Understanding tissue context influences on intratumour heterogeneity

Franziska Michor and Valerie M. Weaver

Although human cancers exhibit intratumour heterogeneity, the influence of the tumour environment on this property is unclear. Single basal-like mammary epithelial cells are now shown to engage a dynamic TGFBR3–JUND signalling circuit in an extracellular-matrix-dependent manner. Cell transition between the distinct gene expression states underlying this circuit alters their properties and may modulate their propensity to malignancy.

Intratumor heterogeneity is a widespread phenomenon in human cancers that can significantly impact metastatic potential and compromise treatment response[1,2]. Cells within a tumour frequently display significant differences in cell size and nuclear morphology, as well as proliferative, apoptotic, migratory and metastatic capacity, and therapeutic sensitivity[3,4]. Tumour variability could be generated by an intrinsic stochasticity in gene expression programs and by genetic and/or heritable epigenetic differences among cells[5]. Yet tumours are also composed of an evolving cellular stroma that is characterized by a progressive remodelling of the extracellular matrix (ECM)[6]. Indeed, cell–ECM adhesion can profoundly modify cell shape and tissue organization, and can dramatically regulate gene expression and cell behaviour[7]. In addition, the interactions of stromal and tumour cells are frequently altered in cancer[8]. Nevertheless, whether modified cell–ECM interactions could also contribute to intratumour heterogeneity, and how this might be achieved, had yet to be explored. In this issue, Janes and colleagues[9] investigate the interplay between the ECM, cell adhesion and heterogeneous gene expression. They demonstrate that basal-like mammary epithelial cells can exist in two distinct ECM-dependent gene expression states that are defined by TGFBR3 (transforming growth factor β receptor 3) and JUND (jun D proto-oncogene), and which can modulate cellular properties and may function in premalignancy.

The authors used three-dimensional (3D) organotypic cultures of reconstituted basement membrane and basal-like mammary epithelial cells (MECs), which typically form multicellular acinus-like tissue structures. Using transcriptomic profiling, they observed a dynamic gene expression heterogeneity among MECs that were attached to the ECM, particularly as the cells assembled acinar structures. They determined that this dynamic heterogeneity was generated by a gene expression circuit composed of two anti-correlated transcriptional programs that establish two states characterized by TGFBR3 and JUND. They also observed that these circuits are dampened by negative autoregulatory feedback mechanisms — in which TGFBR3 represses *JUND* mRNA levels and JUND itself represses *TGFBR3* and *JUND* mRNA levels (Fig. 1) — and used computational modelling to describe the dynamic coupling of the JUND- and TGFBR3-defined expression circuits. According to these findings, intracellular heterogeneity arises when this circuit is spontaneously excited, causing ECM-attached cells to oscillate transiently and asynchronously between states. These oscillations are perceived statically as gene expression heterogeneity within the acinar tissue-like structures (Fig. 1). Importantly, this oscillatory behaviour is absolutely critical for normal acinus morphogenesis, as repressing expression of TGFBR3 circuit members or enhancing JUND circuit molecules perturbs tissue architecture and leads to the formation of aberrant tissue-like structures reminiscent of high-grade premalignant mammary lesions resembling ductal carcinoma *in situ* (DCIS).

Janes and colleagues[9] noted that loss of cell adhesion in these MEC structures is associated with expression of the diagnostic cytokeratin KRT5 (keratin 5), whose heterogeneous expression is a hallmark of high-grade DCIS lesions[10]. Surprisingly, although KRT5 expression correlated with JUND in ECM-attached acinar structures, the authors detected a switch in the KRT5 and JUND co-expression in both ECM-deprived cells *in vitro* and also in a limited set of human basal-like premalignancy samples. They observed that chronic loss of cell adhesion dampens the oscillatory network and frequently results in cell death or keratinization of cells with high KRT5 and no JUND expression. They further demonstrated that the keratinization process was triggered by loss of phosphorylated RPS6 (ribosomal protein S6), shown here to promote the detachment-dependent upregulation of KRT5, and by loss of JUND, which was shown to reduce detachment-induced keratinization without directly affecting KRT5 levels. Nevertheless, they found that some cells were able to survive by engaging a juxtacrine tenascin C (TNC) deposition mechanism (Fig. 1). The authors used *in vitro* 3D cultures and computational modelling to demonstrate that TNC stabilizes the heterogeneous JUND–KRT5 expression observed

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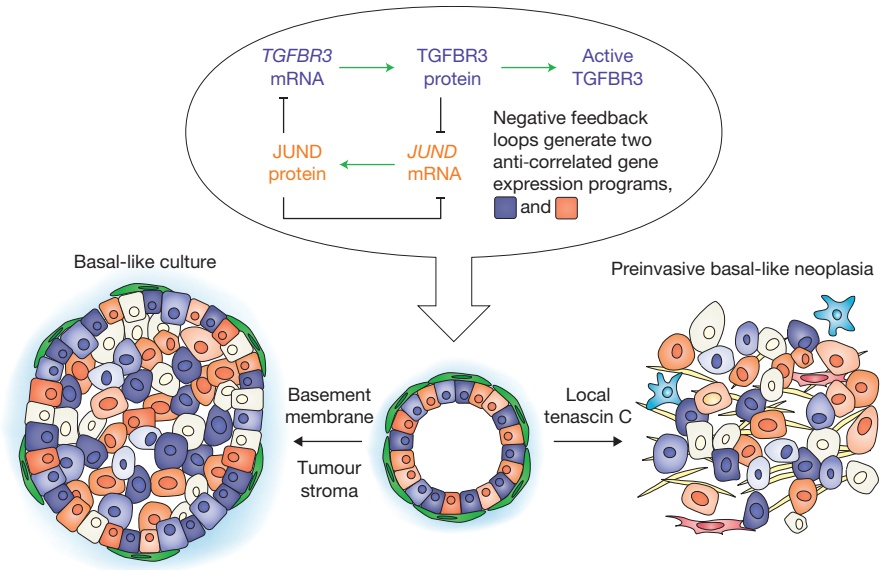

Figure 1 Tissue context and intratumour heterogeneity. Top: dynamic heterogeneity in premalignant breast cells is generated by a gene expression circuit composed of two anti-correlated transcriptional programs that establish two states characterized by TGFBR3 and JUND. Bottom: the transformation of these cells is aided by expression of tenascin C in a juxtracrine manner, which provides a critical survival signal for cells that would otherwise undergo cell death or keratinization. Different shades of purple and orange denote the extents to which cells express the TGFBR3 and JUND states, respectively. Green and blue shapes: stromal cells. Yellow and red elongated shapes: extracellular matrix.

between different cells, and performed mouse xenograft experiments to show that TNC represents a crucial survival signal for cells that would otherwise be subject to keratinization or anoikis.

Taken together, these findings raise the possibility that dynamic oscillatory gene expression circuits might be important for normal tissue morphogenesis, and also suggest that the regulatory network identified here might be modified during cancer progression. Although additional work is needed to establish the clinical significance of these findings, further delineation of these gene oscillatory mechanisms and their effects on cell behaviours such as proliferation, drug sensitivity and metastatic propensity could potentially enable the design of more effective diagnosis and intervention strategies to improve the survival of cancer patients. Moreover, such studies might further our understanding of developmental biology issues such as the intracellular signal transduction pathways regulating multicellularity and the mechanisms of generating and maintaining specific and highly specialized cell states.

Intriguingly, the findings of Janes and colleagues[9] indicate that the heterogeneous KRT5 detected in high-grade human DCIS lesions is not necessarily due to genetic selection; rather, it may reflect loss of tissue-level control of gene oscillatory networks. This suggests that efforts aimed at understanding how gene oscillatory networks are regulated may provide insight into the origins and treatment of intratumour heterogeneity. Indeed, tumours consist of individual cells organized into multicellular aggregates, and tumour cell shape and architecture together with a small coterie of molecular markers are traditionally used as pathological criteria to clinically type and grade human cancers[11]. The present work[9] raises the intriguing possibility that gene oscillatory networks might arise through and reflect the tissue context and organization of tumour structures. Such a concept would inform and impact the treatment of cancer as a tissue-level disease, and will be an interesting topic for future work.

Altered levels and expression of ECM proteins are a hallmark of many aggressive tumours, and cells that engage autocrine ECM pathways are often more metastatic[12]. The observation of Janes and colleagues that TNC promotes tumour cell survival is in line with a study finding that metastasizing breast cancer cells express TNC to support their own metastasis-initiating characteristics[12]. In this system, TNC increases the expression of stem cell signalling proteins, and metastatic outgrowth remains dependent on TNC expression derived from cancer cells until the tumour stroma takes over as a source of TNC. Taken together, these findings[9,12] strongly suggest that TNC plays an essential role in the metastatic process, which is regulated by both cancer cells themselves and the supporting microenvironment. Thus TNC might represent a promising therapeutic target for inhibiting not only early metastatic outgrowth, but also the aberrant signalling landscape between tumour stroma and cancer cells.

The present study also provides important insights into the neo-Darwinian somatic evolution of cancer cells[13]. Current thinking, which has led to many large-scale cancer genome profiling studies such as those by The Cancer Genome Atlas[14], is based on the idea that tumours initiate when a sufficiently large number of genetic driver events has arisen in a cell. Janes and colleagues paint a different, potentially complementary picture: premalignant cells can dynamically toggle between different gene expression programs that are linked by negative feedback loops. These programs, when correctly regulated, maintain normal tissue function and prevent cell outgrowth and migration. However, when dysregulated, for example by aberrant ECM signalling, this system leads cells to evade keratinization and anoikis and allows them to metastasize. Neoplastic transformation thus seems to be more intricately based on highly sensitive gene expression states that are regulated by the local microenvironment than previously thought, and provides potential novel avenues of research to better understand the natural history of human tumours.

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COMPETING FINANCIAL INTERESTS
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