

News & views

Tumour biology

How cancer invasion takes shape

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Skin cancers resulting from distinct mutations have characteristic tissue forms and different disease outcomes. Analysing the architecture of benign and aggressive tumours reveals how mechanical forces drive these patterns.

The interplay between form and function is a cornerstone of biology, and the dismantling of normal tissue organization is a hallmark of many diseases. A long-standing question is whether changes in tissue architecture are merely a by-product of destructive diseases such as cancer, or whether they actively influence disease progression. Distinct types of skin cancer are driven by specific genetic abnormalities and give rise to distinctive tumour shapes. However, how these structures arise, and whether their specific forms affect the different outcomes of benign and malignant cancers, has been unclear. Writing in *Nature*, Fiore *et al.*¹ report an analysis of skin cancer in mice that uncovers some of the key principles involved.

The skin's outer region, called the epidermis, is made of layers of epithelial cells. Down in the basal layer at the bottom of the epidermis, stem cells divide to self-renew their population and to generate cells of the suprabasal layers above, each layer of which represents a further-differentiated state. The final stage of differentiation generates a layer of dead cells on the skin's surface, which are continually shed. The constant need to replace these dying cells creates high demand for the basal stem cells to divide and produce differentiated cells. Owing to their potency and long lifetime, these stem cells, which frequently acquire cancer-causing mutations, are the cells of origin for two common types of skin cancer. One is basal cell carcinoma (BCC), a benign tumour that does not usually spread into other tissues, and the second is squamous cell carcinoma (SCC), which is more aggressive and invasive^{2,3}.

Fiore and colleagues engineered mouse embryonic skin cells to express cancer-causing mutations. A mutation in the gene *SmoM2*

that activates the Sonic Hedgehog signalling pathway produced 'budding' skin conformations, characteristic of BCC (Fig. 1). By contrast, a mutation in the gene *HRas* that causes hyperactivity in the RAS–MAPK pathway generated skin 'folds' similar to those found in SCC. Both types of mutation caused cancer cells to proliferate faster than did their surrounding normal cells, but the mechanical properties of the

tumour environment differed profoundly between the two tumour types.

Using an impressively broad selection of methods and combining theoretical and experimental approaches, Fiore *et al.* demonstrated that the two cancer-promoting mutations had different effects on the production, turnover and stiffness of the basement membrane. This is a thin layer of specialized extracellular matrix material that separates the epidermal cells from the rest of the skin, such as the adjacent compartment below called the dermis. The authors report that the BCC-like tumours actively produced and remodelled the basement membrane, and the resulting extracellular matrix had low stiffness and was malleable in its response to forces generated by the cancer cells. By contrast, the SCC-like cells produced less basement membrane, and the absence of remodelling made the underlying extracellular matrix comparatively stiffer.

As the BCC-like tumour expanded, the compressive forces exerted by the rapidly dividing and thus crowded pool of cancer cells caused buckling of the epidermis and basement membrane, resulting in the growth

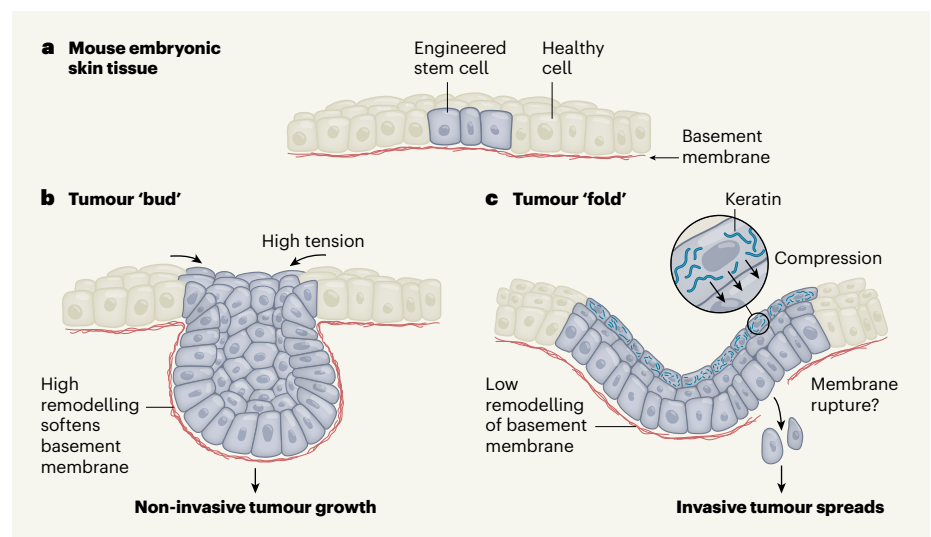


Figure 1 | Constructing the cellular architectures of cancer. **a**, Fiore *et al.*¹ engineered stem cells in embryonic mouse skin to have cancer-promoting mutations in the genes *SmoM2* or *HRas*. These mutant stem cells lie above a layer of extracellular matrix material called the basement membrane. **b**, The tumours in embryos with a *SmoM2* mutation resembled a benign, non-invasive cancer called basal cell carcinoma. These mutant cells actively produced and remodelled the basement membrane, rendering it elastic. The cancer cells generated forces as a result of cellular overcrowding, which buckled the basement membrane, creating a bud-shaped tumour, and produced tension at the boundary with the non-mutant cells. **c**, The tumours in embryos with an *HRas* mutation resembled a malignant, invasive cancer termed squamous cell carcinoma. These *HRas*-mutant cells produced less basement membrane than did the *SmoM2* mutant cells, and the membrane was rigid. The production of higher-than-normal levels of the protein keratin stiffened an upper layer of cells. Sandwiched between these two inflexible layers, the tumour could not easily dissipate the compressive forces exerted, producing an architecture of wave-like folds. The authors suggest that these forces might rupture the basement membrane, enabling invasion of the underlying tissue.

of tumour buds. However, in SCC-like tumours, the same type of force generated by proliferation and cellular crowding exerted towards the stiffer basement membrane did not result in such tissue deformation, and instead the tumour formed wave-like folds. Importantly, Fiore and colleagues report that experimentally altering the basement membrane to mimic high remodelling forced a switch from the formation of tumour folds to buds.

The authors observed specific differences between the two tumour types in the distribution of the actin and myosin protein machinery that generates cellular contractility and tension: the BCC-like cells exhibited high tension at the cellular boundary between the cancer and the neighbouring healthy tissue, however, such boundary tension was not observed for SCC-like cells. Surprisingly, however, these differences were not decisive factors in driving tumour shape. The *HRas* mutation in the SCC-like tumours caused stiffening of the skin's outermost cellular layer by generating higher-than-normal levels of keratin proteins, a hallmark of this cancer. These keratin-rich cells were stiffer than the basal stem cells^{4,5}, and sandwiched the rapidly dividing SCC-like tumour cells between this stiff layer and the rigid basement membrane. The authors showed that both of these adjacent, rigid structures were needed to produce SCC architecture (Fig. 1).

The crucial role of mechanical force in generating biological structures has been highlighted in many contexts, including in the generation of various folds of epithelial cells. In particular, epithelial cells apply actin- and myosin-based contraction to engage in a tug-of-war against the underlying basement membrane. Depending on the mechanical properties of the surrounding structures and the amount of force generated by the epithelial cells, this results in either passive buckling or active folding of the epithelial

tissue⁶. An exciting advance made with Fiore and colleagues' study is the merging of these models into a process that could be described as active buckling, in which cells exert specific contractile forces on their surroundings but also actively influence the mechanics of the underlying basement membrane to produce a specific tumour pattern.

The effect of mechanical forces on cancer has been addressed previously in other work. For example, in tubes formed of epithelial cells in the pancreas, tissue curvature is the key determinant that influences whether cancer grows inwards or outwards from such tubes⁷. One intriguing aspect of the work by Fiore *et al.* is their finding that a single cancer-promoting mutation suffices to orchestrate a stereotypical tumour architecture.

Some questions remain to be answered. What are the signalling mechanisms responsible for changes in the production of basement membrane or the generation of a stiffness gradient in the multi-layered epidermis? Human tumours have complex mutational landscapes, so it will be interesting to assess what effect other genes that promote or hinder tumour development have on the processes that influence tumour shape.

Previous studies^{8,9} of other systems provide clues about how physical changes can integrate with cellular signalling. During the development of chick feather follicle structures, mechanical compression triggers the movement of the protein β -catenin to the nucleus, where it drives a transcriptional response that enables cellular differentiation⁸. In mouse hair follicles, remodelling of the basement membrane modulates the Wnt and TGF- β signalling pathways needed to regulate stem-cell proliferation and subsequent tumour formation⁹. Thus, it is highly probable that, in cancers, mechanical forces are embedded within networks of biochemical signals, in which forces and signalling molecules might

provide constant bidirectional feedback. It will be interesting to learn to what extent such feedback loops, if present, are similar in the context of normal tissue development and cancer.

The precise functional consequences of specific tumour architectures should be a key avenue for future research. Fiore and colleagues suggest that rupture of the basement membrane as a result of tissue forces, possibly accompanied by digestion of the extracellular matrix driven by protease enzymes, is responsible for the invasion of other tissues by SCC tumours. This fascinating hypothesis could have crucial implications if alterations in tumour architecture or basement membrane stiffness could provide early signs of invasion that might be used to predict the outcome of human cancers.

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