Teaching Lecture

E11. The tumour microenvironment in breast cancer

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Tumours are aberrant organs that contain host cells and non-cellular components in addition to the cancer cells [1]. The tumour microenvironment consists of extracellular matrix (ECM) components, fibroblasts, adipocytes, cells of the vascular system, cells of the immune system, growth and differentiation factors, chemokines, cytokines and metabolites. The cancer cells themselves have a major influence on the local environment. The composition of the microenvironment varies with tumour stage and influences functions of the cancer and stromal cells. The stromal components may have tumour inhibiting or promoting functions, or both, depending on the context. Indeed, a breast cancer stroma-derived gene expression signature is associated significantly with prognosis [2].

Carcinoma-associated fibroblasts (CAFs) stimulate cancer cell growth, inflammation, angiogenesis and invasion and share characteristics with embryonic fibroblasts or mesenchymal progenitors. Such activated fibroblasts are largely responsible for tumour-associated changes in the ECM, which are caused by increased synthesis and additional post-translational modifications of ECM molecules and by increased remodelling of ECM proteins by matrix metalloproteinases (MMPs) [3]. For example, increased biosynthesis and remodelling of the fibrillar collagen type I is a consistent feature of tumours and is necessary for angiogenesis [4]. The architecture of fibrillar collagen also changes: Whereas collagen fibres are orientated in parallel to normal epithelial cells, in tumours the fibres are mostly perpendicular to the tumour border. This altered architecture of the collagen fibres enables cells to migrate along the collagen fibres, promoting cell invasion. Fibronectin is alternatively spliced in tumours, leading to expression of developmental isoforms. These changes in the ECM result in increased striffness in tumours compared to normal tissues. Thus tumours can be detected as hard nodules distinct from the normal surrounding tissue. Recent research indicates that the relative stiffness of a tissue has major effects on cellular functions: as the tension of non-malignant, mammary epithelial cells approaches that of tumours, these normal cells disorganize and initiate invasive programs. Moreover, collagen cross-linking produces ECM stiffening that together with oncogenes promote invasive behavior.

Immune-cell infiltrates are present in most, if not all, solid tumours. This indicates that the genetic events that lead to cancer also trigger immune responses [5]. Indeed, oncogene activation hijacks the immune and inflammation-based program nodes for tissue remodeling and regeneration. The most abundant infiltrating cells are myeloid cells that regulate metastasis and angiogenesis, in addition to down-regulating the immune response against the cancer cells. Tumour-associated macrophages (TAMs) resemble M2 macrophages, which promote tumour growth, invasion, metastasis and angiogenesis, and suppress cytotoxic T cell activity against tumours by releasing cytokines, growth factors, matrix-degrading enzymes and angiogenic factors. Accumulation of TAMs is associated with poor prognosis. The so-called myeloid-derived suppressor cells (MDSCs), which consist of immature and activated neutrophils and monocyte/macrophages, accumulate systemically, in the bone marrow, spleen, and peripheral blood, and at the invasive edge of tumours with increasing tumour progression. MDSCs also may promote angiogenesis and promote resistance to anti-angiogenic therapy. They also influence the anti-tumour immune response by producing reactive oxygen species that inhibit proliferation and function of dendritic cells, natural killer cells, B cells and cytotoxic T lymphocytes and by inducing regulatory T cells (TRegs) [6,7].

Tumour blood vessels are disorganised. Instead of clear arterioles, capillaries and venules, the tumour vasculature is irregular, dilated, and can have dead ends, with loosely associated perivascular cells. These changes result in abnormal blood flow and increased vascular permeability. The onset of tumour angiogenesis, known as the 'angiogenic switch', ensures tumour growth, but can occur at different tumour progression stages. Although cancer cells can secrete pro-angiogenic factors, the tumour-infiltrating myeloid-derived cells are an important source. These cells are recruited to tumours by factors secreted by hypoxic cancer cells, including vascular endothelial growth factor (VEGF) and stroma-derived factor 1/chemokine CXC ligand 12 (SDF1/CXCL12). Myeloid-derived cells (including monocytes, macrophages, neutrophils, mast cells, immature dendritic cells and MDSCs), which are often located in close proximity to tumour blood vessels, are implicated in tumour angiogenesis.

Specialized microenvironments within tumours, often referred to as 'niches', confer distinct functions to the

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cancer cells. Endothelial cells likely are important in forming tumour-promoting niches. There is also strong evidence that primary tumours set up the distant microenvironment for colonisation, primarily through recruitment of bone marrow-derived cells and ECM remodeling. Just as there are tumour-promoting microenvironments, or niches, there is also evidence that certain microenvironments may promote tumour dormancy. Thus, macromolecules that are involved in maintaining normal tissue architecture and keeping cancer cells quiescent functionally behave as "tumour suppressors". Moreover, organ specificity of metastasis not only is specified by specific gene expression of the disseminating cancer cells, but also reflects the microenvironments of certain organs that fit the requirements of specific cancer cells.

Cancer cells frequently show marked genetic instability, opening up the opportunity for different microenvironments to select for cells with distinct genotypes [8]. Exposure to a variety of stromal components (e.g., oxygen levels or metabolites, as well as adhesion molecules, ECM components, growth factors and chemokines) may also alter gene expression in cancer cells. In particular, microenvironments with low oxygen or low pH or the margin where the tumour cells interface with stroma edge appear to be capable of inducing or selecting for cancer cells with highly invasive properties. The abnormal organization of the tumour tissue also reduces the access of anticancer drugs to the cancer cells. Indeed, cancer cells in hypoxic areas of tumours are often refractory to therapy. This may be the result of anti-angiogenic therapy, that paradoxically can result in recurrent tumors that are even more aggressive. Whereas, in normal tissues, cells are within a few tens of microns from a blood capillary that enables efficient drug access, under conditions of rapid cancer-cell proliferation, cells may outgrow the accompanying angiogenesis, resulting in increased distance from the blood vessels and impaired drug delivery. Overcoming chemoresistance can be achieved through targeting of the stroma [9], resulting in better drug delivery. Experiments in mouse models of human cancer suggest these alterations in the microenvironment result in normalisation rather than destruction of the tumour vasculature.

Conflict of interest statement

None declared.

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References

[1] Egeblad, M., Nakasone, E.S., Werb, Z. (2010). Tumors as organs: complex tissues that interface with the entire organism. Dev Cell 18, 884–901.

[2] Finak, G., Bertos, N., Pepin, F., Sadekova, S., Souleimanova, M., Zhao, H., Chen, H., Omeroglu, G., Meterissian, S., Omeroglu, A., *et al.* (2008). Stromal gene expression predicts clinical outcome in breast cancer. Nat Med 14, 518–527.

[3] Kessenbrock, K., Plaks, V., and Werb, Z. (2010). Matrix metalloproteinases: Proteolytic regulators of the tumor microenvironment. Cell 141, 52–67.

[4] Erler, J.T., and Weaver, V.M. (2009). Three-dimensional context regulation of metastasis. Clin Exp Metastasis 26, 35–49.

[5] Mantovani, A., Allavena, P., Sica, A., and Balkwill, F. (2008). Cancer-related inflammation. Nature 454, 436–444.

[6] Colotta, F., Allavena, P., Sica, A., Garlanda, C., and Mantovani, A. (2009). Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis 30, 1073–1081.

[7] Gabrilovich, D.I., and Nagaraj, S. (2009). Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 9, 162–174.

[8] Polyak, K., Haviv, I., and Campbell, I.G. (2009). Co-evolution of tumor cells and their microenvironment. Trends Genet 25, 30–38.

[9] Nakasone, E., Askautrud, H.A., Kees, T., Plaks, V., Ewald, A.J., Rasch, M.G., Tan, Y.X., Qin, J., Fein, M., Park, J., Sinha, P., Bissell, M.J., Frengen, E., Werb, Z. and Egeblad, M. (2012). Imaging tumor–stroma interactions during chemotherapy reveals microenvironmental contributions to chemoresistance. Cancer Cell, in press.