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Review

Neutrophil-mediated tumour angiogenesis: Subversion of immune responses to promote tumour growth

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ABSTRACT

Neutrophils are rapidly responding, phagocytes that are an essential part of the host innate immune response to invading micro-organisms. Along with other leucocytes they also play a key role in directing repair at sites of tissue damage. Neutrophils accomplish many of their biological functions by releasing enzymes, anti-microbial agents and cytokines when stimulated to degranulate. There is now increasing evidence to show that tumours are able to recruit neutrophils by secreting a number of tumour cell or stromal-derived chemoattractants. Once within the tumour microenvironment neutrophils, like macrophages, are polarised into a pro-tumour phenotype that can foster tumour growth by secreting factors that directly influence tumour cell proliferation, drive immunosuppression and promote tumour angiogenesis. In this review we discuss the likely mechanisms by which neutrophils are recruited into the tumour and then elaborate on how these cells may induce tumour vascularisation by the secretion of powerful pro-angiogenic factors.

1. Introduction

Tumours generally arise from genetically modified cells undergoing uncontrolled cell division. Eventually, these cells form a small tumour mass that is unable to grow beyond the size of a few millimetres because the cell growth out-strips the supply of nutrients and oxygen, and the removal of waste metabolites. In order to grow and expand further the tumour must redirect the existing vasculature to ensure a plentiful supply of nourishment and removal of catabolites. The process of generating new blood vessels from an existing vascular bed is known as angiogenesis, and this occurs in such normal physiological processes as menstruation and wound healing where an increase in tissue vascularisation is required. Angiogenesis is a complex, multi-stage process that involves the dissolution of the existing vasculature and surrounding extracellular matrix (ECM), proliferation and movement of endothelial cells and pericytes towards an avascular site, followed by ECM and vascular remodelling, stabilisation and maturation to form a functional blood vessel. This process is regulated by a number of important pro- or anti-angiogenic factors, and it is the balance between these factors that ultimately controls the angiogenic switch.

Data accumulating over many years have revealed a number of mechanisms that tumours employ to initiate and direct angiogenesis (reviewed in [1]). However, it is the role that tumour-associated myeloid cells play in this process that has gained considerable attention, in particular that of tumour-associated macrophages (TAM) [2]. In contrast, the roles that neutrophils contribute to this process have, until recently, been largely ignored. Like macrophages, neutrophils are phagocytes that are key players in the innate immune response. Neutrophils are the most numerous leucocyte found in the body and are considered the first line of defence against invading organisms. They can be likened to military rapid-response units and are quickly mobilised to sites of infection or damage to combat pathogens or restore tissue homeostasis. Neutrophils are packed with granules that contain a plethora of enzymes, anti-microbial agents, cytokines and growth factors, and upon degranulation this arsenal of molecules mediates microbial killing, tissue remodelling and repair [3].

An increase in number of peripheral blood neutrophils in cancer patients compared to healthy controls has been observed in several clinical studies. This may not be surprising given the inflammatory nature of the tumour microenvironment and the increased expression of circulating colony-stimulating factors that mediate leucocyte release from the bone marrow (reviewed in [4,5]). However, it is only relatively recently that tumour-associated neutrophils (TAN) were identified and found to impact on disease progression and severity. Early studies reported increased numbers of neutrophils in biopsies of patients with colon adenocarcinoma, myxofibrosarcoma, glioma and bronchioloalveolar carcinoma compared to the surrounding non-diseased tissue or from that of control subjects [6–9]. Moreover, Bellocq et al. found that the elevated number of neutrophils in bronchioloalveolar carcinoma positively

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Table 1
Association of TAN with clinical outcome in human cancers.

Tumour type	Neutrophil marker	Clinical outcome	Mechanism of recruitment	Reference
Colorectal carcinoma	CD66b	Shortened patient survival	ND	[105]
Hepatocellular carcinoma	CD66b CD66b CD15	Poor patient survival	CXCL16 CXCL5 CXCL1, CXCL2, CXCL3, and CXCL8	[106][31][83]
Melanoma	CD66b	Poor prognosis	Expression of pSTAT3	[107]
Head and neck squamous cell carcinoma (HNSCC)	CD66b MPO	Poor patient survival in advanced disease	CXCL8 MIF	[10] [37]
Non-small cell lung cancer (NSCLC)	CD66b	Poor clinical outcome	ND	[108]
Broncheoalveolar adenocarcinoma	Morphological identification	Poor patient survival	CXCL8	[8]
Renal cell carcinoma	CD66b	Increased tumour size and short recurrence-free, cancer-specific and overall survival	ND	[109]
Non-Hodgkins lymphoma	CD15, elastase, nuclear morphology	Indirect link, neutrophils were main source of APRIL which is linked to patient survival	ND	[110]
Gastric adenocarcinoma	CD15	Lymph node/distant metastasis, tumour stage and patient prognosis	ND	[11]

ND, not determined.

correlated with poor patient survival, suggesting that the presence of intratumoural neutrophils may have a profound effect on clinical outcome [8]. Similar findings have recently been reported for other tumours. Using immunohistochemical analysis for the neutrophil-specific marker CD66b, Trellakis et al., observed marked neutrophil infiltration into head and neck squamous cell carcinomas (HNSCC) which was significantly associated with poor survival in patients with advanced disease [10]. In addition, Zhao et al. found elevated numbers of CD15-positive neutrophils in gastric adenocarcinoma that correlated with poor prognosis and patient outcome [11]. High levels of intratumoural neutrophils have been observed in clear cell renal carcinoma, non-small-cell lung carcinoma, hepatocellular and colorectal carcinoma, melanoma and non-Hodgkin lymphomas; all of which showed TAN as an independent prognostic factor linked with poor patient survival or relapse (see Table 1).

An increasing number of studies using diseased human tissue, a variety of tumour-bearing animal models and multi-cell-based *in vitro* techniques (Fig. 1) have been used to investigate the role of TAN in tumour progression, and there is now much evidence to implicate TAN in influencing several key aspects of tumourigenesis and tumour growth [4,5]. Moreover, it now appears that the phenotype of TAN can be polarised into two distinct sub-groups (N1 and N2) similar to that described for TAM [12]. Fridlender et al., recently showed that the tumour-derived cytokine TGF-β skews neutrophils towards a tumour-promoting (N2) phenotype whilst IFN-β supports development of an anti-tumour (N1) phenotype [12]. It is becoming increasingly clear that TAN significantly contribute to tumour progression. This review will firstly discuss the current literature on the likely factors and mechanisms that tumours employ to recruit neutrophils to the tumour site, and will then progress to focus on TAN and their role in promoting tumour angiogenesis.

2. Tumour-derived factors mediating neutrophil recruitment to tumours

Neutrophils are highly responsive motile cells that are drawn across the vasculature towards diseased sites by chemotactic cues. By far the most studied of tumour-derived chemoattractants are the CXCL chemokines that possess the ELR (Glu-Leu-Arg) tri-peptide motif and that bind to the G-protein-coupled receptors CXCR1 and CXCR2 expressed on the cell surface of neutrophils. CXCL6 (GCP-2) and CXCL8 (IL-8) bind to both CXCR1 and CXCR2, whereas CXCL1-3 (Gro-α, β and γ), CXCL5 (ENA-78) and CXCL7 bind exclusively to CXCR2 [13,14].

Expression of CXCL8, the most studied neutrophil-attracting chemokine, is increased in a multitude of different human carcinomas and tumour cell lines such as colon, breast, lung, cervical, brain, prostate and ovarian carcinomas to name only a few (reviewed by [15]). Moreover, chemokine expression can be up-regulated further by the potent pro-inflammatory cytokine TNF-α which is frequently found within the micro-environment of many tumours [16], or by other tumour micro-environmental conditions such as low nutrient levels, increased acidity and hypoxia [17–19]. There is compelling evidence to show that CXCL8 secreted by tumour cells derived from numerous types of carcinomas or from cells within the tumour stroma direct the recruitment of tumour infiltrating neutrophils, and this has been comprehensively reviewed elsewhere [20–22]. However, in recent years there have been a number of studies describing the role of other ELR-positive chemokines and also non-chemokine neutrophil chemoattractants in driving neutrophil recruitment into tumours.

In early studies increased expression of CXCL1 was detected in gastric, intestinal and colon carcinomas compared to normal tissue [23,24] but correlation of CXCL1 expression to neutrophil influx was not performed. However, Wallace et al., have recently shown that activation of the prostaglandin F receptor on endometrial adenocarcinoma explants by prostaglandin F2α increased the expression of tumour cell-derived CXCL1. Elevated numbers of neutrophils were observed in endometrial tumours compared with normal endometrium. Moreover, xenograft tumours comprising of prostaglandin F receptor-positive cells in nude mice showed increased neutrophil infiltration in a CXCL1-dependent manner compared with tumours arising from wild-type cells [25].

The contribution of CXCL5 to neutrophil recruitment has been more intensely studied. Scheingart et al. correlated increased expression of CXCL5 and neutrophil recruitment in human adrenocortical carcinoma, and in murine experimental tumours using an adrenocortical cell line (RL-251) [26]. High levels of CXCL5 expression were detected in pancreatic and prostate cancer and this correlated with an increased inflammatory cell infiltrate and tumour progression [27–29]. Similarly, Okabe et al. observed a significant relationship between high CXCL5 levels and neutrophil recruitment into intrahepatic cholangiocarcinoma. Furthermore,