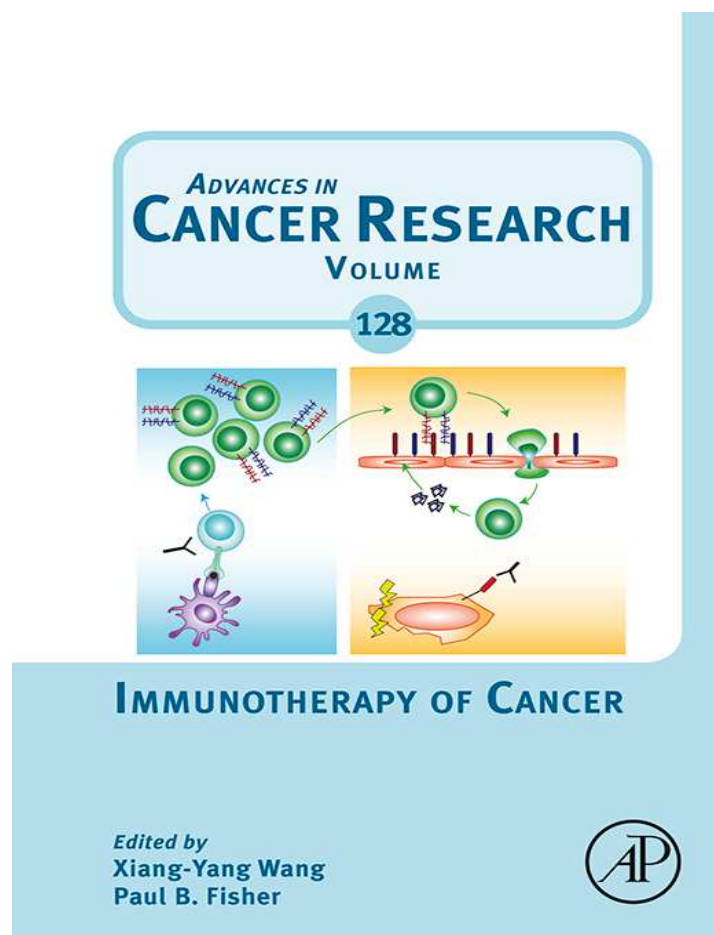


**Provided for non-commercial research and educational use only.
Not for reproduction, distribution or commercial use.**

This chapter was originally published in the book *Advances in Cancer Research, Vol. 128* published by Elsevier, and the attached copy is provided by Elsevier for the author's benefit and for the benefit of the author's institution, for non-commercial research and educational use including without limitation use in instruction at your institution, sending it to specific colleagues who know you, and providing a copy to your institution's administrator.



All other uses, reproduction and distribution, including without limitation commercial reprints, selling or licensing copies or access, or posting on open internet sites, your personal or institution's website or repository, are prohibited. For exceptions, permission may be sought for such use through Elsevier's permissions site at:

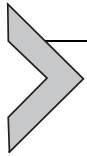
<http://www.elsevier.com/locate/permissionusematerial>

From Eduardo Bonavita, Maria Rosaria Galdiero, Sebastien Jaillon and Alberto Mantovani, Phagocytes as Corrupted Policemen in Cancer-Related Inflammation. In: Xiang-Yang Wang and Paul B. Fisher, editors, *Advances in Cancer Research, Vol. 128*, Burlington: Academic Press, 2015, pp. 141-171.

ISBN: 978-0-12-802316-7

© Copyright 2015 Elsevier Inc.

Academic Press



Phagocytes as Corrupted Policemen in Cancer-Related Inflammation

Eduardo Bonavita^{*,1}, Maria Rosaria Galdiero^{*,†,1}, Sebastien Jaillon^{*,1},
Alberto Mantovani^{*,‡,2}

*IRCCS Istituto Clinico Humanitas, Rozzano, Italy

†Division of Clinical Immunology and Allergy, University of Naples Federico II, Naples, Italy

‡Humanitas University, Rozzano, Italy

²Corresponding author: e-mail address: alberto.mantovani@humanitasresearch.it

Contents

1. Introduction	142
2. Origin and Functions of TAMs	143
3. Macrophages in Complement-Mediated, PTX3-Regulated Tumor Promotion	149
4. The Yin Yang of TAMs in Anticancer Therapy	153
5. Neutrophils and Cancer	154
5.1 Neutrophil Recruitment and Their Prognostic Significance in Tumors	154
5.2 Neutrophils in Tumor Initiation and Progression	156
5.3 Neutrophils in Tumor Progression: Angiogenesis and Metastatic Behavior Modulation	157
5.4 Neutrophil Plasticity and Heterogeneity in Cancer	158
5.5 Neutrophils, TANs, and MDSCs	158
6. Concluding Remarks	160
Acknowledgment	160
References	161

Abstract

Inflammation is a key component of the tumor microenvironment. Tumor-associated macrophages (TAMs) and tumor-associated neutrophils (TANs) are prototypic inflammatory cells in cancer-related inflammation. Macrophages provide a first line of resistance against infectious agents but in the ecological niche of cancer behave as corrupted policemen. TAMs promote tumor growth and metastasis by direct interactions with cancer cells, including cancer stem cells, as well as by promoting angiogenesis and tissue remodeling and suppressing effective adaptive immunity. In addition,

¹ E.B., M.R.G., and S.J. have equally contributed to this review and are in alphabetical order. Specifically, E.B. contributed to the field of macrophage and complement and M.R.G. and S.J. to the neutrophils section.

the efficacy of chemotherapy, radiotherapy, and checkpoint blockade inhibitors is profoundly affected by regulation of TAMs. In particular, TAMs can protect and rescue tumor cells from cytotoxic therapy by orchestrating a misguided tissue repair response. Following extensive preclinical studies, there is now proof of concept that targeting tumor-promoting macrophages by diverse strategies (e.g., Trabectedin, anti-colony-stimulating factor-1 receptor antibodies) can result in antitumor activity in human cancer and further studies are ongoing. Neutrophils have long been overlooked as a minor component of the tumor microenvironment, but there is evidence for an important role of TANs in tumor progression. Targeting phagocytes (TAMs and TANs) as corrupted policemen in cancer may pave the way to innovative therapeutic strategies complementing cytoreductive therapies and immunotherapy.



1. INTRODUCTION

Epidemiological, genetic, and experimental evidence demonstrate that chronic nonresolving inflammation can increase cancer risk and promotes cancer progression (Coussens, Zitvogel, & Palucka, 2013; Mantovani & Allavena, 2015; Mantovani, Allavena, Sica, & Balkwill, 2008). Tumor-promoting inflammation is now recognized as a key component of cancer (Hanahan & Weinberg, 2011). A link between chronic inflammation and cancer has long been suspected, but only recently the cellular and molecular mechanisms have in part been disclosed.

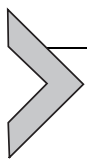
Epidemiological studies indicate that the risk of carcinogenesis increases under conditions of persistent nonresolving inflammation. Estimate suggests that chronic infections are at the basis of 15–20% of all cancers developed. Examples include viral infections with hepatitis B and C for liver cancer, papilloma virus for cervix carcinoma; bacterial infections, such as *Helicobacter pylori* for gastric cancer or lymphoma; parasites, such as *Schistosoma* for bladder cancer.

Chronic inflammation can also be triggered by noninfectious agents including irritants such as tobacco smoke, asbestos, silica, gastric reflux, chronic inflammatory disorders of the gastrointestinal tract, and autoimmune diseases can promote cancer development and metabolic dysfunctions, obesity in particular, are associated with a state of low-grade inflammation and increased cancer risk. Long-term use of nonsteroidal antiinflammatory drugs aspirin in particular reduces the risk of carcinogenesis and tumor progression.

Another line of evidence is provided by the composition of the tumor microenvironment, where inflammatory leukocytes and many inflammatory mediators (cytokines, chemokines, enzymes) are present. Inflammatory

cells and mediators are an essential constituent of the tumor microenvironment (Coussens et al., 2013; Hanahan & Weinberg, 2011; Mantovani et al., 2008). Cells of the monocyte–macrophage lineage are major components of the host cell infiltrate of tumors that can reach up to 50% of the total mass. The analysis of the function of leukocyte infiltrate has paved the way to the dissection of tumor-promoting inflammatory mechanisms in cancer (De Palma & Lewis, 2013; Mantovani, Bottazzi, Colotta, Sozzani, & Ruco, 1992; Mantovani, Sozzani, Locati, Allavena, & Sica, 2002; Noy & Pollard, 2014). Indeed, the observation of leukocyte infiltration in tumors was first made by the German pathologist Rudolf Virchow in the nineteenth century, who postulated that cancer may arise in chronically inflamed tissues (Balkwill & Mantovani, 2001; Mantovani et al., 1992).

Not all the tumors have an underlying cause of infection or chronic inflammation, but also in these tumors a reactive inflammatory microenvironment and inflammatory cell infiltration have been described. Inflammation, in these cases, is triggered by the activation of oncogenes (e.g., Ras, Myc, BRAF) and/or the inactivation of tumor-suppressor genes (e.g., p53, PTEN), that in addition to promote cell proliferation, also stimulate the transcription of inflammatory genes, including cytokines and chemokines that recruit circulating leukocytes to the tumor tissues and further fuel the inflammatory response. Several lines of evidence indicate that macrophages have the potential to kill tumor cells and to elicit tumor-destructive reactions. Tumor-associated macrophages (TAMs) are drivers of tumor progression in established tumors, promoting cancer cell proliferation and survival, angiogenesis and lymphoangiogenesis, skewing and taming effective T-cell responses. There is also evidence that inflammatory cells may mediate tumor initiation and promote genetic instability (Mantovani et al., 2008; Noy & Pollard, 2014). Thus, extrinsic causes of inflammation (infections, irritants) and intrinsic causes (oncogene-activated inflammatory response in cancer cells) both concur to build up an inflammatory tumor microenvironment (Mantovani & Allavena, 2015; Mantovani et al., 2008). Here, we will review the role of phagocytes (macrophages and neutrophils) in tumor progression and their connection with humoral innate immunity, prompted by recent evidence (Bonavita et al., 2015).



2. ORIGIN AND FUNCTIONS OF TAMs

Tissue-resident macrophages, characterized in mice by the expression of the chemokine receptor CX3CR1, protect tissues and maintain

homeostasis, whereas inflammatory macrophages, characterized by the expression of CCR2, are recruited at inflammatory sites and contribute to the inflammatory response. Mouse-resident macrophages (Kupffer cells in liver, microglia in brain, Langerhans cells in the skin, and alveolar macrophages in lung) develop in the embryo (Gomez Perdiguero et al., 2015). During this process, progenitors colonize peripheral tissues and differentiate into resident macrophages which will self-maintain throughout life (De Kleer et al., 2014). On the other hand, inflammatory macrophages derive from adult bone marrow-derived monocytes. However, resident macrophages in the gut, heart, and dermis originally derive from the yolk sac, but during adult life are replenished by bone marrow progenitors (Bain et al., 2014; McGovern et al., 2014; Molawi et al., 2014; Wynn et al., 2013). In tumors, TAMs mainly originate from bone marrow monocytes (Franklin et al., 2014; Mantovani et al., 1992; Noy & Pollard, 2014; Shand et al., 2014). In some mouse tumors, local proliferation does occur (Bottazzi et al., 1990; Tymoszek et al., 2014), but recent evidence suggests that, in general, recruitment of circulating monocytes is essential for TAMs accumulation (Franklin et al., 2014; Noy & Pollard, 2014). Chemokines (e.g., CCL2, CCL5, and CXCL12) and the growth factor CSF-1 (M-CSF) play a major role in monocyte infiltration in tumors. Recently, components of the Complement cascade have also been described to play a role in macrophage recruitment (e.g., Bonavita et al., 2015). Incoming blood monocytes preferentially localize in hypoxic or necrotic areas within tumor stroma; they are profoundly influenced by the tumor environment and rapidly differentiate into tumor-conditioned macrophages. Among chemokines, CCL5/RANTES, CXCL12/SDF-1, and CXCL3/fractalkine, for instance, were found in neoplastic tissues and contribute to macrophage recruitment and tumor promotion (Balkwill, 2004; Bottazzi et al., 1983; Mantovani et al., 2004; Reed et al., 2012; Ueno et al., 2000). In addition to chemokines and growth factors, noncanonical chemotactic peptides also produced by stromal and tumor cells, such as vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF- β), basic fibroblast growth factor (bFGF), macrophage colony-stimulating factor (M-CSF/CSF-1), urokinase plasminogen activator (uPa), the antimicrobial peptide β -defensin-3, and the lectin Reg3 β (Allavena & Mantovani, 2012; Brierie & Moses, 2010; Gironella et al., 2013; Jin et al., 2010; Lin, Gouon-Evans, Nguyen, & Pollard, 2002; Linde et al., 2012; Reed et al., 2012; Zhang, Sud, Mizutani, Gyetko, & Pienta, 2011), caused monocyte recruitment and macrophage differentiation. CXC chemokines (CXCL8, CXCL1,

CXCL2, CXCL3, CXCL5), known for their role in neutrophil recruitment in both physiological and pathological conditions and involved in cancer progression, are also produced in tumor-associated inflammation. This favors tumor angiogenesis and metastasis (Keeley, Mehrad, & Strieter, 2010; Lazennec & Richmond, 2010; Mantovani, Cassatella, Costantini, & Jaillon, 2011).

Plasticity and diversity are key properties of cells of the monocyte–macrophage lineage (Biswas & Mantovani, 2010; Mosser & Edwards, 2008; Sica & Mantovani, 2012). In primary tumors and in metastatic sites, TAMs are involved in complex bidirectional interactions with tumor cells; cancer stem cells (CSCs); fibroblasts; mesenchymal stem cells; endothelial cells; and T, B, and NK cells. Macrophages can undergo polarized classical M1 activation in response to interferon- γ (IFN- γ) and Lipopolysaccharide (LPS), or alternative M2 activation driven by IL-4 or IL-13. M1- and M2-polarized macrophages are extremes of a continuum in a universe of functional states. The molecular mechanisms and functional properties of polarized macrophages have recently been reviewed (Mantovani & Allavena, 2015; Murray et al., 2014). In many mouse and human tumors, TAMs have a frank M2 phenotype or properties which are to some extent shared with M2-polarized cells. In general, TAMs promote tumor growth and metastasis, angiogenesis, and subversion of effective antitumor immunity (Biswas & Mantovani, 2010; Coussens et al., 2013; Sica & Mantovani, 2012). Signals derived from tumors and host cells shape the functional phenotype of TAMs. In different tumor and tissue contexts, these functional determinants include hypoxia, cytokines (e.g., TGF- β and CSF-1), and metabolic products of cancer cells (e.g., lactic acid); IL-4 and IL-13 produced by Th2 cells; and IL-10 produced by Treg cells, B cells, and immune complexes (Colegio et al., 2014; Coussens et al., 2013; De Palma & Lewis, 2013; Mantovani & Allavena, 2015; Mantovani et al., 2008; Noy & Pollard, 2014; Ruffell, Affara, & Coussens, 2012; Sica & Mantovani, 2012). Within the cancer tissue, there can be microanatomical diversity of TAMs function with accumulation of M2-like cells in hypoxic areas (Movahedi et al., 2010). Moreover, inflammatory components and pathways of orchestration differ in tumors originating in distinct anatomical sites (Ruffell et al., 2012).

There is strong evidence that macrophages can be “reprogrammed” by some immunological stimuli, such as IFN- γ or IFN- α , from immunosuppressive M2 macrophages into immunostimulatory cells (De Palma & Lewis, 2013; Duluc et al., 2009). At the clinical level, it has been reported that IFN- γ -driven intratumoral microenvironment exhibits superior

prognostic effect compared with an IFN- α -driven microenvironment in patients with colon carcinoma. This gives a successful proof of principle that complex cytokine interaction networks can be found and dissected in human tissues (Grenz et al., 2013). Moreover, a Th1-dominated tumor microenvironment is strongly associated with a positive prognosis in CRC (Camus et al., 2009; Galon et al., 2006; Naschberger et al., 2008). Several lines of evidence indicate that macrophages infiltrating the tumor take part in the inflammatory process, favoring tumor formation and progression and a M2-like phenotype for TAMs has been reported in several studies (Biswas et al., 2006). The M2-like phenotype can be induced by the tumor cells. Katara et al. reported that vacuolar ATPase (V-ATPase) produced by tumor cells can promote tumor survival and growth. In particular, cancer tissues and cells overexpress the $\alpha 2$ isoform of V-ATPase ($\alpha 2V$). The relevance of the $\alpha 2V$ role has been tested in *in vitro* studies, exposing macrophages to the cleaved N-terminal domain of $\alpha 2V$. In these conditions, macrophages express and secrete TAM-associated molecules such as mannose receptor-1, arginase-1, interleukin-10, TGF- β , MMP-9, and VEGF (Katara et al., 2014). A member of the TGF- β family has recently been reported to promote M2-like polarization of TAMs and to inhibit IL-12 (Wang et al., 2014).

During tumor growth and progression, functions of TAMs include extracellular matrix remodeling, promotion of tumor cell invasion and metastasis, angiogenesis, lymphangiogenesis, and immune suppression (Mantovani et al., 2002). In fact, TAMs produce a number of proteolytic molecules, such as plasmin, urokinase-type plasminogen activator, cathepsin B, and matrix metalloproteases (MMPs) which may directly remodel the extra cellular matrix (ECM) (Gocheva et al., 2010; Nagakawa, Aoki, Kasuya, Tsuchida, & Koyanagi, 2002; Wang et al., 2011). The role of MMPs in tumor progression has been suggested by their capacity to degrade the basement membrane to activate growth factors and to enhance angiogenesis (Huang et al., 2002; Stetler-Stevenson & Yu, 2001; Wang, So, Reierstad, & Fishman, 2005). Invasiveness of cancer cells is facilitated by TAMs expression of nonproteolytic molecules. For instance, expression of chemokines that bind CXCR2 was increased in macrophages exposed to conditioned media from mammary epithelial cells containing FGF receptor 1-induced soluble factors. In turn, these chemokines induced migration of primary and tumoral mammary epithelial cells (Bohrer & Schwertfeger, 2012). In mice injected subcutaneously with pancreatic cancer cells, expression of

scavenger-receptor A in hematopoietic cells, consistent with its expression on macrophages, was required for cancer metastasis (Neyen et al., 2013). In glioma stem-like cells, the expression of MMP-9 promoted by macrophages-derived TGF- β 1 increased the invasiveness of tumor cells (Ye et al., 2012). Finally, tumor-derived versican V1 enhanced the expression of the antimicrobial peptide hCAP18/LL37 in macrophages, which in turn contributed to ovarian tumor cell proliferation and invasion (Li et al., 2013).

Macrophages have been described to be associated with the metastatic potential of several tumors (Lin, Li, Tadashi, & Dong, 2011; Qing et al., 2012). In classical experiments of Gorelik and coworkers, it was described that transfer of thioglycollate-elicited peritoneal macrophages in mice increased by up to 100-fold the number of metastatic lung nodules induced by the intravenous injection of melanoma or Lewis lung carcinoma tumor cells (Gorelik, Wiltrout, Brunda, Holden, & Herberman, 1982). In a mouse model of breast cancer, IL-4-treated macrophages upregulated the expression of cysteine protease cathepsin B, which promoted lung metastasis (Vasiljeva et al., 2006). Moreover, M2-polarizing cytokines or tumor cell-conditioned media cause macrophages expression of a truncated fibronectin isoform, namely migration-stimulating factor, that is a potent chemotactic factor for tumor cells (Solinas et al., 2010). Depletion studies in experimental animals cause reduced incidence of metastasis, giving further support to the prometastatic function of TAMs (DeNardo et al., 2009; Joyce & Pollard, 2009).

TAMs are associated with tumor angiogenesis and lymphangiogenesis: TAMs express mediators such as TGF- β , VEGF-A, VEGF-C, PDGF, MMP-9, thymidine phosphorylase, and chemokines (e.g., CXCL8/IL-8) which are directly or indirectly involved in new vessel formation and sprouting (Granata et al., 2010; Hotchkiss et al., 2003; Murdoch, Giannoudis, & Lewis, 2004; Schmidt & Carmeliet, 2010; Schoppmann, Horvat, & Birner, 2002). TAMs-derived MMP-9 induces the release of heparin-bound growth factors, particularly VEGF-A, crucial for the angiogenic switch (Ebrahim et al., 2010). VEGF-recruited monocytes improve their performance as angiogenic cells (Avraham-Davidi et al., 2013). Recruited monocytes derive from the pool of circulating Ly6Chi monocytes that undergo phenotypic and functional changes upon entry in the VEGF-rich environment. These recruited monocytes acquire enhanced proangiogenic capabilities and, importantly, a markedly increased capacity to remodel existing blood vessels.

In the tumor microenvironment, low-oxygen tension increases the expression levels of Hypoxia-inducible factor (HIF) -1 and HIF-2, which trigger a proangiogenic program in macrophages characterized by high expression levels of VEGF, bFGF, CXCL8/IL-8, and glycolytic enzymes (Murdoch et al., 2004). In the tumor microenvironment, local hypoxia causes high levels of adenosine that stimulate angiogenic and lymphangiogenic factors released by human (Granata et al., 2010). Casazza and coworkers recently reported that the Sema3A/neuropilin-1 signaling axis controls TAMs localization into hypoxic tumor areas. If TAMs are confined inside normoxic regions by blunting the Sema3A/neuropilin-1 pathway, anti-tumor immunity is restored and angiogenesis abated, and consequently tumor growth and metastasis are inhibited. Thus, cancer cell-derived Sema3A, not VEGF, is responsible for TAMs entry into hypoxic niches through neuropilin-1 signaling, where TAMs escape antitumor immunity and promote vascularization (Casazza et al., 2013). Modulating TAMs localization and thus their phenotype can be a new approach to guide TAMs activities against cancer. Moreover, Laoui et al. reported that hypoxia is not a major driver of the TAMs subset differentiation found in tumor infiltrate, namely CD11b^{hi}F4/80^{hi}Ly6C^{lo} MHC-II^{lo} or MHC-II^{hi} TAMs, both of which derived from tumor-infiltrating Ly6Chi monocytes, but rather specifically fine-tunes the phenotype of M2-like MHC-II^{lo} TAMs, that as a consequence contain higher mRNA levels for hypoxia-regulated genes than their MHC-II^{hi} counterparts (Laoui et al., 2014).

TAMs also express immunosuppressive potential, secreting or expressing a wide range of molecules, such as TGF- β , iNOS, arginase-1, IDO, and IL-10, known for their immunosuppressive role (Hagemann et al., 2006; Mantovani & Sica, 2010; Sica et al., 2000; Zhao et al., 2012). In murine models of breast cancer, TAMs suppress T-cell functions through their metabolic activities, expressing arginase-1 or iNOS (Bronte & Zanovello, 2005; Chang, Liao, & Kuo, 2001; Doedens et al., 2010; Movahedi et al., 2010). However and particularly in humans, TAMs-mediated T-cell suppression may also occur irrespective of L-arginine metabolism (Kryczek et al., 2006). For instance, TAMs have been shown to express the immunosuppressive molecule B7-H1 in hepatocellular carcinoma (HCC), B7-H4 in ovarian and lung cancer, and B7-H3 in lung cancer (Chen et al., 2012, 2013; Kryczek et al., 2006; Kuang et al., 2009). In addition, TAMs have the capacity to induce the expression of these molecules on cancer cell surface, thus providing a novel mechanism by which cancer cells escape the immune surveillance (Chen et al., 2013).

3. MACROPHAGES IN COMPLEMENT-MEDIATED, PTX3-REGULATED TUMOR PROMOTION

The physiological functions of the Complement system include defence against microbial infections, and disposal of immune complexes and products of inflammatory injury (Ricklin & Lambris, 2013). The Complement system also controls different immunological and inflammatory processes. The latter include enhancement of humoral immunity, regulation of adaptive immunity, apoptotic cell clearance, angiogenesis, cellular regeneration, and growth (Ricklin, Hajishengallis, Yang, & Lambris, 2010). The interaction of Complement components with receptors present on macrophages leads to modulation of cytokine production and induction of inflammatory responses. The myelomonocytic cell lineage expresses Complement receptors which mediate pathogen phagocytosis (e.g., C1qR (s), CR1, CR3, CR4, and CR1g) or induce inflammatory responses (e.g., C3aR, C5aR1, and C5aR2; Bohlsion, O’Conner, Hulsebus, Ho, & Fraser, 2014; Fig. 1). C3a and C5a mediate macrophage activation through different signaling mechanisms. For instance, C3a activates NLRP3 inflammasome

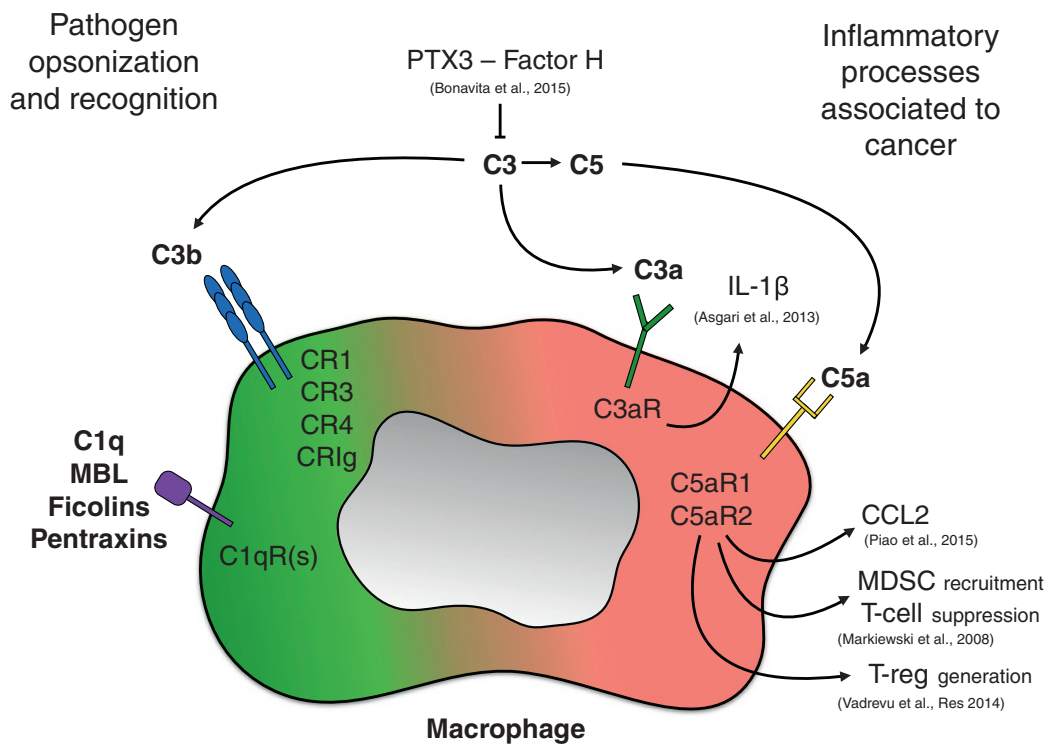


Figure 1 Macrophages and the interplay with humoral innate immunity in the regulation of inflammation and cancer. For explanation, see text.

increasing ATP release and favoring IL-1 β production (Asgari et al., 2013). C5a has been correlated with IL-6 induction and development of inflammatory Th17 response (Fang, Zhang, Miwa, & Song, 2009).

During neoplastic transformation, tumor cells can acquire new morphological changes that render them susceptible to Complement attack. The high number of genetic alterations associated with carcinogenesis dramatically changes the composition of the cell membrane. For example, an altered glycosylation is considered a hallmark of cancer cells (Hanahan & Weinberg, 2011), and progression of epithelial cells from a normal to malignant phenotype is associated with an aberrant metabolism of membrane phospholipids affecting signal transduction pathways (Pio, Corrales, & Lambris, 2014). However, in comparison to normal counterpart, tumor cells have also been shown to express higher levels of membrane-bound regulatory proteins and soluble Complement inhibitors, including CD21, CD35, CD46, CD55, CD59, and Factor H, which could be responsible of hindered Complement cytotoxicity (Bellone et al., 2012; Gelderman, Tomlinson, Ross, & Gorter, 2004; Hörl et al., 2013).

Although no formal evidence supports the existence of an effective immune surveillance mediated by Complement during carcinogenesis, changes in the composition of cell membrane may target tumor cells for Complement recognition. Several observations support Complement-mediated recognition of malignant cells and Complement activation in many cancers. Elevated levels of C3a are present in the ascitic fluid of patients with ovarian cancer (Bjorge et al., 2005). C3c and C4 levels are elevated in lung cancer patients and their concentration is directly correlated with tumor volume (Ajona et al., 2013, 2015). The lectin pathway of Complement is more activated in colorectal cancer patients in comparison to healthy individuals, and systemic levels of MASP-2 have been reported to be an independent prognostic marker for poor survival (Ytting, Jarle Christensen, Thiel, Jensenius, & Nielsen, 2005). The activation of the classical pathway of Complement has also been found in patients affected by mucosa-associated lymphoid tissue lymphoma (Bu, Zheng, Wang, & Yu, 2007). Moreover, Complement-dependent cytotoxicity was necessary for immunotherapeutic response to rituximab in central nervous system (CNS) lymphomas (Kadoch et al., 2014) and chronic lymphocytic leukemia (Middleton et al., 2015).

In recent years, many studies have identified new and unexpected roles for Complement activation within the tumor microenvironment challenging the classical view of the Complement system as an anticancer mechanism (Bonavita et al., 2015) Complement elements can promote growth of

transplanted tumors in the context of chronic inflammation (Markiewski et al., 2008). Notably, mice deficient in C3 or C5aR show decreased tumor growth in models of transplantable tumors, in comparison to wild-type mice, suggesting that the Complement system somehow promotes tumor growth (Rutkowski, Sughrue, Kane, Mills, & Parsa, 2010). In line with this view, several studies have demonstrated a protumorigenic role for activated Complement components in all stages of carcinogenesis. The genetic abrogation of C3 significantly reduced tumor incidence in models of 3-methylcholanthrene- and 7,12-dimethylbenz [a] anthracene/terephthalic acid (DMBA/TPA)-induced carcinogenesis (Bonavita et al., 2015). Moreover C3 deficiency was associated with reduced tumor macrophages infiltration (Bonavita et al., 2015). Complement activation promoted azoxymethane/dextran sodium sulphate (AOM/DSS)-induced carcinogenesis in IL-1 β /IL-17A-dependent manner and C3-deficient mice developed significantly less colonic lesions (Ning et al., 2015). Complement can suppress antitumoral immunity via C5a that is a potent chemoattractant for myeloid-derived suppressor cells (MDSCs), which inhibit cytotoxic T lymphocytes (CTL) (Markiewski et al., 2008). In a preclinical model of breast cancer, C5aR engagement facilitated metastasis by suppressing effectors CD8 and CD4 T-cell responses in the lungs (Vadrevu et al., 2014). In addition, C5a favored liver metastasis by promoting tumor inflammation. Indeed, genetic deficiency of C5aR leads to impaired production of CCL2 (Piao et al., 2015). Finally, data obtained studying pathologies not related to cancer raise the possibility that Complement proteins may enhance Epithelial-mesenchymal transition (EMT), provide chemotactic stimuli (i.e., C5a and C3a; Pasinetti et al., 1996), and induce production of growth factors (i.e., VEGF and TGF- β ; Nozaki et al., 2006), which prime and encourage tumor invasion and migration (Christofori, 2006).

Although several lines of evidence sustain a protumoral role for Complement, this system can play different roles in different tumor contexts. For instance, C3 deficiency did not affect tumor incidence in a model of skin carcinogenesis driven by HPV16 (de Visser, Korets, & Coussens, 2004), or even promoted tumor formation in the case of Her2/neu breast tumors (Bandini et al., 2013). Collectively, these data suggest that Complement activation has a dual role in cancer: it has the potential to kill cancer cells, but Complement elements can modulate macrophage functions promoting cancer-related inflammation and tumor progression.

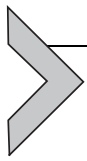
Modulation of Complement activation is a common feature of pentraxins. The short pentraxins C-reactive protein (CRP) and serum amyloid P component recognize different Complement components.

The interaction between CRP and C1q leads to the formation of C3 convertase and thus to the activation of the classical pathway (Sjoberg, Trouw, McGrath, Hack, & Blom, 2006). Surface-bound CRP inhibits alternative pathway amplification through a specific interaction with Factor H, the main soluble regulator of this pathway. In addition, CRP and SAP bind C4b-binding protein (C4BP), a soluble regulator of the classical and lectin pathways (Inforzato et al., 2013).

Similarly, the prototype of long pentraxins PTX3 has a dual role in Complement activation. The first protein identified as PTX3 ligand was C1q, the activator of the classical pathway (Bottazzi et al., 1997). The interaction between PTX3 and the globular head of C1q occurs in a calcium-dependent manner and depending on the way it is presented leads either to activation or inhibition of the Complement cascade (Doni et al., 2012). PTX3 has also been shown to interact with three members of the lectin pathway, namely ficolin-1, ficolin-2, and mannose-binding lectin (MBL; Gout et al., 2011). PTX3 enhances ficolin-1, ficolin-2, and MBL-dependent Complement deposition on the surface of *Aspergillus fumigatus* and *Candida albicans*, respectively, favoring Complement-mediated innate immune responses (Ma et al., 2013). In addition, the formation of a complex ficolin-1/PTX3 on the surface of apoptotic cell facilitated the clearance of apoptotic cells down-regulating in parallel the release of IL-8 by macrophages (Ma et al., 2013). Finally, PTX3 interacts with Factor H, favoring its deposition on PTX3-coated surface and limiting an exacerbated activation of the Complement cascade (Deban et al., 2008). In atypical hemolytic uremic syndrome, mutations observed in Factor H reduced the interaction with PTX3 and lead to enhanced inflammation and Complement-mediated damage (Okemefuna, Nan, Miller, Gor, & Perkins, 2010). PTX3 has also been shown to interact with C4BP, which inhibits Complement activation by acting as a cofactor for factor I in the cleavage and inactivation of C4b (Braunschweig & Jozsi, 2011). This interaction promoted the recruitment of C4BP on late-apoptotic cells and extracellular matrix, suggesting negative modulation of local Complement activation that would otherwise lead to inflammation and tissue damage.

In a model of 3-methylcholanthrene-induced carcinogenesis, PTX3 deficiency was associated to increased susceptibility to cancer, higher proinflammatory mediator release (i.e., CCL2), and gene instability. Tumor tissues from PTX3-deficient mice were characterized by significantly higher C3 deposition in comparison to wild-type tumors because of defective Factor H recruitment. Deficiency of C3 in PTX3 gene-targeted mice was sufficient to rescue the increased susceptibility to tumor growth and

macrophage recruitment. Higher tumor incidence in PTX3-deficient mice was also associated with increased C5a levels and the pharmacological blocking of C5aR *in vivo* reduced tumor frequency (Bonavita et al., 2015). Thus, PTX3 deficiency unleashes unrestrained Complement activation with production of C5a, CCL2, and enhanced recruitment of tumor-promoting macrophages. These results indicate that an essential component of the humoral arm of innate immunity and regulator of Complement activation acts as an extrinsic oncosuppressor by acting at the level of Complement-mediated, macrophage-sustained, tumor-promoting inflammation.



4. THE YIN YANG OF TAMs IN ANTICANCER THERAPY

The evidence and consensus about the role of TAMs in tumor-promoting inflammation (Hanahan & Weinberg, 2011) raise the issue of their involvement in current treatment modalities and of their potential as therapeutic targets. In general, two main approaches have been used: direct depletion of macrophages or inhibition of monocyte recruitment and restimulation of their cytotoxic function (reeducation of TAMs; Beatty et al., 2011; Edwards & Emens, 2010; Germano et al., 2013; Mantovani & Allavena, 2015; Rozel et al., 2009; Xin et al., 2009). As mentioned above, cancer cell-centered therapeutic strategies and immunotherapy profoundly influence the function of TAMs by directly modulating their function or by affecting components of the tumor microenvironment (e.g., effective adaptive immune responses). In turn, TAMs can contribute to the ultimate efficacy of anticancer strategies or retain and amplify their tumor-promoting function by orchestrating a misdirected tissue repair response. The role of TAMs in anticancer therapy has recently been reviewed (Mantovani & Allavena, 2015). Evidence suggests that in conventional cytotoxic therapeutic strategies (chemotherapy and radiotherapy), TAMs can have a dual role. Chemotherapy and radiotherapy can elicit a misdirected macrophage-orchestrated tissue repair response and thus rescue and protect tumor cells including CSCs. On the other hand, TAMs can contribute to the antitumor activity of selected anticancer drugs and low-dose radiotherapy (Mantovani & Allavena, 2015). Moreover, TAMs may play a role in targeted therapies and in checkpoint blockade inhibiting antibodies (Mantovani & Allavena, 2015). Finally, following extensive preclinical testing, there is now proof of principle that targeting TAMs can have antitumor activity in human tumors (Germano et al., 2013). In particular, there is evidence that Trabectedin, approved for clinical use in Europe for sarcomas and ovarian

carcinoma, acts at least in part by depleting tumor-promoting monocytes (Germano et al., 2013).



5. NEUTROPHILS AND CANCER

Neutrophils represent the most abundant leukocyte subpopulation in human peripheral blood and play a primary role in host defence against pathogens during the earliest phases of the inflammatory responses. The role of neutrophils in tumor development has long been underestimated due to their short half-life and terminally differentiated phenotype. In the last decade, the advent of new technical tools allowed to better characterize these cells, thus challenging this limited classical point of view. Indeed, evidences propose emerging roles for neutrophils in coordinating many aspects of the inflammatory response and tumor development. Similarly to TAMs, tumor-associated neutrophils (TANs) can exert both antitumoral and protumoral functions and experimental animal models suggest that neutrophils are characterized by a surprising plasticity (Fridlender et al., 2009; Mantovani, 2009; Fig. 2).

5.1 Neutrophil Recruitment and Their Prognostic Significance in Tumors

Within the tumor microenvironment, a number of CXC chemokines (e.g., CXCL1, CXCL2, CXCL3, CXCL5, CXCL8), known for their neutrophil

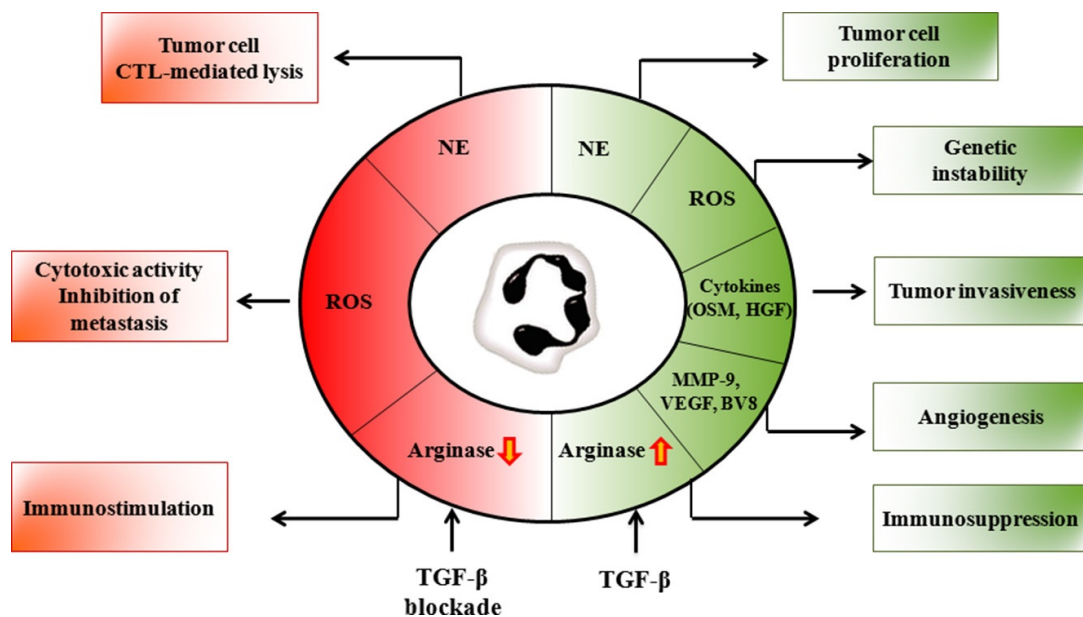


Figure 2 Neutrophils potentially impact key aspects of cancer. For explanation, see text.

chemoattractant properties, are produced by tumor and stromal cells and have been related to cancer initiation, to the promotion of tumor angiogenesis, and metastasis (Keeley et al., 2010; Lazenec & Richmond, 2010; Mantovani et al., 2011). For example, evidence derived from murine models described an important role for the CXCR2 signaling pathway in lung and pancreatic cancer promotion (Ijichi et al., 2011; Keane, Belperio, Xue, Burdick, & Strieter, 2004). In various murine models of cancers (inflammation-associated skin cancer, colitis-associated or spontaneous intestinal cancer), CXCR2 abrogation or neutrophil depletion inhibited both inflammation-induced and spontaneous carcinogenesis (Jamieson et al., 2012). Moreover, in a murine model of graft tumor, CXCL17 promoted the recruitment of myeloid CD11b⁺Gr1⁺F4/80⁻ cells within the tumor, favoring tumor growth, angiogenesis, and metastatic behavior (Matsui et al., 2012). In humans, HCC cells and head and neck squamous cell carcinoma (HNSCC) cell lines recruited neutrophils in a CXCR2-dependent manner through the production of CXCL8 (Kuang et al., 2011) and macrophage-inhibiting factor (MIF; Dumitru et al., 2011; Trellakis, Farjah, et al., 2011). Moreover, in a wide cohort of HCC tumors, correlations between increased CXCL5 expression, neutrophil infiltration, and poor patients' survival were found (Zhou et al., 2012). In addition, in a murine model of lung cancer determined by K-ras activation and p53 abrogation, TAM and TAN precursors relocated from the spleen to the tumor and splenectomy significantly reduced the infiltration of myeloid cells within the tumor (Cortez-Retamozo et al., 2012). In addition, Angiotensin II was identified as a pivotal factor in the amplification of hematopoietic self-renewal (Cortez-Retamozo et al., 2013).

Various epidemiological evidences described a negative correlation between TANs and patient clinical outcome in metastatic and localized renal cell carcinoma, bronchioloalveolar carcinoma, HCC, colorectal cancer, and head and neck cancer (Donskov, 2013; Jensen et al., 2009; Kuang et al., 2011; Rao et al., 2012; Trellakis, Bruderek, et al., 2011; Wislez et al., 2003). Moreover, higher tumor-infiltrating neutrophil density was associated with higher histological grade in glioma (Fossati et al., 1999) and more aggressive pancreatic cancer (Reid et al., 2011). In contrast, the association between neutrophil infiltration and patients' clinical outcome remains controversial for some tumor types, such as gastric and colorectal cancer (Caruso et al., 2002; Hirt et al., 2013). These controversial evidences may be due to variability in the methods used to identify neutrophils within tumors (e.g., immunohistochemistry, hematoxylin–eosin staining), as well as the choice of patient datasets and outcomes.

5.2 Neutrophils in Tumor Initiation and Progression

The association between neutrophil-derived reactive oxygen species (ROS) and carcinogenesis has been described already 30 years ago (Weitzman, Weitberg, Clark, & Stossel, 1985). Accordingly, neutrophil-derived ROS and related products, such as myeloperoxidase-mediated HOCl, induced genetic instability, an emerging hallmark of cancer, due to DNA point mutations (Gungor et al., 2010; Hanahan & Weinberg, 2011).

Neutrophil-derived granule proteins can also play dual roles in tumor progression. For instance, neutrophil elastase (NE) can favor tumor cell proliferation via the alteration of the platelet-derived growth factor receptor (PDGFR) intracellular signaling and epithelial-to-mesenchymal transition (Grosse-Steffen et al., 2012; Houghton et al., 2010). In contrast, NE can be taken up by cancer cells, leading to alteration of self-antigens and activation of a CTL-mediated antitumor response (Mittendorf et al., 2012).

Neutrophils also produce a number of cytokines, which play important roles in cancer (Tecchio, Scapini, Pizzolo, & Cassatella, 2013). For instance, stimulated neutrophils secrete Oncostatin M, which stimulates cancer cells to produce VEGF, thus enhancing tumor cell invasive behavior (Queen, Ryan, Holzer, Keller-Peck, & Jorczyk, 2005). In addition, neutrophil-derived hepatocyte growth factor (HGF) promoted the invasive behavior of cholangiocellular and hepatocellular cell lines *in vitro* (Imai et al., 2005). In bronchoalveolar carcinoma patients, an association between neutrophil infiltration, poor patients' prognosis, and levels of HGF in bronchoalveolar lavage fluid was described (Wislez et al., 2003). In HNSCC patients, a correlation between tumor-infiltrating neutrophils and the expression of CORTACTIN, a protein involved in cellular migration, was found (Dumitru et al., 2013). Moreover, tumor-infiltrating neutrophils and CORTACTIN were associated with poor patients' outcome (Dumitru et al., 2013). In contrast, neutrophil-derived molecules can also display antitumoral functions. For instance, neutrophils are an important source of TNF-related apoptosis-inducing ligand (TRAIL), which displays antitumoral activities (Cassatella, 2006; Hewish, Lord, Martin, Cunningham, & Ashworth, 2010). Indeed, *Mycobacterium bovis* Bacillus Calmette–Guerin (BCG) induced the release of TRAIL from neutrophils, suggesting a role for neutrophils in mediating the anticancer effects of BCG in bladder cancer (Kemp et al., 2005). Moreover, neutrophil-derived TRAIL promoted apoptosis of leukemic cells in chronic myeloid leukemia patients (Tanaka, Ito, Kyo, & Kimura, 2007; Tecchio et al., 2004). In

addition, in lung cancer patients, TANs present an activated phenotype, characterized by high expression levels of proinflammatory mediators (i.e., CCL2, CCL3, CXCL8). These activated TANs efficiently stimulate T-cell proliferation and IFN- γ release through a cell contact-dependent manner (Eruslanov et al., 2014). This cross-talk enhanced the expression of costimulatory molecules in neutrophils, sustaining a positive-feedback loop and supporting an antitumoral role for TANs in early stages of human lung cancers (Eruslanov et al., 2014).

5.3 Neutrophils in Tumor Progression: Angiogenesis and Metastatic Behavior Modulation

Neutrophils play a dual role in modulating angiogenesis and metastatic behavior of tumors. Neutrophils express various angiogenic factors, such as VEGF-A, which is also the main mediator of the CXCL1-induced angiogenic activity (Scapini et al., 2004). In murine models of subcutaneous melanoma and fibrosarcoma, in the absence of IFN- β , TANs acquired proangiogenic features, such as increased expression of CXCR4, VEGF-A, and MMP-9 (Jablonska, Leschner, Westphal, Lienenklaus, & Weiss, 2010). MMP-9 is a well-known proangiogenic factor, inducing the release of the active form of VEGF-A from the ECM (Nozawa, Chiu, & Hanahan, 2006).

Bv8 (also known as prokineticin-2) is known to promote neutrophil mobilization and angiogenesis. In a tumor xenograft model, G-CSF induced the expression of Bv8 in neutrophils and blocking Bv8 impaired neutrophil recruitment, tumor growth, and angiogenesis (Shojaei et al., 2007). Interestingly, tumors resistant to anti-VEGF therapy displayed high neutrophil infiltration, and resistance to anti-VEGF treatment was due to G-CSF-induced Bv8 expression. Indeed, blocking G-CSF or Bv8 impaired tumor growth and angiogenesis (Shojaei, Singh, Thompson, & Ferrara, 2008; Shojaei et al., 2009). In contrast, neutrophils also display antiangiogenic properties. For instance, NE itself degraded VEGF and FGF-2 and *in vitro*-generated angiostatin-like fragments from plasminogen, which suppressed VEGF- and FGF-2-mediated angiogenesis (Ai et al., 2007; Scapini et al., 2002). Neutrophils play many roles in modifying the tumor metastatic behavior. Melanoma-derived CXCL8 increased the expression of β_2 -integrin on neutrophils, which engaged ICAM-1 expressed on melanoma cells, thus favoring the interaction between neutrophils and melanoma cells. This dangerous interaction allowed melanoma cells to transit across the endothelium,

giving rise to distant metastasis (Huh, Liang, Sharma, Dong, & Robertson, 2010). In addition, neutrophil extracellular traps were able to capture circulating tumor cells and promoted their engraftment to distant organ sites (Cools-Lartigue et al., 2013). In contrast, in an *in vivo* model of breast cancer, under the influence of G-CSF and tumor-derived CCL2, neutrophils accumulated in the premetastatic lung and inhibited metastatic engraftment through the release of H₂O₂. Accordingly, following neutrophil depletion, the metastatic load was significantly enhanced (Granot et al., 2011). Recently, a role for type I IFN signaling in reducing the metastatic load has been described. More in detail, in a model of breast cancer, *Ifnar1*-deficient mice displayed an increased metastatic load together with increased neutrophil infiltration in the premetastatic lung, compared to the wild-type mice. *Ifnar1*^{-/-} neutrophils displayed altered killing activity and increased CXCR2 expression, responsible for their homing in the premetastatic lungs (Wu et al., 2015).

5.4 Neutrophil Plasticity and Heterogeneity in Cancer

In contrast with the classical point of view, neutrophils appear as cells endowed with unsuspected plasticity. In murine models of mesothelioma and lung cancer, neutrophils acquired a protumoral phenotype under the influence of TGF- β (Fridlender et al., 2009). Accordingly, neutrophils recruited in TGF- β -blocking conditions displayed increased antitumor cytotoxic activity, high expression of TNF- α , CCL3, and ICAM-1, and low levels of arginase-1, a well-known T-cell inhibitory factor. TGF- β neutralization also enhanced a T-cell mediated antitumor response, in which neutrophils played a role as effector cells (Fridlender et al., 2009). In contrast, type I interferon signaling has been involved in the acquisition of an antitumoral phenotype in neutrophils. Therefore, in mice lacking type I IFN signals, neutrophils displayed proangiogenic and prometastatic features (Jablonska et al., 2010; Wu et al., 2015). Thus, similarly to the Th1–Th2 and M1–M2 paradigms, a new paradigm has been proposed in which neutrophils can be polarized toward an antitumor N1 or a protumor N2 phenotype in response to signals derived from the microenvironment.

5.5 Neutrophils, TANs, and MDSCs

During cancer development, a heterogeneous population of myeloid cells appears in peripheral blood of tumor-bearing mice and cancer patients. These cells, namely MDSCs, display immunosuppressive and cancer-promoting

properties and are divided into monocytic (Mo-MDSCs) and granulocytic (G-MDSCs) cells, on the basis of distinct morphological and phenotypical aspects (Youn & Gabrilovich, 2010).

The distinction between G-MDSCs and TANs is not so clear. Indeed, neutrophils and G-MDSCs display the same membrane markers (CD11b, Gr1, and Ly6G), similar morphology, and immunosuppressive properties via arginase-1 production (Gabrilovich, Ostrand-Rosenberg, & Bronte, 2012). Accordingly and recently, in a murine model of breast cancer, atypical CD11b⁺Ly6G⁺Rb1^{low} neutrophils appeared during tumor progression in peripheral tissues, but not in the primary tumors. This neutrophil subpopulation suppressed T-cell-mediated immune response through the production of ROS. Hematopoietic stem cell differentiation toward the myeloid lineage in bone marrow was found to be driven by tumor-derived G-CSF (Casbon et al., 2015).

In patients with renal cancer, a subset of activated neutrophils in peripheral blood was identified, able to induce T-cell immunosuppression through the production of arginase-1 (Rodriguez et al., 2009; Schmielau & Finn, 2001). Therefore, in this view, these activated neutrophils were considered as G-MDSCs due to their immunosuppressive phenotype. In contrast, MDSCs have been also referred as immature neutrophils (Solito et al., 2011; Trellakis, Farjah, et al., 2011). Indeed, in a genetic conditional lung adenocarcinoma model, TAN precursors physically relocated from spleen to tumors and, since MDSCs accumulated in the spleen of tumor-bearing animals, TAN activities were at least in part attributed to MDSCs (Cortez-Retamozo et al., 2012). Accordingly, G-MDSCs acquired phenotypical and functional aspects of neutrophils, under the influence of GM-CSF, supporting the theory by which G-MDSCs are immature neutrophils (Youn, Collazo, Shalova, Biswas, & Gabrilovich, 2012). Immature neutrophilic MDSCs have also been described in peripheral blood of cancer patients and correlated with poor clinical outcome (Trellakis, Farjah, et al., 2011).

In contrast with these evidences, Fridlender and colleagues performed a transcriptomic analysis on peripheral neutrophils, TANs, and G-MDSCs in tumor-bearing mice, and found that TANs and G-MDSCs are distinct populations of cells and that naïve neutrophils and G-MDSCs are more closely related to each other than to TANs (Fridlender et al., 2012). Accordingly and quite recently, a heterogeneous population of low-density neutrophils (LDNs) has been identified in peripheral blood of tumor-bearing mice and cancer patients (Sagiv et al., 2015). Compared to mature high-density neutrophils (HDNs), LDNs displayed reduced chemotactic activity,

phagocytosis, and oxidative burst as well as lower expression of chemokines (i.e., CXCL1, CXCL2, CXCL10) and chemokine receptors (i.e., CXCR2). From the functional point of view, LDNs impaired CD8⁺ T-cell proliferation. Thus, in contrast to HDNs, LDNs displayed protumoral activities, which were mainly driven by TGF- β . In addition, within LDNs, two populations of neutrophils were identified, which displayed similar immunosuppressive properties, but different maturation stages. Thus, finally, three distinct populations of neutrophils can be distinguished. The first one consists of HDNs, previously referred as N1 neutrophils, which displayed a mature phenotype together with cytotoxic and antitumor activities. The second and third populations are found within LDNs and consist of immature cells, previously described as G-MDSCs and mature cells, previously described as N2 neutrophils, both sharing immunosuppressive and tumor-promoting functions (Sagiv et al., 2015). Therefore, this increasing body of evidence emphasizes the high versatility of neutrophils in different pathophysiological settings and paves the way for new therapeutic approaches based on their multifaceted biological aspects.



6. CONCLUDING REMARKS

Cells of the myelomonocytic lineage have emerged as a key feature of cancer-related inflammation. They are important players both in the extrinsic pathway connecting inflammation and cancer, consisting of inflammatory conditions which predispose to cancerogenesis, and of the oncogene-driven tumorigenesis process. Macrophage and neutrophils are a major source of humoral fluid-phase pattern recognition molecules such as the long pentraxin PTX3, and their recruitment and function is regulated by the humoral arm of innate immunity. Recent work has highlighted (Bonavita et al., 2015) that Complement and its regulation by PTX3 are an important component of the inflammatory microenvironment and that PTX3 acts as a *bona fide* cancer suppressor gene in mouse and human tumors. There is evidence that targeting TAMs has antitumor activity in human cancer and these preclinical and clinical results are likely to pave the way to innovative therapeutic strategies.

ACKNOWLEDGMENT

Supported by Associazione Italiana per la Ricerca sul Cancro and Italian Ministry of Health.

REFERENCES

- Ai, S., Cheng, X. W., Inoue, A., Nakamura, K., Okumura, K., Iguchi, A., et al. (2007). Angiogenic activity of bFGF and VEGF suppressed by proteolytic cleavage by neutrophil elastase. *Biochemical and Biophysical Research Communications*, *364*, 395–401.
- Ajona, D., Pajares, M. J., Corrales, L., Perez-Gracia, J. L., Agorreta, J., Lozano, M. D., et al. (2013). Investigation of complement activation product C4d as a diagnostic and prognostic biomarker for lung cancer. *Journal of the National Cancer Institute*, *105*, 1385–1393.
- Ajona, D., Razquin, C., Pastor, M. D., Pajares, M. J., Garcia, J., Cardenal, F., et al. (2015). Elevated levels of the complement activation product C4d in bronchial fluids for the diagnosis of lung cancer. *PloS One*, *10*, e0119878.
- Allavena, P., & Mantovani, A. (2012). Immunology in the clinic review series; focus on cancer: Tumour-associated macrophages: Undisputed stars of the inflammatory tumour microenvironment. *Clinical and Experimental Immunology*, *167*, 195–205.
- Asgari, E., Le Friec, G., Yamamoto, H., Perucha, E., Sacks, S. S., Köhl, J., et al. (2013). C3a modulates IL-1 β secretion in human monocytes by regulating ATP efflux and subsequent NLRP3 inflammasome activation. *Blood*, *122*, 3473–3481.
- Avraham-Davidi, I., Yona, S., Grunewald, M., Landsman, L., Cochain, C., Silvestre, J. S., et al. (2013). On-site education of VEGF-recruited monocytes improves their performance as angiogenic and arteriogenic accessory cells. *The Journal of Experimental Medicine*, *210*, 2611–2625.
- Bain, C. C., Bravo-Blas, A., Scott, C. L., Gomez Perdiguero, E., Geissmann, F., Henri, S., et al. (2014). *Nature Immunology*, *15*, 929–937.
- Balkwill, F. (2004). Cancer and the chemokine network. *Nature Reviews. Cancer*, *4*, 540–550.
- Balkwill, F., & Mantovani, A. (2001). Inflammation and cancer: Back to Virchow? *Lancet*, *357*, 539–545.
- Bandini, S., Curcio, C., Macagno, M., Quaglino, E., Arigoni, M., Lanzardo, S., et al. (2013). Early onset and enhanced growth of autochthonous mammary carcinomas in C3-deficient Her2/neu transgenic mice. *Oncoimmunology*, *2*, e26137.
- Beatty, G. L., Chiorean, E. G., Fishman, M. P., Saboury, B., Teitelbaum, U. R., Sun, W., et al. (2011). CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science*, *331*, 1612–1616.
- Bellone, S., Roque, D., Cocco, E., Gasparrini, S., Bortolomai, I., Buza, N., et al. (2012). Downregulation of membrane complement inhibitors CD55 and CD59 by siRNA sensitises uterine serous carcinoma overexpressing Her2/neu to complement and antibody-dependent cell cytotoxicity in vitro: Implications for trastuzumab-based immunotherapy. *British Journal of Cancer*, *106*, 1543–1550.
- Bierie, B., & Moses, H. L. (2010). Transforming growth factor beta (TGF-beta) and inflammation in cancer. *Cytokine & Growth Factor Reviews*, *21*, 49–59.
- Biswas, S. K., Gangi, L., Paul, S., Schioppa, T., Saccani, A., Sironi, M., et al. (2006). A distinct and unique transcriptional program expressed by tumor-associated macrophages (defective NF-kappaB and enhanced IRF-3/STAT1 activation). *Blood*, *107*, 2112–2122.
- Biswas, S. K., & Mantovani, A. (2010). Macrophage plasticity and interaction with lymphocyte subsets: Cancer as a paradigm. *Nature Immunology*, *11*, 889–896.
- Bjorge, L., Hakulinen, J., Vintermyr, O. K., Jarva, H., Jensen, T. S., Iversen, O. E., et al. (2005). Ascitic complement system in ovarian cancer. *British Journal of Cancer*, *92*, 895–905.
- Bohlon, S. S., O'Conner, S. D., Hulsebus, H. J., Ho, M.-M., & Fraser, D. A. (2014). Complement, C1q, and C1q-related molecules regulate macrophage polarization. *Frontiers in Immunology*, *5*, 402.
- Bohrer, L. R., & Schwertfeger, K. L. (2012). Macrophages promote fibroblast growth factor receptor-driven tumor cell migration and invasion in a CXCR2-dependent manner. *Molecular Cancer Research: MCR*, *10*, 1294–1305.

- Bonavita, E., Gentile, S., Rubino, M., Maina, V., Papait, R., Kunderfranco, P., et al. (2015). PTX3 is an extrinsic oncosuppressor regulating complement-dependent inflammation in cancer. *Cell*, *160*, 700–714.
- Bottazzi, B., Erba, E., Nobili, N., Fazioli, F., Rambaldi, A., & Mantovani, A. (1990). A paracrine circuit in the regulation of the proliferation of macrophages infiltrating murine sarcomas. *Journal of Immunology*, *144*, 2409–2412.
- Bottazzi, B., Polentarutti, N., Acero, R., Balsari, A., Boraschi, D., Ghezzi, P., et al. (1983). Regulation of the macrophage content of neoplasms by chemoattractants. *Science*, *220*, 210–212.
- Bottazzi, B., Vouret-Craviari, V., Bastone, A., De Gioia, L., Matteucci, C., Peri, G., et al. (1997). Multimer formation and ligand recognition by the long pentraxin PTX3. Similarities and differences with the short pentraxins C-reactive protein and serum amyloid P component. *The Journal of Biological Chemistry*, *272*, 32817–32823.
- Braunschweig, A., & Jozsi, M. (2011). Human pentraxin 3 binds to the complement regulator C4b-binding protein. *PloS One*, *6*, e23991.
- Bronte, V., & Zanovello, P. (2005). Regulation of immune responses by L-arginine metabolism. *Nature Reviews. Immunology*, *5*, 641–654.
- Bu, X., Zheng, Z., Wang, C., & Yu, Y. (2007). Significance of C4d deposition in the follicular lymphoma and MALT lymphoma and their relationship with follicular dendritic cells. *Pathology, Research and Practice*, *203*, 163–167.
- Camus, M., Tosolini, M., Mlecnik, B., Pages, F., Kirilovsky, A., Berger, A., et al. (2009). Coordination of intratumoral immune reaction and human colorectal cancer recurrence. *Cancer Research*, *69*, 2685–2693.
- Caruso, R. A., Bellocco, R., Pagano, M., Bertoli, G., Rigoli, L., & Inferrera, C. (2002). Prognostic value of intratumoral neutrophils in advanced gastric carcinoma in a high-risk area in northern Italy. *Modern Pathology: An Official Journal of the United States and Canadian Academy of Pathology, Inc*, *15*, 831–837.
- Casazza, A., Laoui, D., Wenes, M., Rizzolio, S., Bassani, N., Mambretti, M., et al. (2013). Impeding macrophage entry into hypoxic tumor areas by Sema3A/Nrp1 signaling blockade inhibits angiogenesis and restores antitumor immunity. *Cancer Cell*, *24*, 695–709.
- Casbon, A. J., Reynaud, D., Park, C., Khuc, E., Gan, D. D., Schepers, K., et al. (2015). Invasive breast cancer reprograms early myeloid differentiation in the bone marrow to generate immunosuppressive neutrophils. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, E566–E575.
- Cassatella, M. A. (2006). On the production of TNF-related apoptosis-inducing ligand (TRAIL/Apo-2L) by human neutrophils. *Journal of Leukocyte Biology*, *79*, 1140–1149.
- Chang, C. I., Liao, J. C., & Kuo, L. (2001). Macrophage arginase promotes tumor cell growth and suppresses nitric oxide-mediated tumor cytotoxicity. *Cancer Research*, *61*, 1100–1106.
- Chen, C., Qu, Q. X., Shen, Y., Mu, C. Y., Zhu, Y. B., Zhang, X. G., et al. (2012). Induced expression of B7-H4 on the surface of lung cancer cell by the tumor-associated macrophages: A potential mechanism of immune escape. *Cancer Letters*, *317*, 99–105.
- Chen, C., Shen, Y., Qu, Q. X., Chen, X. Q., Zhang, X. G., & Huang, J. A. (2013). Induced expression of B7-H3 on the lung cancer cells and macrophages suppresses T-cell mediating anti-tumor immune response. *Experimental Cell Research*, *319*, 96–102.
- Christofori, G. (2006). New signals from the invasive front. *Nature*, *441*, 444–450.
- Colegio, O. R., Chu, N. Q., Szabo, A. L., Chu, T., Rhebergen, A. M., Jairam, V., et al. (2014). Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature*, *513*, 559–563.

- Cools-Lartigue, J., Spicer, J., McDonald, B., Gowing, S., Chow, S., Giannias, B., et al. (2013). Neutrophil extracellular traps sequester circulating tumor cells and promote metastasis. *The Journal of Clinical Investigation*, *123*, 3446–3458.
- Cortez-Retamozo, V., Etzrodt, M., Newton, A., Rauch, P. J., Chudnovskiy, A., Berger, C., et al. (2012). Origins of tumor-associated macrophages and neutrophils. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 2491–2496.
- Cortez-Retamozo, V., Etzrodt, M., Newton, A., Ryan, R., Pucci, F., Sio, S. W., et al. (2013). Angiotensin II drives the production of tumor-promoting macrophages. *Immunity*, *38*, 296–308.
- Coussens, L. M., Zitvogel, L., & Palucka, A. K. (2013). Neutralizing tumor-promoting chronic inflammation: A magic bullet? *Science*, *339*, 286–291.
- De Palma, M., & Lewis, C. E. (2013). Macrophage regulation of tumor responses to anti-cancer therapies. *Cancer Cell*, *23*, 277–286.
- de Visser, K. E., Korets, L. V., & Coussens, L. M. (2004). Early neoplastic progression is complement independent. *Neoplasia*, *6*, 768–776.
- Deban, L., Jarva, H., Lehtinen, M. J., Bottazzi, B., Bastone, A., Doni, A., et al. (2008). Binding of the long pentraxin PTX3 to factor H: Interacting domains and function in the regulation of complement activation. *Journal of Immunology*, *181*, 8433–8440.
- De Kleer, I., Willems, F., Lambrecht, B., & Goriely, S. (2014). *Frontiers in Immunology*, *5*, 423.
- DeNardo, D. G., Barreto, J. B., Andreu, P., Vasquez, L., Tawfik, D., Kolhatkar, N., et al. (2009). CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. *Cancer Cell*, *16*, 91–102.
- Doedens, A. L., Stockmann, C., Rubinstein, M. P., Liao, D., Zhang, N., DeNardo, D. G., et al. (2010). Macrophage expression of hypoxia-inducible factor-1 alpha suppresses T-cell function and promotes tumor progression. *Cancer Research*, *70*, 7465–7475.
- Doni, A., Garlanda, C., Bottazzi, B., Meri, S., Garred, P., & Mantovani, A. (2012). Interactions of the humoral pattern recognition molecule PTX3 with the complement system. *Immunobiology*, *217*, 1122–1128.
- Donskov, F. (2013). Immunomonitoring and prognostic relevance of neutrophils in clinical trials. *Seminars in Cancer Biology*, *23*, 200–207.
- Duluc, D., Corvaisier, M., Blanchard, S., Catala, L., Descamps, P., Gamelin, E., et al. (2009). Interferon-gamma reverses the immunosuppressive and protumoral properties and prevents the generation of human tumor-associated macrophages. *International Journal of Cancer. Journal International du Cancer*, *125*, 367–373.
- Dumitru, C. A., Bankfalvi, A., Gu, X., Eberhardt, W. E., Zeidler, R., Lang, S., et al. (2013). Neutrophils activate tumoral CORTACTIN to enhance progression of oropharynx carcinoma. *Frontiers in Immunology*, *4*, 33.
- Dumitru, C. A., Gholaman, H., Trellakis, S., Bruderek, K., Dominas, N., Gu, X., et al. (2011). Tumor-derived macrophage migration inhibitory factor modulates the biology of head and neck cancer cells via neutrophil activation. *International Journal of Cancer Journal International du Cancer*, *129*, 859–869.
- Ebrahim, Q., Chaurasia, S. S., Vasanji, A., Qi, J. H., Klenotic, P. A., Cutler, A., et al. (2010). Cross-talk between vascular endothelial growth factor and matrix metalloproteinases in the induction of neovascularization in vivo. *The American Journal of Pathology*, *176*, 496–503.
- Edwards, J. P., & Emens, L. A. (2010). The multikinase inhibitor sorafenib reverses the suppression of IL-12 and enhancement of IL-10 by PGE(2) in murine macrophages. *International Immunopharmacology*, *10*, 1220–1228.
- Eruslanov, E. B., Bhojnagarwala, P. S., Quatromoni, J. G., Stephen, T. L., Ranganathan, A., Deshpande, C., et al. (2014). Tumor-associated neutrophils stimulate T cell responses in early-stage human lung cancer. *The Journal of Clinical Investigation*, *124*, 5466–5480.

- Fang, C., Zhang, X., Miwa, T., & Song, W.-C. (2009). Complement promotes the development of inflammatory T-helper 17 cells through synergistic interaction with Toll-like receptor signaling and interleukin-6 production. *Blood*, *114*, 1005–1015.
- Fossati, G., Ricevuti, G., Edwards, S. W., Walker, C., Dalton, A., & Rossi, M. L. (1999). Neutrophil infiltration into human gliomas. *Acta Neuropathologica*, *98*, 349–354.
- Franklin, R. A., Liao, W., Sarkar, A., Kim, M. V., Bivona, M. R., Liu, K., et al. (2014). The cellular and molecular origin of tumor-associated macrophages. *Science*, *344*, 921–925.
- Fridlender, Z. G., Sun, J., Kim, S., Kapoor, V., Cheng, G., Ling, L., et al. (2009). Polarization of tumor-associated neutrophil phenotype by TGF-beta: “N1” versus “N2” TAN. *Cancer Cell*, *16*, 183–194.
- Fridlender, Z. G., Sun, J., Mishalian, I., Singhal, S., Cheng, G., Kapoor, V., et al. (2012). Transcriptomic analysis comparing tumor-associated neutrophils with granulocytic myeloid-derived suppressor cells and normal neutrophils. *PloS One*, *7*, e31524.
- Gabrivovich, D. I., Ostrand-Rosenberg, S., & Bronte, V. (2012). Coordinated regulation of myeloid cells by tumours. *Nature Reviews. Immunology*, *12*, 253–268.
- Galon, J., Costes, A., Sanchez-Cabo, F., Kirilovsky, A., Mlecnik, B., Lagorce-Page, C., et al. (2006). Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science*, *313*, 1960–1964.
- Gelderman, K. A., Tomlinson, S., Ross, G. D., & Gorter, A. (2004). Complement function in mAb-mediated cancer immunotherapy. *Trends in Immunology*, *25*, 158–164.
- Germano, G., Frapolli, R., Belgiovine, C., Anselmo, A., Pesce, S., Liguori, M., et al. (2013). Role of macrophage targeting in the anti-tumor activity of Trabectedin. *Cancer Cell*, *23*, 249–262.
- Gironella, M., Calvo, C., Fernandez, A., Closa, D., Iovanna, J. L., Rosello-Catafau, J., et al. (2013). Reg3beta deficiency impairs pancreatic tumor growth by skewing macrophage polarization. *Cancer Research*, *73*, 5682–5694.
- Gocheva, V., Wang, H. W., Gadea, B. B., Shree, T., Hunter, K. E., Garfall, A. L., et al. (2010). IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion. *Genes & Development*, *24*, 241–255.
- Gomez Perdiguero, E., Klapproth, K., Schulz, C., Busch, K., Azzoni, E., Crozet, L., et al. (2015). *Nature*, *518*, 547–551.
- Gorelik, E., Wiltrout, R. H., Brunda, M. J., Holden, H. T., & Herberman, R. B. (1982). Augmentation of metastasis formation by thioglycollate-elicited macrophages. *International Journal of Cancer. Journal International du Cancer*, *29*, 575–581.
- Gout, E., Moriscot, C., Doni, A., Dumestre-Perard, C., Lacroix, M., Perard, J., et al. (2011). M-ficolin interacts with the long pentraxin PTX3: A novel case of cross-talk between soluble pattern-recognition molecules. *Journal of Immunology*, *186*, 5815–5822.
- Granata, F., Frattini, A., Loffredo, S., Staiano, R. I., Petraroli, A., Ribatti, D., et al. (2010). Production of vascular endothelial growth factors from human lung macrophages induced by group IIA and group X secreted phospholipases A2. *Journal of Immunology*, *184*, 5232–5241.
- Granot, Z., Henke, E., Comen, E. A., King, T. A., Norton, L., & Benezra, R. (2011). Tumor entrained neutrophils inhibit seeding in the premetastatic lung. *Cancer Cell*, *20*, 300–314.
- Grenz, S., Naschberger, E., Merkel, S., Britzen-Laurent, N., Schaal, U., Konrad, A., et al. (2013). IFN-gamma-driven intratumoral microenvironment exhibits superior prognostic effect compared with an IFN-alpha-driven microenvironment in patients with colon carcinoma. *The American Journal of Pathology*, *183*, 1897–1909.
- Grosse-Steffen, T., Giese, T., Giese, N., Longerich, T., Schirmacher, P., Hansch, G. M., et al. (2012). Epithelial-to-mesenchymal transition in pancreatic ductal adenocarcinoma

- and pancreatic tumor cell lines: The role of neutrophils and neutrophil-derived elastase. *Clinical & Developmental Immunology*, 2012, 720768.
- Gungor, N., Knaapen, A. M., Munnia, A., Peluso, M., Haenen, G. R., Chiu, R. K., et al. (2010). Genotoxic effects of neutrophils and hypochlorous acid. *Mutagenesis*, 25, 149–154.
- Hagemann, T., Wilson, J., Burke, F., Kulbe, H., Li, N. F., Pluddemann, A., et al. (2006). Ovarian cancer cells polarize macrophages toward a tumor-associated phenotype. *Journal of Immunology*, 176, 5023–5032.
- Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell*, 144, 646–674.
- Hewish, M., Lord, C. J., Martin, S. A., Cunningham, D., & Ashworth, A. (2010). Mismatch repair deficient colorectal cancer in the era of personalized treatment. *Nature Reviews. Clinical Oncology*, 7, 197–208.
- Hirt, C., Eppenberger-Castori, S., Sconocchia, G., Iezzi, G., Tornillo, L., Terracciano, L., et al. (2013). Colorectal carcinoma infiltration by myeloperoxidase-expressing neutrophil granulocytes is associated with favorable prognosis. *Oncoimmunology*, 2, e25990.
- Hörl, S., Bánki, Z., Huber, G., Ejaz, A., Windisch, D., Muellauer, B., et al. (2013). Reduction of complement factor H binding to CLL cells improves the induction of rituximab-mediated complement-dependent cytotoxicity. *Leukemia*, 27, 2200–2208.
- Hotchkiss, K. A., Ashton, A. W., Klein, R. S., Lenzi, M. L., Zhu, G. H., & Schwartz, E. L. (2003). Mechanisms by which tumor cells and monocytes expressing the angiogenic factor thymidine phosphorylase mediate human endothelial cell migration. *Cancer Research*, 63, 527–533.
- Houghton, A. M., Rzymkiewicz, D. M., Ji, H., Gregory, A. D., Egea, E. E., Metz, H. E., et al. (2010). Neutrophil elastase-mediated degradation of IRS-1 accelerates lung tumor growth. *Nature Medicine*, 16, 219–223.
- Huang, S., Van Arsdall, M., Tedjarati, S., McCarty, M., Wu, W., Langley, R., et al. (2002). Contributions of stromal metalloproteinase-9 to angiogenesis and growth of human ovarian carcinoma in mice. *Journal of the National Cancer Institute*, 94, 1134–1142.
- Huh, S. J., Liang, S., Sharma, A., Dong, C., & Robertson, G. P. (2010). Transiently entrapped circulating tumor cells interact with neutrophils to facilitate lung metastasis development. *Cancer Research*, 70, 6071–6082.
- Ijichi, H., Chytil, A., Gorska, A. E., Aakre, M. E., Bierie, B., Tada, M., et al. (2011). Inhibiting Cxcr2 disrupts tumor-stromal interactions and improves survival in a mouse model of pancreatic ductal adenocarcinoma. *The Journal of Clinical Investigation*, 121, 4106–4117.
- Imai, Y., Kubota, Y., Yamamoto, S., Tsuji, K., Shimatani, M., Shibatani, N., et al. (2005). Neutrophils enhance invasion activity of human cholangiocellular carcinoma and hepatocellular carcinoma cells: An in vitro study. *Journal of Gastroenterology and Hepatology*, 20, 287–293.
- Inforzato, A., Doni, A., Barajon, I., Leone, R., Garlanda, C., Bottazzi, B., et al. (2013). PTX3 as a paradigm for the interaction of pentraxins with the complement system. *Seminars in Immunology*, 25, 79–85.
- Jablonska, J., Leschner, S., Westphal, K., Lienenklaus, S., & Weiss, S. (2010). Neutrophils responsive to endogenous IFN- β regulate tumor angiogenesis and growth in a mouse tumor model. *The Journal of Clinical Investigation*, 120, 1151–1164.
- Jamieson, T., Clarke, M., Steele, C. W., Samuel, M. S., Neumann, J., Jung, A., et al. (2012). Inhibition of CXCR2 profoundly suppresses inflammation-driven and spontaneous tumorigenesis. *The Journal of Clinical Investigation*, 122, 3127–3144.
- Jensen, H. K., Donskov, F., Marcussen, N., Nordmark, M., Lundbeck, F., & von der Maase, H. (2009). Presence of intratumoral neutrophils is an independent prognostic

- factor in localized renal cell carcinoma. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, 27, 4709–4717.
- Jin, G., Kawsar, H. I., Hirsch, S. A., Zeng, C., Jia, X., Feng, Z., et al. (2010). An antimicrobial peptide regulates tumor-associated macrophage trafficking via the chemokine receptor CCR2, a model for tumorigenesis. *PloS One*, 5, e10993.
- Joyce, J. A., & Pollard, J. W. (2009). Microenvironmental regulation of metastasis. *Nature Reviews. Cancer*, 9, 239–252.
- Kadoch, C., Li, J., Wong, V. S., Chen, L., Cha, S., Munster, P., et al. (2014). Complement activation and intraventricular rituximab distribution in recurrent central nervous system lymphoma. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, 20, 1029–1041.
- Katara, G. K., Jaiswal, M. K., Kulshrestha, A., Kolli, B., Gilman-Sachs, A., & Beaman, K. D. (2014). Tumor-associated vacuolar ATPase subunit promotes tumorigenic characteristics in macrophages. *Oncogene*, 33, 5649–5654.
- Keane, M. P., Belperio, J. A., Xue, Y. Y., Burdick, M. D., & Strieter, R. M. (2004). Depletion of CXCR2 inhibits tumor growth and angiogenesis in a murine model of lung cancer. *Journal of Immunology*, 172, 2853–2860.
- Keeley, E. C., Mehrad, B., & Strieter, R. M. (2010). CXC chemokines in cancer angiogenesis and metastases. *Advances in Cancer Research*, 106, 91–111.
- Kemp, T. J., Ludwig, A. T., Earel, J. K., Moore, J. M., Vanoosten, R. L., Moses, B., et al. (2005). Neutrophil stimulation with Mycobacterium bovis bacillus Calmette-Guerin (BCG) results in the release of functional soluble TRAIL/Apo-2L. *Blood*, 106, 3474–3482.
- Kryczek, I., Zou, L., Rodriguez, P., Zhu, G., Wei, S., Mottram, P., et al. (2006). B7-H4 expression identifies a novel suppressive macrophage population in human ovarian carcinoma. *The Journal of Experimental Medicine*, 203, 871–881.
- Kuang, D. M., Zhao, Q., Peng, C., Xu, J., Zhang, J. P., Wu, C., et al. (2009). Activated monocytes in peritumoral stroma of hepatocellular carcinoma foster immune privilege and disease progression through PD-L1. *The Journal of Experimental Medicine*, 206, 1327–1337.
- Kuang, D. M., Zhao, Q., Wu, Y., Peng, C., Wang, J., Xu, Z., et al. (2011). Peritumoral neutrophils link inflammatory response to disease progression by fostering angiogenesis in hepatocellular carcinoma. *Journal of Hepatology*, 54, 948–955.
- Laoui, D., Van Overmeire, E., Di Conza, G., Aldeni, C., Keirsse, J., Morias, Y., et al. (2014). Tumor hypoxia does not drive differentiation of tumor-associated macrophages but rather fine-tunes the M2-like macrophage population. *Cancer Research*, 74, 24–30.
- Lazennec, G., & Richmond, A. (2010). Chemokines and chemokine receptors: New insights into cancer-related inflammation. *Trends in Molecular Medicine*, 16, 133–144.
- Li, D., Wang, X., Wu, J. L., Quan, W. Q., Ma, L., Yang, F., et al. (2013). Tumor-produced versican V1 enhances hCAP18/LL-37 expression in macrophages through activation of TLR2 and vitamin D3 signaling to promote ovarian cancer progression in vitro. *PloS One*, 8, e56616.
- Lin, E. Y., Gouon-Evans, V., Nguyen, A. V., & Pollard, J. W. (2002). The macrophage growth factor CSF-1 in mammary gland development and tumor progression. *Journal of Mammary Gland Biology and Neoplasia*, 7, 147–162.
- Lin, J. Y., Li, X. Y., Tadashi, N., & Dong, P. (2011). Clinical significance of tumor-associated macrophage infiltration in supraglottic laryngeal carcinoma. *Chinese Journal of Cancer*, 30, 280–286.
- Linde, N., Lederle, W., Depner, S., van Rooijen, N., Gutschalk, C. M., & Mueller, M. M. (2012). Vascular endothelial growth factor-induced skin carcinogenesis depends on recruitment and alternative activation of macrophages. *The Journal of Pathology*, 227, 17–28.

- Ma, Y. J., Doni, A., Romani, L., Jurgensen, H. J., Behrendt, N., Mantovani, A., et al. (2013). Ficolin-1-PTX3 complex formation promotes clearance of altered self-cells and modulates IL-8 production. *Journal of Immunology*, *191*, 1324–1333.
- Mantovani, A. (2009). The yin-yang of tumor-associated neutrophils. *Cancer Cell*, *16*, 173–174.
- Mantovani, A., & Allavena, P. (2015). The interaction of anticancer therapies with tumor-associated macrophages. *The Journal of Experimental Medicine*, *212*, 435–445. <http://dx.doi.org/10.1084/jem.20150295>.
- Mantovani, A., Allavena, P., Sica, A., & Balkwill, F. (2008). Cancer-related inflammation. *Nature*, *454*, 436–444.
- Mantovani, A., Allavena, P., Sozzani, S., Vecchi, A., Locati, M., & Sica, A. (2004). Chemokines in the recruitment and shaping of the leukocyte infiltrate of tumors. *Seminars in Cancer Biology*, *14*, 155–160.
- Mantovani, A., Bottazzi, B., Colotta, F., Sozzani, S., & Ruco, L. (1992). The origin and function of tumor-associated macrophages. *Immunology Today*, *13*, 265–270.
- Mantovani, A., Cassatella, M. A., Costantini, C., & Jaillon, S. (2011). Neutrophils in the activation and regulation of innate and adaptive immunity. *Nature Reviews. Immunology*, *11*, 519–531.
- Mantovani, A., & Sica, A. (2010). Macrophages, innate immunity and cancer: Balance, tolerance, and diversity. *Current Opinion in Immunology*, *22*, 231–237.
- Mantovani, A., Sozzani, S., Locati, M., Allavena, P., & Sica, A. (2002). Macrophage polarization: Tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends in Immunology*, *23*, 549–555.
- Markiewski, M. M., DeAngelis, R. A., Benencia, F., Ricklin-Lichtsteiner, S. K., Koutoulaki, A., Gerard, C., et al. (2008). Modulation of the antitumor immune response by complement. *Nature Immunology*, *9*, 1225–1235.
- Matsui, A., Yokoo, H., Negishi, Y., Endo-Takahashi, Y., Chun, N. A., Kadouchi, I., et al. (2012). CXCL17 expression by tumor cells recruits CD11b+Gr1 high F4/80-cells and promotes tumor progression. *PloS One*, *7*, e44080.
- McGovern, N., Schlitzer, A., Gunawan, M., Jardine, L., Shin, A., Poyner, E., V., et al. (2014). *Immunity*, *41*, 465–477.
- Middleton, O., Cosimo, E., Dobbin, E., McCaig, A. M., Clarke, C., Brant, A. M., et al. (2015). Complement deficiencies limit CD20 monoclonal antibody treatment efficacy in CLL. *Leukemia*, *29*, 107–114.
- Mittendorf, E. A., Alatrash, G., Qiao, N., Wu, Y., Sukhumalchandra, P., St John, L. S., et al. (2012). Breast cancer cell uptake of the inflammatory mediator neutrophil elastase triggers an anticancer adaptive immune response. *Cancer Research*, *72*, 3153–3162.
- Molawi, K., Wolf, Y., Kandalla, P. K., Favret, J., Hagemeyer, N., Frenzel, K., et al. (2014). *The Journal of Experimental Medicine*, *211*, 2151–2158.
- Mosser, D. M., & Edwards, J. P. (2008). Exploring the full spectrum of macrophage activation. *Nature Reviews Immunology*, *8*, 958–969.
- Movahedi, K., Laoui, D., Gysemans, C., Baeten, M., Stange, G., Van den Bossche, J., et al. (2010). Different tumor microenvironments contain functionally distinct subsets of macrophages derived from Ly6C(high) monocytes. *Cancer Research*, *70*, 5728–5739.
- Murdoch, C., Giannoudis, A., & Lewis, C. E. (2004). Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues. *Blood*, *104*, 2224–2234.
- Murray, P. J., Allen, J. E., Biswas, S. K., Fisher, E. A., Gilroy, D. W., Goerdt, S., et al. (2014). Macrophage activation and polarization: Nomenclature and experimental guidelines. *Immunity*, *41*, 14–20.
- Nagakawa, Y., Aoki, T., Kasuya, K., Tsuchida, A., & Koyanagi, Y. (2002). Histologic features of venous invasion, expression of vascular endothelial growth factor and matrix

- metalloproteinase-2 and matrix metalloproteinase-9, and the relation with liver metastasis in pancreatic cancer. *Pancreas*, *24*, 169–178.
- Naschberger, E., Croner, R. S., Merkel, S., Dimmler, A., Tripal, P., Amann, K. U., et al. (2008). Angiostatic immune reaction in colorectal carcinoma: Impact on survival and perspectives for antiangiogenic therapy. *International Journal of Cancer. Journal International du Cancer*, *123*, 2120–2129.
- Neyen, C., Pluddemann, A., Mukhopadhyay, S., Maniati, E., Bossard, M., Gordon, S., et al. (2013). Macrophage scavenger receptor a promotes tumor progression in murine models of ovarian and pancreatic cancer. *Journal of Immunology*, *190*, 3798–3805.
- Ning, C., Li, Y. Y., Wang, Y., Han, G. C., Wang, R. X., Xiao, H., et al. (2015). Complement activation promotes colitis-associated carcinogenesis through activating intestinal IL-1 β /IL-17A axis. *Mucosal Immunology*. <http://dx.doi.org/10.1038/mi.2015.18>, 2015 March 4.
- Noy, R., & Pollard, J. W. (2014). Tumor-associated macrophages: From mechanisms to therapy. *Immunity*, *41*, 49–61.
- Nozaki, M., Raisler, B. J., Sakurai, E., Sarma, J. V., Barnum, S. R., Lambris, J. D., et al. (2006). Drusen complement components C3a and C5a promote choroidal neovascularization. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 2328–2333.
- Nozawa, H., Chiu, C., & Hanahan, D. (2006). Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 12493–12498.
- Okemefuna, A. I., Nan, R., Miller, A., Gor, J., & Perkins, S. J. (2010). Complement factor H binds at two independent sites to C-reactive protein in acute phase concentrations. *The Journal of Biological Chemistry*, *285*, 1053–1065.
- Pasinetti, G. M., Tocco, G., Sakhi, S., Musleh, W. D., DeSimoni, M. G., Mascarucci, P., et al. (1996). Hereditary deficiencies in complement C5 are associated with intensified neurodegenerative responses that implicate new roles for the C-system in neuronal and astrocytic functions. *Neurobiology of Disease*, *3*, 197–204.
- Piao, C., Cai, L., Qiu, S., Jia, L., Song, W., & Du, J. (2015). Complement 5a enhances hepatic metastases of colon cancer via monocyte chemoattractant protein-1-mediated inflammatory cell infiltration. *Journal of Biological Chemistry*, *290*, 10667–10676, 2015 March 4, pii: jbc.M114.612622.
- Pio, R., Corrales, L., & Lambris, J. (2014). The role of complement in tumor growth. In C. Koumenis, E. Hammond, & A. Giaccia (Eds.), *Tumor microenvironment and cellular stress* (pp. 229–262). New York: Springer.
- Qing, W., Fang, W. Y., Ye, L., Shen, L. Y., Zhang, X. F., Fei, X. C., et al. (2012). Density of tumor-associated macrophages correlates with lymph node metastasis in papillary thyroid carcinoma. *Thyroid: Official Journal of the American Thyroid Association*, *22*, 905–910.
- Queen, M. M., Ryan, R. E., Holzer, R. G., Keller-Peck, C. R., & Jorcyk, C. L. (2005). Breast cancer cells stimulate neutrophils to produce oncostatin M: Potential implications for tumor progression. *Cancer Research*, *65*, 8896–8904.
- Rao, H. L., Chen, J. W., Li, M., Xiao, Y. B., Fu, J., Zeng, Y. X., et al. (2012). Increased intratumoral neutrophil in colorectal carcinomas correlates closely with malignant phenotype and predicts patients' adverse prognosis. *PloS One*, *7*, e30806.
- Reed, J. R., Stone, M. D., Beadnell, T. C., Ryu, Y., Griffin, T. J., & Schwertfeger, K. L. (2012). Fibroblast growth factor receptor 1 activation in mammary tumor cells promotes macrophage recruitment in a CX3CL1-dependent manner. *PloS One*, *7*, e45877.
- Reid, M. D., Basturk, O., Thirabanjasak, D., Hruban, R. H., Klimstra, D. S., Bagci, P., et al. (2011). Tumor-infiltrating neutrophils in pancreatic neoplasia. *Modern Pathology: An Official Journal of the United States and Canadian Academy of Pathology, Inc.* *24*, 1612–1619.

- Ricklin, D., Hajishengallis, G., Yang, K., & Lambris, J. D. (2010). Complement: A key system for immune surveillance and homeostasis. *Nature Immunology*, *11*, 785–797.
- Ricklin, D., & Lambris, J. D. (2013). Complement in immune and inflammatory disorders: Pathophysiological mechanisms. *Journal of Immunology*, *190*, 3831–3838.
- Rodriguez, P. C., Ernstoff, M. S., Hernandez, C., Atkins, M., Zabaleta, J., Sierra, R., et al. (2009). Arginase I-producing myeloid-derived suppressor cells in renal cell carcinoma are a subpopulation of activated granulocytes. *Cancer Research*, *69*, 1553–1560.
- Rozel, S., Galban, C. J., Nicolay, K., Lee, K. C., Sud, S., Neeley, C., et al. (2009). Synergy between anti-CCL2 and docetaxel as determined by DW-MRI in a metastatic bone cancer model. *Journal of Cellular Biochemistry*, *107*, 58–64.
- Ruffell, B., Affara, N. I., & Coussens, L. M. (2012). Differential macrophage programming in the tumor microenvironment. *Trends in Immunology*, *33*, 119–126.
- Rutkowski, M. J., Sughrue, M. E., Kane, A. J., Mills, S. A., & Parsa, A. T. (2010). Cancer and the complement cascade. *Molecular Cancer Research: MCR*, *8*, 1453–1465.
- Sagiv, J. Y., Michaeli, J., Assi, S., Mishalian, I., Kisos, H., Levy, L., et al. (2015). Phenotypic diversity and plasticity in circulating neutrophil subpopulations in cancer. *Cell Reports*, *10*, 562–573.
- Scapini, P., Morini, M., Tecchio, C., Minghelli, S., Di Carlo, E., Tanghetti, E., et al. (2004). CXCL1/macrophage inflammatory protein-2-induced angiogenesis in vivo is mediated by neutrophil-derived vascular endothelial growth factor-A. *Journal of Immunology*, *172*, 5034–5040.
- Scapini, P., Nesi, L., Morini, M., Tanghetti, E., Belleri, M., Noonan, D., et al. (2002). Generation of biologically active angiostatin kringle 1–3 by activated human neutrophils. *Journal of Immunology*, *168*, 5798–5804.
- Schmidt, T., & Carmeliet, P. (2010). Blood-vessel formation: Bridges that guide and unite. *Nature*, *465*, 697–699.
- Schmielau, J., & Finn, O. J. (2001). Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of t-cell function in advanced cancer patients. *Cancer Research*, *61*, 4756–4760.
- Schoppmann, S. F., Horvat, R., & Birner, P. (2002). Lymphatic vessels and lymphangiogenesis in female cancer: Mechanisms, clinical impact and possible implications for anti-lymphangiogenic therapies (Review). *Oncology Reports*, *9*, 455–460.
- Shand, F. H. W., Ueha, S., Otsuji, M., Koid, S. S., Shichino, S., Tsukui, T., et al. (2014). *Proceedings of the National Academy of Sciences*, *111*, 7771–7776.
- Shojaei, F., Singh, M., Thompson, J. D., & Ferrara, N. (2008). Role of Bv8 in neutrophil-dependent angiogenesis in a transgenic model of cancer progression. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 2640–2645.
- Shojaei, F., Wu, X., Qu, X., Kowanetz, M., Yu, L., Tan, M., et al. (2009). G-CSF-initiated myeloid cell mobilization and angiogenesis mediate tumor refractoriness to anti-VEGF therapy in mouse models. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 6742–6747.
- Shojaei, F., Wu, X., Zhong, C., Yu, L., Liang, X. H., Yao, J., et al. (2007). Bv8 regulates myeloid-cell-dependent tumour angiogenesis. *Nature*, *450*, 825–831.
- Sica, A., & Mantovani, A. (2012). Macrophage plasticity and polarization: In vivo veritas. *The Journal of Clinical Investigation*, *122*, 787–795.
- Sica, A., Saccani, A., Bottazzi, B., Polentarutti, N., Vecchi, A., van Damme, J., et al. (2000). Autocrine production of IL-10 mediates defective IL-12 production and NF-kappa B activation in tumor-associated macrophages. *Journal of Immunology*, *164*, 762–767.
- Sjoberg, A. P., Trouw, L. A., McGrath, F. D., Hack, C. E., & Blom, A. M. (2006). Regulation of complement activation by C-reactive protein: Targeting of the inhibitory activity of C4b-binding protein. *Journal of Immunology*, *176*, 7612–7620.

- Solinas, G., Schiarea, S., Liguori, M., Fabbri, M., Pesce, S., Zammataro, L., et al. (2010). Tumor-conditioned macrophages secrete migration-stimulating factor: A new marker for M2-polarization, influencing tumor cell motility. *Journal of Immunology*, *185*, 642–652.
- Solito, S., Falisi, E., Diaz-Montero, C. M., Doni, A., Pinton, L., Rosato, A., et al. (2011). A human promyelocytic-like population is responsible for the immune suppression mediated by myeloid-derived suppressor cells. *Blood*, *118*, 2254–2265.
- Stetler-Stevenson, W. G., & Yu, A. E. (2001). Proteases in invasion: Matrix metalloproteinases. *Seminars in Cancer Biology*, *11*, 143–152.
- Tanaka, H., Ito, T., Kyo, T., & Kimura, A. (2007). Treatment with IFN α in vivo up-regulates serum-soluble TNF-related apoptosis inducing ligand (sTRAIL) levels and TRAIL mRNA expressions in neutrophils in chronic myelogenous leukemia patients. *European Journal of Haematology*, *78*, 389–398.
- Tecchio, C., Huber, V., Scapini, P., Calzetti, F., Margotto, D., Todeschini, G., et al. (2004). IFN α -stimulated neutrophils and monocytes release a soluble form of TNF-related apoptosis-inducing ligand (TRAIL/Apo-2 ligand) displaying apoptotic activity on leukemic cells. *Blood*, *103*, 3837–3844.
- Tecchio, C., Scapini, P., Pizzolo, G., & Cassatella, M. A. (2013). On the cytokines produced by human neutrophils in tumors. *Seminars in Cancer Biology*, *23*, 159–170.
- Trellakis, S., Bruderek, K., Dumitru, C. A., Gholaman, H., Gu, X., Bankfalvi, A., et al. (2011). Polymorphonuclear granulocytes in human head and neck cancer: Enhanced inflammatory activity, modulation by cancer cells and expansion in advanced disease. *International Journal of Cancer. Journal International du Cancer*, *129*, 2183–2193.
- Trellakis, S., Farjah, H., Bruderek, K., Dumitru, C. A., Hoffmann, T. K., Lang, S., et al. (2011). Peripheral blood neutrophil granulocytes from patients with head and neck squamous cell carcinoma functionally differ from their counterparts in healthy donors. *International Journal of Immunopathology and Pharmacology*, *24*, 683–693.
- Tymoszuk, P., Evens, H., Marzola, V., Wachowicz, K., Wasmer, M. H., Datta, S., et al. (2014). In situ proliferation contributes to accumulation of tumor-associated macrophages in spontaneous mammary tumors. *European Journal of Immunology*, *44*, 2247–2262.
- Ueno, T., Toi, M., Saji, H., Muta, M., Bando, H., Kuroi, K., et al. (2000). Significance of macrophage chemoattractant protein-1 in macrophage recruitment, angiogenesis, and survival in human breast cancer. *Clinical Cancer Research*, *8*, 3282–3289.
- Vadrevu, S. K., Chintala, N. K., Sharma, S. K., Sharma, P., Cleveland, C., Riediger, L., et al. (2014). Complement C5a receptor facilitates cancer metastasis by altering T-cell responses in the metastatic niche. *Cancer Research*, *74*, 3454–3465.
- Vasiljeva, O., Papazoglou, A., Kruger, A., Brodoefel, H., Korovin, M., Deussing, J., et al. (2006). Tumor cell-derived and macrophage-derived cathepsin B promotes progression and lung metastasis of mammary cancer. *Cancer Research*, *66*, 5242–5250.
- Wang, F. Q., So, J., Reierstad, S., & Fishman, D. A. (2005). Matrilysin (MMP-7) promotes invasion of ovarian cancer cells by activation of progelatinase. *International Journal of Cancer. Journal International du Cancer*, *114*, 19–31.
- Wang, X. F., Wang, H. S., Zhang, F., Guo, Q., Wang, H., Wang, K. F., et al. (2014). Nodal promotes the generation of M2-like macrophages and downregulates the expression of IL-12. *European Journal of Immunology*, *44*, 173–183.
- Wang, R., Zhang, J., Chen, S., Lu, M., Luo, X., Yao, S., et al. (2011). Tumor-associated macrophages provide a suitable microenvironment for non-small lung cancer invasion and progression. *Lung Cancer*, *74*, 188–196.
- Weitzman, S. A., Weitberg, A. B., Clark, E. P., & Stossel, T. P. (1985). Phagocytes as carcinogens: Malignant transformation produced by human neutrophils. *Science (New York, N.Y.)*, *227*, 1231–1233.

- Wislez, M., Rabbe, N., Marchal, J., Milleron, B., Crestani, B., Mayaud, C., et al. (2003). Hepatocyte growth factor production by neutrophils infiltrating bronchioloalveolar subtype pulmonary adenocarcinoma: Role in tumor progression and death. *Cancer Research*, *63*, 1405–1412.
- Wu, C. F., Andzinski, L., Kasnitz, N., Kroger, A., Klawonn, F., Lienenklaus, S., et al. (2015). The lack of type I interferon induces neutrophil-mediated pre-metastatic niche formation in the mouse lung. *International Journal of Cancer. Journal International du Cancer*. <http://dx.doi.org/10.1002/ijc.29444>.
- Wynn, T. A., Chawla, A., & Pollard, J. W. (2013). *Nature*, *496*, 445–455.
- Xin, H., Zhang, C., Herrmann, A., Du, Y., Figlin, R., & Yu, H. (2009). Sunitinib inhibition of Stat3 induces renal cell carcinoma tumor cell apoptosis and reduces immunosuppressive cells. *Cancer Research*, *69*, 2506–2513.
- Ye, X. Z., Xu, S. L., Xin, Y. H., Yu, S. C., Ping, Y. F., Chen, L., et al. (2012). Tumor-associated microglia/macrophages enhance the invasion of glioma stem-like cells via TGF-beta1 signaling pathway. *Journal of Immunology*, *189*, 444–453.
- Youn, J. I., Collazo, M., Shalova, I. N., Biswas, S. K., & Gabrilovich, D. I. (2012). Characterization of the nature of granulocytic myeloid-derived suppressor cells in tumor-bearing mice. *Journal of Leukocyte Biology*, *91*, 167–181.
- Youn, J. I., & Gabrilovich, D. I. (2010). The biology of myeloid-derived suppressor cells: The blessing and the curse of morphological and functional heterogeneity. *European Journal of Immunology*, *40*, 2969–2975.
- Ytting, H., Jarle Christensen, I., Thiel, S., Jensenius, J. C., & Nielsen, H. J. (2005). Serum mannan-binding lectin-associated serine protease 2 levels in colorectal cancer: Relation to recurrence and mortality. *Clinical Cancer Research*, *11*, 1441–1446.
- Zhang, J., Sud, S., Mizutani, K., Gyetko, M. R., & Pienta, K. J. (2011). Activation of urokinase plasminogen activator and its receptor axis is essential for macrophage infiltration in a prostate cancer mouse model. *Neoplasia*, *13*, 23–30.
- Zhao, Q., Kuang, D. M., Wu, Y., Xiao, X., Li, X. F., Li, T. J., et al. (2012). Activated CD69+ T cells foster immune privilege by regulating IDO expression in tumor-associated macrophages. *Journal of Immunology*, *188*, 1117–1124.
- Zhou, S. L., Dai, Z., Zhou, Z. J., Wang, X. Y., Yang, G. H., Wang, Z., et al. (2012). Overexpression of CXCL5 mediates neutrophil infiltration and indicates poor prognosis for hepatocellular carcinoma. *Hepatology*, *56*, 2242–2254.