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Review

# On the cytokines produced by human neutrophils in tumors

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ABSTRACT

Although traditionally viewed as short-lived innate immunity cells, only playing a crucial role in host defense toward infections, neutrophils have recently become subject of a new wave of research in diverse areas including in tumors. Indeed, increasing experimental evidence indicate that neutrophils may directly or indirectly influence the tumor fate through the release of a wide array of molecules able to exert either pro-tumor or anti-tumor functions depending on the microenvironment milieu, including cytokines. This review therefore attempts to uncover the role that neutrophils play during the different steps of tumor development (from promotion to progression), as well as in anti-tumor responses, *via* cytokine production.



## 1. Introduction

Tumors consist not only of neoplastic cells but also of a microenvironment composed by stromal cells and infiltrating leukocytes engaging continuous interactions with both neoplastic cells and with each other [1]. Although accumulating genetic alterations are the primary and critical events, increasing evidence point for the involvement of also tumor-associated leukocytes in tumor establishment and progression, for their capacity to exert either stimulatory or inhibitory functions toward neoplastic cell growth. In addition, the crosstalk established between infiltrating leukocytes belonging to the innate and adaptive immunity, may indirectly influence the tumor fate [2], as demonstrated, for instance, by the immunosuppressive activity elicited by the so-called myeloid-derived suppressor cells (MDSCs) toward the T-cell mediated anti-tumor immune response [3].

Among the tumor-infiltrating, mature myeloid cells, tumor-associated macrophages (TAM) have been extensively characterized as either tumor-promoting (M2) or tumor-inhibiting (M1) cells, depending on microenvironmental milieu in which they act [4]. By contrast, tumor-associated polymorphonuclear neutrophils (TAN) have received less attention, likely because granulocytes have been traditionally viewed as short-lived cells, only playing a crucial role in host defense toward microorganisms for their capacity to release a battery of proteases and bactericidal substances, as well as to generate reactive oxygen species [5]. However, recent studies uncovering the capability of neutrophils to transcribe cytokine- and chemokine-encoding genes [6] have greatly broadened our knowledge on their potential functional role, even in unsuspected pathological processes such as in tumor [7]. Consistently, neutrophils have been often found as components of the inflammatory infiltrate characterizing many models of human and murine cancers [8] in which neutrophil-attracting CXC-chemokines [9] and/or pro-survival factors [10] are constitutively produced by tumor and surrounding stroma cells [9,11]. Although the significance of these findings remains to be fully clarified, the wide array of cytokines that can be potentially released by neutrophils under different conditions allow them to interplay with neoplastic cells either directly (as pro-tumor or anti-tumor effectors) or indirectly (*e.g.*, by regulating angiogenesis, tumor growth and anti-tumor immune response) [7]. Noteworthy, although most of the reports herein described generically refer to the term “neutrophil”, original studies have demonstrated, at least in mouse, the existence of potential neutrophil subsets [12]. Such a notion has recently lead to the identification – similarly to the macrophage paradigm – of a tumor-promoting (N2) or tumor-inhibiting (N1) phenotype of tumor-infiltrating mouse neutrophils, with transforming growth factor-β (TGF-β) being an important player in determining or not this polarization [13]. In addition, transcriptomic analyses have identified, in tumor-bearing mice, three distinct populations of neutrophils, *i.e.*, naïve neutrophils, granulocytic MDSC and TAN, each one characterized by a specific mRNA profile [14]. Even though strictly human counterparts for these populations have not been clearly identified, increasing experimental evidence indicate the existence of neutrophil subsets also

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in patients affected by tumors [15]. For instance, a population of activated neutrophils has been recently proposed as the human correlate of mouse granulocytic MDSC [16]. Concerning neutrophils, it is important to mention that an additional level of complexity to keep in mind – often rendering difficult the data interpretation – is that many studies referring to neutrophils or polymorphonuclear neutrophils (PMN) actually relate to populations of not very highly purified cells, which therefore may encompass different leukocyte types or be functionally influenced by the contaminating cells.

In this review, we will focus on the current information related to the production of cytokines by human neutrophils in function of the different steps of tumor development, from promotion to angiogenesis and progression. We will contextualize the potential biological significance of neutrophil-derived cytokines as determined by their pro/anti-tumor activities. When appropriate, supporting observations generated in the mouse system will be also mentioned. We apologize to those authors whose excellent work could not be cited due to space limitation.

## 2. Neutrophil-derived cytokines involved in tumor promotion

Tumor promotion is defined as the process in which existing tumors are stimulated to grow. Since unsuspected times, inflammatory cells have been viewed as tumor promoters [17]. Interestingly, although macrophages have been long credited to play the most important role in this scenario, a growing number of experimental evidence currently indicate that also neutrophils may exert analogous tumor-promoting functions. For instance, the pro-tumoral role of neutrophils is supported by observational studies showing that, in patients with different tumor types, increased numbers of intratumoral or circulating neutrophils are often associated with a poor prognosis [18]. Moreover, in the last years it has been demonstrated that neutrophils, *via* cytokine production, may exert a tumor-nursing function which can be switched on by the stimuli prevailing within the naïve tumor microenvironment [19]. In the latter case, the two closely related tumor-necrosis factor (TNF) superfamily members, namely a proliferation-inducing ligand (APRIL) and (to a lesser extent) B-cell-activating factor (BAFF)/B lymphocyte stimulator (BLyS), have emerged as important neutrophil-derived mediators of tumor promotion [20], even though such an assumption is only based on a number of indirect evidence, as described below.

### 2.1. APRIL

APRIL, also termed TRDL-1α, TALL-2, zTNF2 or TNFSF13, is a cytokine initially produced as a homotrimeric type II transmembrane protein, and then cleaved in the Golgi apparatus by furin-like proteases for its extracellular release [21]. It binds to two receptors shared with BAFF/BLyS, namely B cell maturation antigen (BCMA) and transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) [22], as well as to heparan sulfate proteoglycans (HSPGs) [23,24], as coreceptors. *In vitro*, APRIL augments B-cell antigen presentation, co-stimulates B-cell proliferation, mediates immunoglobulins switch recombination, enhances B-cell survival, regulates B-cell tolerance, induces plasma cells survival, co-stimulates activated T cells and promotes proliferation and survival of solid and B cell tumors [21,24,25]. APRIL-transgenic mice develop a B-1-cell associated neoplasia, which is reminiscent of human B chronic lymphocytic leukemia (B-CLL) [25]. Besides being broadly expressed in normal tissue and tumor cells from diverse origin [26], APRIL has been also detected in peripheral blood neutrophils from healthy subjects at both mRNA and protein levels [27], as well as in neutrophils infiltrating human mucosa-associated lymphoid tissue (MALT) [28]. Most of the evidence suggesting a role for neutrophils as a source of APRIL in normal and neoplastic tissues derive from *in situ* studies in which two specific anti-APRIL antibodies, discriminating APRIL-binding (Aprily-2) and APRIL-producing cells (Stalk-1), were used [27]. Accordingly, Mhawech-Fauceglia and colleagues [29] first demonstrated, by double immunofluorescence-staining experiments, the presence of Stalk-1$^+$–, CD15$^+$–, and elastase$^+$–stromal neutrophils in approximately two-thirds of more than 2000 tissue sections prepared from a variety of human tumors from diverse cells origin. Importantly, in the same specimens, Aprily-2$^+$ depots were observed in close contacts with tumor nests, thus evidencing the local HSPGs-mediated retention of neutrophil-derived APRIL [29]. Tumor-infiltrating neutrophils resulted Stalk-1$^+$ also in a subsequent study performed by the same authors on a series of 406 carcinoma specimens, in which Aprily-2 and HSPGs staining also co-localized in close contact with tumor cells in a consistent proportion of cases [30]. Additionally, neutrophils turned-out to be a relevant source of APRIL also in some B-cell derived malignancies [27] in a study analyzing 144 B cell non-Hodgkin lymphoma (NHL) biopsy samples, in which a strong Aprily-2/HSPGs-associated staining was noticed in about half of diffuse large B cell lymphoma (DLBCL) specimens. Accordingly, tissue samples from such NHL subtype displayed abundant APRIL producing cells (Stalk-1$^+$) identified as CD15$^+$–, elastase$^+$–neutrophils. Interestingly, DLBCL specimens with high APRIL expression belonged to patients with a significantly impaired survival, thus supporting a tumor-promoting role for APRIL, and in turn, for neutrophils, at least in this NHL subtype [27]. Similarly, a consistent percentage of 285 classical Hodgkin lymphomas (cHL) specimens, analyzed by the same group [31], showed a HSPGs-mediated accumulation of secreted APRIL in malignant cells, while Stalk-1$^+$ cells were identified, again, as CD15$^+$–, elastase$^+$–neutrophils. Furthermore, in a retrospective study on 107 HL biopsy specimens, *in situ* APRIL up-regulation was found to increase with the stage of disease, with neutrophils again identified as the main source of APRIL [32]. Nonetheless, the intensity of APRIL expression in HL tissue sections did not correlate with the prognosis, but instead with the levels of serum acute phase proteins, thus suggesting that APRIL release could be part of an inflammatory reaction creating a promoting environment for HL [32]. Finally, an enhanced APRIL expression has been also detected in unstimulated peripheral blood neutrophils of patients with oral cavity squamous cell carcinoma (OSCC), that the authors, however, correlated more with tumor progression than with tumor promotion [33].

### 2.2. BAFF/BLyS

With regard to BAFF/BLyS, a 285 aa cytokine existing both as type II membrane and soluble molecule [34] also known as TALL-1 or THAN, it regulates B cell physiology, including differentiation, proliferation and immunoglobulin production, by interacting with its specific receptor, known as BAFF-R, but also with BCMA and TACI [22]. Usually, BAFF/BLyS gene is expressed by circulating monocytes [34], and by *in vitro* derived macrophages and dendritic cells, especially after exposure to interferon-γ (IFN-γ), lipopolysaccharide (LPS) and (to a lesser extent) interleukin-10 (IL-10) [35]. Importantly, BAFF/BLyS transgenic mice show a high incidence of lymphomas in the absence of TNF-α [36], while *in vitro* experiments have demonstrated that human recombinant BAFF/BLyS enhances the survival and/or attenuates apoptosis of malignant B cells, such as those derived from NHL, B-CLL, HL, multiple myeloma (MM) and Waldenstrom Macroglobulinemia [37]. Also neutrophils have been shown to express high levels of BAFF/BLyS upon *in vitro* incubation with IFN-γ, or – to a higher extent – following granulocyte-colony stimulating factor (G-CSF)-exposure,

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