whole-body expression	Pancreatic ductal adenocarcinoma	Caerulein			165,166
Hras	G12V	Doxycycline-inducible expression	Cutaneous squamous cell carcinoma	None	TGFβ, leptin, blood vessels	**ERK, MAPK,** LEPR–PI3K–AKT–mTOR	167
Myc	Overexpression	Immunoglobulin enhancer-induced	Lymphoma	None	Unknown	**FAK, CDKs, AMPK**	168,169
		Mammary-specific MMTV promoter	Mammary adenocarcinoma				170,171
		Tyrosine hydroxylase promoter	Neuroblastoma				172
		Surfactant protein C promoter	Lung adenocarcinoma				173
Pik3ca	H1047R	MMTV-Cre-dependent expression	Mammary adenosquamous carcinoma or adenomyoepithelioma	None	Unknown	**AKT–mTOR,**	174

[a] Primary (boldface) and secondary signalling pathways affected by the indicated mutations. AMPK, AMP-activated protein kinase; *Apc*, adenomatosis polyposis coli; CDKs, cyclin-dependent kinases; DSS, dextran sodium sulfate; *Egfr*, epidermal growth factor receptor; FAK, focal adhesion kinase; IL-1β, interleukin-1β; JAK, Janus kinase; LEPR, leptin receptor; MMTV, mouse mammary tumour virus; T_{reg} cells, regulatory T cells; TGFβ, transforming growth factor-β.