The power of diversity: subclonal cooperation rewrites metastatic potential.

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A key feature of tumours is the high level of intratumoral heterogeneity. While recent evidence demonstrated that different sub-clones co-operate to support heterogeneity and boost tumour growth, little is known about their effect on metastatic progression. A new study now shows that minor cancer cell sub-clones may represent non-cell autonomous drivers in metastasis and instigate growth at distant sites of otherwise non-metastatic cancer cells. This phenomenon is shown to primarily be mediated systemically by perturbation in the metastatic tissue.

Genetic mutations in driver oncogenes are at the origin of cancer, but during their growth, cancer cells undergo genetic and epigenetic evolution resulting in substantial subclonal heterogeneity within tumours. Understanding how such heterogeneity originates and impacts on tumour behaviour, will have important implications for cancer treatment[1]. Recently, growing attention has been dedicated to the investigation of co-operative behaviour of different cancer cell sub-clones to influence the characteristics of the entire tumour[2-4]. In a previous study, Polyak and colleagues[5], using a human breast cancer model, elegantly demonstrated that tumour growth can be fostered by minor tumour cell subpopulations driving tumour growth by inter-clonal non-cell-autonomous mechanisms. The experimental approach was to force heterogeneity in a human breast cancer cell line, which normally gives rise to indolent low proliferative tumours when injected in the fat pad of mice. This cell line, was used to generate different sub-lines, then combined to create polyclonal tumours. The authors found that minor sub-clones in polyclonal tumours, particularly those expressing interleukin 11 (IL11) and FOS-induced growth factor (FIGF), were drivers of non-cell-autonomous proliferation in all cancer cells. They exploited their dominant driving effect by sustaining cancer cell heterogeneity rather than generating a cell-

autonomous expansion. This work uncovered co-operative interactions between cancer cells with different properties and demonstrate how the presence of minor sub-clones drives tumour growth. Polyclonal tumours also showed metastatic behaviour, revealing that minor sub-clones not only sustained cancer cell diversity but additionally generated new phenotypic trends.

In this issue of NCB, in a follow-up study from the same group, Janiszawska et al., dissected the origin of the dominant non-cell-autonomous switch to metastatic behaviour observed in polyclonal tumours, driven by the presence of minor IL11 and FIGF sub-clones of cancer cells. The authors thoroughly show how the non-cell autonomous driving of polyclonal tumours causes metastatic behaviour of all cancer cells by orchestrating the systemic activation and recruitment of inflammatory cells at distant metastatic sites.

Janiszewska et al., initially took advantage of the same experimental setting of the previous study[5] to generate polyclonal tumours from the non-metastatic MDA-MB-468 human breast cancer cell line. Tumours containing only 5% of IL11+ cells and 5% of FIGF+ cells turned into aggressive cancers, giving rise to polyclonal metastases also harbouring otherwise non-metastatic cells. To investigate the mechanisms by which the minor subclones instigate metastatic behaviour, they performed RNA-seq analysis of the different cancer cells clones, the IL11+ and FIGF+ driver subclones as well as labelled neutral subclones growing either as monoclonal or together in polyclonal tumours. They also analysed the tumour stroma from parental monoclonal or polyclonal primary tumours as well as the metastatic lung tissue. The results pointed to clear alterations in the local tumour microenvironment of polyclonal tumours. The fact that the IL11/FIGF clones did not expand during primary tumour growth highlights a non-cell-autonomous mechanism of promoting metastasis via changes in the local microenvironment. Interestingly, tumours containing the IL11/FIGF clones and therefore capable of seeding metastases, were also shown to trigger important changes within the lung tissue mainly linked to immune related pathways and harboured an increased number of neutrophils.

Next, Janiszewska and colleagues took advantage of an elegant experimental setting to specifically address the functional relevance of the systemic changes induced by polyclonal tumours. Here, a mixture of IL11 and FIGF doxycycline-inducible MDA-MB-

468 cells were orthotopically grown in the mammary gland, while slow growing non-metastatic patient-derived (PD) xenograft cells were grafted in the contralateral mammary gland. Only upon doxycycline administration, inducing IL11 and FIGF expression, metastases were observed, but importantly, despite showing no effect on the primary PD xenograft growth, metastatic nodules also contained patient-derived tumour cells. These data show that IL11 and FIGF from one tumour act systemically to instigate metastatic growth in otherwise non-metastatic cells from a different tumour. Since neutrophils, which are extensively reported to support metastasis[6,7], were enriched in the lungs of polyclonal tumours, the authors used a blocking antibody *in vivo* to test their contribution to the metastatic process. Neutrophil depletion decreased the number of metastases in the lungs of doxycycline treated animals, pointing at these inflammatory cells as one of the effectors of the metastatic behaviour of polyclonal tumours.

IL11, which has been previously reported as a pro-tumorigenic cytokine[8], signals on target cells through its unique IL11Rα receptor and the GP130 co-receptor leading to the activation of the JAK-STAT3 pathway. However, when the authors characterized the immune-landscape of the metastatic tissue by scRNA-seq of purified CD45+ immune cells, no expression of IL11Ra was detected on lung neutrophils. Despite the absence of the receptor, the neutrophil signature from doxycycline treated mice shows dramatic changes, pointing at an increased pro-tumorigenic activity. The scRNA-seq identifies a small pool of IL11Ra positive lung mesenchymal cells among the CD45+ pool, termed by the authors as mesenchymal stromal cells (MStrCs). This suggests a lung stromal component as a potential target of IL11 signalling. Indeed, MStrCs from lungs of DOX-induced primary tumours upregulated many neutrophils modulators such as CXCL1, CXCL12 and CXCL14. Therefore, tissue lung stromal cells may represent the link between factors released by the primary tumour and the pro-metastatic switch of neutrophils at the distant site.

This exciting work represents an important addition to the field of clonal cooperation. It shows that the creation of a metastatic tumour does not simply require changes within the primary tumour, but it orchestrates long distant perturbation of the metastatic environment where non-cell-autonomous co-operation of driver cancer cell clones allows non-metastatic cells to grow.

Janiszewska et al., also describes the presence of a previously uncharacterized pool of CD45 positive mesenchymal cells (MStrCs), potentially mediating the interaction between primary tumour and immune cells at the metastatic site. These intriguing stromal cells deserve further investigation to better understand their origin and the physiological role they might have in the tissue.

The authors used one model of breast tumour for their experimental system and more investigations are needed to understand how applicable these findings are to different cancer types. However, the fact that the mouse neutrophil and MStrCs signatures increased in both IL11 high-expressing breast cancer metastases and in metastases versus primary tumours, argue for a potential clinical relevance of the mechanism described in this study.

Figure 1: Polyclonal tumours containing IL11 expressing subclone gain metastatic potential.

Monoclonal tumours generated by the human breast cancer cell line MDA-MB-468, normally give rise to indolent low proliferative tumours. Forcing heterogeneity within cancer cells by creating different sub-clones expressing IL11 and FIGF, produces polyclonal tumours, which are now able to metastasize to the lung. A significant contribution to this polyclonal tumour feature is shown to be mediated systemically by perturbation within the metastatic tissue. Particularly, lung stromal cells expressing the IL11 receptor (IL11Ra) are shown to change their gene expression profile in presence of polyclonal tumours and release neutrophils stimulating factors. In turn, lung neutrophils from mice harbouring polyclonal tumours acquire a pro-tumorigenic signature and support metastatic growth. These changes promote the metastatic outgrowth of polyclonal metastases also including otherwise not metastatic breast tumour cells.

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