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A whole range of complex interactions develops between growing malignant neoplastic cells and non-malignant stromal elements of a tumour. The tumour microenvironment includes a number of interrelated elements: fibroblasts, macrophages and dendritic cells, endothelial cells, pericytes, inflammatory cells and extracellular matrix components. All these elements are actively involved in tumour growth and progression and angiogenesis, and can either promote or inhibit these processes. Understanding of the relationship between the various components of the tumour microenvironment and tumour cells may allow the introduction of combination drug therapy having different target points which gives hope of reducing the doses of medicines, and thus reducing their toxicity while increasing their efficiency.

Key words: tumour growth, microenvironment, macrophages, angiogenesis.

The tumour and its microenvironment – a complicated interplay

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Introduction

The emergence and growth of malignant tumour is a sequence of consecutive events which often lead to the development of multiple distant metastases and the patient's death. Tumour formation usually begins as a genetic change in a single cell, such as mutation activating an oncogene or deactivating a tumour suppressor gene. If the mutation is perpetuated, it induces the proliferation of cells with new properties which are no longer subject to the body's control system. The proliferation and spread of cancer cells is closely linked to the effect of stromal elements, i.e. the tumour environment in which it grows. Growing evidence suggests that the tumour microenvironment is more than just a passive support structure for proliferating cancerous cells and, in fact, plays a significant and active role in tumour progression. A better understanding of mutual associations and interdependencies between the cells of the growing tumour and elements of the tumour microenvironment may contribute to the development of improved treatments for cancer patients. Future cancer therapy will most likely be combination treatment based on conventional chemotherapeutic agents possessing a direct cytotoxic effect against tumour cells and substances targeted specifically against stromal elements promoting tumour growth.

Role of the microenvironment in tumour growth

Growing tumour tissue is a complex system incorporating a multitude of relationships and loops between its various elements [1, 2]. Any attempt to explore tumour biology only by investigating the properties of tumour cells is an incomplete and inadequate approach which disregards the significant influence of non-cancerous elements of the tumour's immediate environment on its growth and progression. The tumour microenvironment, i.e. connective tissue and inflowing cells within which tumour grows, is a complex system of different interconnected elements including:

- connective tissue fibroblasts,
- macrophages and dendritic cells,
- endothelial cells and pericytes,
- inflammatory infiltration cells,
- elements of the extracellular matrix.

All the elements are actively involved in tumour growth and progression and may either promote or inhibit the processes enumerated above [1, 2].

Connective tissue fibroblasts

Fibroblasts are the most abundant cell population within the tumour microenvironment. Cancer associated fibroblasts (CAFs) may develop in the tumour growth site either from preexisting fibroblasts or from mesenchymal stem cells (STEM) from the marrow [2]. Cancer associated fibroblasts demonstrate the expression of SMA (smooth muscle actin), i.e. acquire properties similar to myofibroblasts, which play a central role in wound healing. Cancer asso-

ciated fibroblasts are cells promoting tumour growth: they stimulate the proliferation of cancer cells and facilitate tumour progression, as well as being involved in the initiation of invasion. Studies in mice showed that the blockade of the PDGF (platelet-derived growth factor) receptor in stromal fibroblasts in cervical cancer delays cancer progression [3]. Medications acting by inhibition of fibroblast activity may in the future support conventional methods used in the treatment of malignancies.

Macrophages

Macrophages are cells derived from peripheral blood monocytes, present in tissues. Macrophages have cytotoxic and phagocytic properties and participate in inflammatory processes. Macrophages presence outside blood vessels is an effect of local secretion of chemotactic factors in the surrounding tissue. The most important of them are M-CSF (macrophage colony-stimulating factor), VEGF (vascular endothelial growth factor), MCP-1 (monocyte chemotactic protein) and angiopoietin [4, 5]. Under the influence of different signals from the tumour microenvironment, monocytes migrating out of a vessel towards their target tissue site may be differentiated either into M1 macrophages (classic macrophage activation resulting in macrophages with predominantly anti-tumour activity) or M2 macrophages (alternative activation mechanism producing macrophages with pro-tumour activity) [5, 6]. Whether a monocyte is ultimately transformed in the tissue into the M1 anti-tumoural macrophage phenotype or the M2 pro-tumoural phenotype depends on the type of stimulation induced by the tumour microenvironment.

Macrophages with the M1 phenotype develop as a result of activation by selected cytokines, e.g. IFN-γ (interferon γ) secreted during the inflammatory process and inducing classic monocyte/macrophage differentiation. Interferon γ has pro-inflammatory and anti-tumour activity, and the capacity to eliminate pathogenic microorganisms. M1 macrophages:

- secrete pro-inflammatory cytokines: TNF (tumour necrosis factor), IL-1, IL-6 and IL-12 (interleukins 1, 6 and 12), thus promoting inflammatory and anti-tumour responses,
- have an enhanced capacity for generating active oxygen forms,
- exhibit potent cytotoxic properties: have the ability to destroy tumour cells directly due to the so-called "antibody-independent cytotoxicity" mechanism,
- have the capability of eliminating malignant cells via "antibody-dependent cytotoxicity" (antibodies coat tumour cells, while the macrophage binds to the Fc portion of the antibody on the tumour cell). The process of tumour cell elimination via this mechanism is much more efficient than the previous one. Tumour cell destruction causes the release of a range of antigens which are presented by antigen-presenting cells (APCs) in the lymph nodes, which contributes to tumour elimination.

M2 phenotype macrophages (tumour associated macrophages, TAMs) develop as a result of monocyte activation by cytokines, particularly those secreted by tumour cells: IL-4 and IL-10 (interleukins 4 and 10). They mediate the so-called alternative differentiation of monocytes into M2 phenotype macrophages. The cells possess anti-inflammatory properties and cooperate with tumour cells, promoting their growth. M2-type macrophages secrete a range of chemokines and growth factors including PDGF (platelet-derived growth factor), EGF (epidermal growth factor), TGF-β (transforming growth factor β), M-CSF and IL8 [5-7].

Growth factors, especially PDGF and EGF secreted by M2 macrophages, stimulate tumour cells but, at the same time, promote angiogenesis and the proliferation of pericytes around new blood vessels. Pericytes stabilize the structure of new vessels. Moreover, M2 macrophages produce VEGF-C, which plays a part in stimulating proliferative processes.

M2 macrophages produce IL-10, a cytokine suppressing and deregulating the cytotoxic activity of cytotoxic T-cells (CD8+) and natural killer (NK) cells, reducing their anti-tumour action [9]. Their distinctive feature is also the production of proinflammatory cytokines (IL-1, IL-6, IL-12, TNF) in much lower quantities in comparison with M1-type macrophages. They also have a decreased capacity to generate active oxygen forms. Other important properties of M2 macrophages include production and secretion of substances degrading elements of the extracellular matrix, chiefly matrix metalloproteinases 3 and 9 (MMP3 and MMP9) and the plasminogen activator, which can promote tumour growth and the development of metastases.

The final activity of M2 macrophages depends on the modulatory effect of tumour cells. Epidermal growth factor-stimulated tumour cells release M-CSF (CSF-1), which results in continued differentiation of inflowing monocytes into macrophages with the phenotype M2. The process further stimulates tumour growth. Consequently, a specific loop develops between M2 macrophages and tumour cells, intensifying the activity of both cell types.

A crucial factor determining the ultimate effect in the tissue is the M1/M2 macrophage ratio; however, the majority of macrophages within the tumour tissue assume phenotype M2. For some tumour types, an association was established between increased percentages of M2 cells (TAMs) in the tumour tissue and shorter patient survival times.

It is also possible that macrophages infiltrating the tumour can be a part of the tumour's active strategy of avoiding the immune response. As shown in our studies, macrophages of this type demonstrate strong expression of the RCAS1 protein [10]. The protein, as studies by Sonoda *et al*. [11] suggest, may play a major role in suppressing the immune response targeted against the tumour.

Endothelial cells and pericytes

For any tumour to grow to more than several millimetres in diameter, it must create a new vascular network to ensure appropriate supply of oxygen and nutrients to the neoplastic tissue. The growth of new blood vessels, a process called angiogenesis, is induced by the tumour's pro-angiogenic factors including VEGF, angiopoietin 1 and 2 [12, 13], as well as several other important factors: FGF-β (fibroblast growth factor β), TGF-β and IL-8 [14]. In normal physiological conditions the above factors trigger angiogenesis at wound sites and in regenerative processes [1]. New vessel creation also occurs during embryogenesis [15]. In pathological conditions,

besides tumour growth, angiogenesis is an important mechanism in chronic inflammatory processes [2]. Angiogenesis within the tumour tissue is a multi-step process involving vascular endothelium, pericytes, smooth muscle cells and inflowing inflammatory cells, as well as tumour stromal elements [14]. VEGF-A, the most important pro-angiogenic factor, acts via VEGFR1 and R2 receptors. The presence of VEGFR1 additionally stimulates the activity of metalloproteinases which may "loosen" the stroma, thus promoting vascular growth, as well as producing a chemotactic effect on monocytes and inducing their transformation into TAMs (M2-type tumour associated macrophages). Oxygen supply to a specific tumour area is one of the factors determining the type of monocyte activation and, consequently, the number of M2 macrophages in a particular area. M2 macrophages are able to survive in poorly vascularized and oxygen-deficient areas where they produce and secrete cytoki-nes (VEGF and IL-8) and metalloproteinases (MMP-3 and MMP-9), which induce and sustain angiogenesis and lymphangiogenesis [2, 5]. Similar action is performed by neutrophils, which also have the ability to produce pro-angiogenic mediators [2]. It is evident, then, that cells associated with the inflammatory reaction not only fail to suppress the tumour but may even promote its growth by stimulating the process of angiogenesis.

Reduced blood and oxygen supply to tumour cells can slow down neoplastic growth. Attempts are undertaken to interrupt angiogenesis (i.e. new blood vessel growth within the tumour), destroy existing vessels supplying the tumour and reduce the concentration of pro-angiogenic factors within the tumour. There are several substances available for clinical application, including bevacizumab, a monoclonal antibody against VEGF, or small-molecule inhibitors of tyrosine receptor kinases such as sunitinib, sorafenib or pazopanib, whose anti-tumour properties are associated, *inter alia*, with signal blocking via VEGF receptors [13]. Interferon α was also demonstrated to suppress the activity of pro-angiogenic molecules [16]. Anti-vascular action was also confirmed in TNF-α [17].

Drugs inhibiting angiogenesis and lymphangiogenesis, as well as anti-vascular agents, are expected to become a useful adjunct to existing conventional cancer treatments.

Inflammatory infiltration cells

Not so long ago, the presence of inflammatory infiltration within and around the tumour site was believed to be a manifestation of the body's defence against cancer, and as such, a good prognostic indicator. It turned out, however, that the lymphocyte response against the growing tumour is too weak and insufficient to stop tumour growth in the majority of cases. Evidence is mounting that developing tumours are in fact able to use inflammatory cells to promote their own growth. Rapid tumour expansion may lead to the development of necrotic foci within the tumour site. In response to that process, tumour and stromal cells adjacent to necrotic areas may liberate cytokines which exert a chemotactic influence on monocytes and granulocytes (GM-CSF) and factors stimulating tumour growth and angiogenesis, i.e. VEGF, FGF-β, TNF and EGF [2]. Inflammatory cells accumulating in the tumour's vicinity support the inflammatory process and secrete cytokines and growth factors promoting the process of angiogenesis [4]. Inflowing neutrophils and macrophages are the main sources of metalloproteinases (MMP3 and 9) which degrade the tumour stroma and, consequently, facilitate local tumour spread [2]. Eosinophils, cells which are often present in small numbers among other cells of the inflammatory infiltration, are also implicated in the pro-angiogenic mechanism because of their ability to secrete FGF-β, IL-6 and TGF-β.

Elements of the extracellular matrix

Extracellular matrix elements, i.e. proteoglycans, glycosaminoglycans and type-I collagen, are a basis for all other components of the tumour microenvironment and for multiplying tumour cells. They also play an important role in facilitating the diffusion of various substances, drugs in particular, within the tumour site [18]. Loosening of the extracellular matrix structure by enzymatic digestion may be a factor precipitating the distribution of anti-neoplastic agents; on the other hand, it may contribute to the process of angiogenesis within the tumour and to the initiation of tumour cell migration. Enzymes involved in the degradation of the extracellular matrix are stromal metalloproteinases, especially MMP3 and MMP9. They are secreted, among others, by excited M2 macrophages. They cause degradation of the basement membrane and promote monocyte migration, as well as supporting angiogenesis. Urokinase plasminogen activator [19] is also importantly implicated in the formation of metastases.

Summary

The tumour environment comprises multiple interconnected elements which may either promote or suppress neoplastic development. Tumour cells modify the functions of non-tumour elements of the tumour microenvironment: endothelial cells, pericytes, fibroblasts, dendritic cells and inflammatory infiltration cells, macrophages in particular, thus creating favourable conditions for tumour growth. At the same time, modified cells of the microenvironment promote neoplastic growth, tumour infiltration of surrounding tissues and the development of metastases. Therefore, gaining better insight into the tumour microenvironment and mutual relations existing between its elements and tumour cells seems to be an important step towards better understanding of tumour biology. Recognition of these complicated relationships between different elements of the tumour microenvironment and neoplastic cells may potentially contribute to the introduction of combination therapy with drugs targeting various constituents of the tumour tissue. Such treatment gives hope for reducing anti-tumour drug doses, and thus lowering toxic effects, while increasing therapeutic efficacy.

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