Cell. Mol. Life Sci. (2012) 69:1391–1414
DOI 10.1007/s00018-011-0863-7

Cellular and Molecular Life Sciences

REVIEW

Macrophage–tumor crosstalk: role of TAMR tyrosine kinase receptors and of their ligands

Thomas Schmidt · Isabel Ben-Batalla · Alexander Schultze · Sonja Loges

Received: 14 August 2011 / Revised: 14 October 2011 / Accepted: 14 October 2011 / Published online: 11 November 2011

Abstract Ample clinical and preclinical evidence indicates that macrophages interact with tumor cells as well as with virtually all populations of host cells present in the tumor microenvironment. This crosstalk can strongly promote malignancy, but also has in principle the potential to inhibit tumor growth. Thus, it is of the utmost importance to improve our understanding of the mechanisms driving the pro- and antimalignant behavior of tumor-associated macrophages (TAMs) in order to develop better anticancer therapies. In this review, we discuss the biological consequences of reciprocal interactions between TAMs, cancer cells, endothelial cells, fibroblasts and other leukocyte subfractions within tumors. It was recently elucidated that tumors specifically educate macrophages to secrete growth arrest-specific gene 6 (Gas6), the common ligand of the Tyro3, Axl, Mer receptor (TAMR) family. In turn, Gas6 fosters tumor growth by promoting cancer cell proliferation. Therefore, the Gas6–TAMR axis might represent a novel target for disrupting tumor–macrophage crosstalk. We summarize here what is known about TAMR and their ligands in (human) cancer biology. In order to shed more light on the role of macrophages in human cancer, we additionally provide an overview of what is currently known about the prognostic impact of TAMs in human cancer.

Keywords TAM (Tumor-associated macrophages) · Gas6 · TAMR (Tyro3, Axl, Mer receptors) · Tumor-macrophage crosstalk · Cancer · Inflammation

Introduction

Cancer represents a heterogeneous class of diseases originating from neoplastic cells capable of uncontrolled growth. More than 10 years ago, six essential "hallmarks of cancer" were extracted from several decades of research in order to define malignancy [1]: (1) self-sufficiency in growth signals, (2) insensitivity to antigrowth signals, (3) evasion of apoptosis, (4) limitless replicative potential, (5) sustained angiogenesis, and (6) tissue invasion and metastasis. Today, however, this tumor cell-centered picture of cancer represents a rather simplistic view that neglects the complex microenvironment of the host. This microenvironment forms an integral part of every tumor and it is crucially involved in every single step of carcinogenesis, ranging from cancer initiation to metastasis [2, 3]. Cancer cells are surrounded by numerous different stromal cell types, including vascular and lymphatic endothelial cells, pericytes, vascular smooth muscle cells,

Thomas Schmidt and Isabel Ben-Batalla contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00018-011-0863-7) contains supplementary material, which is available to authorized users.

T. Schmidt
Department of General, Visceral and Transplantation Surgery, University of Heidelberg, Heidelberg, Germany

I. Ben-Batalla · A. Schultze · S. Loges (✉)
Department of Hematology and Oncology with Sections BMT and Pneumology, Hubertus Wald Tumorzentrum, University Comprehensive Cancer Center Hamburg, University Medical Center Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany
e-mail: s.loges@uke.uni-hamburg.de

I. Ben-Batalla · A. Schultze · S. Loges
Institute of Tumor Biology, Center of Experimental Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

mesenchymal cells, adipocytes, cancer-associated fibroblasts (CAFs) as well as a large variety of bone marrow-derived cells (BMDCs) [2–5]. Tumor-infiltrating BMDCs comprise a heterogeneous population of leukocytes with immunological properties such as B- and T-lymphocytes, NK cells, macrophages and related myeloid cells, dendritic cells, granulocytes and mast cells [4, 6]. This leukocyte infiltrate varies in size, composition and distribution between different tumor types and stages of progression and is often termed "tumor inflammation" [4, 6]. However, we need to keep in mind that this terminology is misleading because tumor inflammation lacks many of the cardinal features of inflammation sensu strictu including fever, swelling and edema [7]. In this review, we also use "tumor inflammation", but we refer to the smoldering, subacute and chronic inflammation typically found in tumors [8].

Tumor and stroma cells are embedded in an extracellular matrix consisting of integrins, collagens, hyaluron, laminins and proteoglycans amongst others, with which tumor cells intensively communicate via junctions, receptors, growth factors, hormones and other soluble molecules [3, 9]. Thus, cancers represent complex mixtures of malignant and non-malignant (host-derived) cells and components interacting with one another in a reciprocal manner throughout tumor development and progression [2–4, 6]. Unfortunately, in many cases, tumor cells succeed in exploiting the microenvironment for their benefit by creating a supportive environment that promotes cancer initiation and growth, and eventually its progression to fatal disease [2]. For instance, induction of angiogenesis is an extensively studied example of how cancers exploit their host [10–12]. Tumor-infiltrating inflammatory cells were once assumed to inhibit tumor growth or to be a consequence of failed cancer cell destruction. However, in the light of recent data, it is becoming increasingly clear that these cells can play key roles in promoting tumors by multiple mechanisms [2, 4, 6, 7]. Even immunological cell types with potential tumoricidal activity such as macrophages and neutrophils often are converted under the influence of cancer cells into tumor-promoting subpopulations [7, 13]. Macrophages and closely related cell types can even mediate resistance to conventional chemotherapy or targeted antiangiogenic treatment [14, 15].

Thus, cells of the immune system act as "double-edged swords" in the context of tumor biology because they are in principle capable of destroying and promoting cancers. However, in many cases immune cells seem to show pro-tumoral activity [6]. However, because many patients who died of non-malignant causes have been found to host occult carcinomas, for instance in their breast or prostate, which failed to progress to advanced cancer [16], we also need to consider that the microenvironment can and does constrain malignant cells. Obviously, it would be desirable to tilt the microenvironment more towards destruction of tumors. As a consequence, development of anticancer drugs has moved from a traditional cancer cell-centered approach towards increased targeting of the microenvironment, as reflected by development of numerous compounds acting primarily on host-derived cells or structures [16]. Unfortunately, despite tremendous efforts in the field of tumor immunology and immunotherapy, attempts to instruct immune cells to fight the progression of established tumors has had only limited success [17]. Therefore, it is of the utmost importance to better dissect the molecular and cellular basis of these fatal interactions between tumor cells and immune cells in order to improve our knowledge of cancer biology and to develop more effective therapies for cancer patients.

The importance and high priority of this issue are also reflected in the recent appreciation of tumor inflammation as seventh hallmark of cancer [18–20]. Different cell types and mechanisms related to this topic have recently been extensively reviewed elsewhere [4, 6, 7]. In this review, we specifically highlight the bidirectional crosstalk between macrophages and tumors (i.e. tumor cells and microenvironmental host cells) with a special focus on the role of the Tyro3, Axl and Mer (TAM) receptors and of their ligands growth arrest-specific gene 6 (Gas6) and protein S.

In the following section we first summarize the role of macrophages in cancer and then focus on their interaction with different cell types. We mainly discuss preclinical insights, but also provide links to human (clinical) data where appropriate.

Role of macrophages in cancer

Macrophages are differentiated cells of the myelomonocytic lineage capable of phagocytosis. They are important components of the innate immune system. In mice, macrophages express the cell surface markers CD11b, F4/80 and colony-stimulating factor-1 receptor (CSF-1R; CD115), but they do not display the Ly6G epitope of Gr1. In humans, macrophages are characterized by the presence of CD16, CD68, CD163 and CD312 [7]. By combining these cell surface profiles with morphological parameters macrophages can be differentiated from other closely related myeloid cell types with partially overlapping phenotypes such as polynuclear neutrophils and eosinophils [7]. In general, macrophages originate from monocytes, which are recruited from the peripheral blood into tissues, where they differentiate into macrophages. Tissue macrophages adopt various organ-specific phenotypes such as Kupffer cells in the liver, Langerhans cells in the skin, osteoclasts in the bone and microglia in the brain [7]. The details of this process including monocytic lineage differentiation from $CD34^{+}$ hematopoietic stem cells and the

regulation by growth factors have been reviewed elsewhere [21].

TAMs and T cells are the most abundant immune cells in the tumor microenvironment [4, 22]. It is important to note that mononuclear phagocytes exhibit remarkable plasticity and diversity. Besides prototypic macrophages (as described above), subpopulations including a TIE2-expressing monocyte subset (TEMs), myeloid-derived suppressor cells and myeloid dendritic cells occur within the population of myelomonocytes in tumors [4]. These cells share certain phenotypic and functional properties with macrophages such as the cell surface marker CD11b and the ability to promote tumor progression. The precise role of each of these players in cancer biology remains to be determined.

Due to the diversity of macrophage function several attempts were initiated to categorize them, resulting in one commonly used classification, which is based on their immunogenic function [7, 23]. "Classically activated" macrophages are involved in the type I immune response mediated by T helper 1 (Th1) cells and they were therefore coined M1 macrophages. M1 macrophages are activated by microbial products, interferon gamma and by Toll-like receptor signaling [7, 24]. They release high levels of proinflammatory cytokines including IL-1, IL-6, IL-12, IL-23 and tumor necrosis factor alpha (TNFα), and express high levels of major histocompatibility complex molecules [6, 7, 24]. Moreover, M1 macrophages secrete Th1 cell-attracting chemokines including CXCL9 and CXCL10. They also generate reactive oxygen species and nitric oxide [25]. Thus, M1 macrophages exhibit a proinflammatory phenotype able to support antitumoral immune responses by activating other immune cells and by engulfing tumor cells.

In contrast, another subtype of macrophages is termed "alternatively activated" M2 in response to cytokines of T helper 2 (Th2) type, such as IL-4, IL-10 and IL-13. Also alternative mediators, which are abundantly present within the tumor microenvironment, including IL-6, LIF and prostaglandin E2 can induce M2 polarization of macrophages [26, 27]. M2 macrophages express IL-1RA, IL-1decoy receptor and the chemokines CCL17, CCL22 and CCL24. Furthermore, they down-modulate MHC II and IL-12 expression, and thus have a less inflammatory and immunoactivating phenotype than M1 macrophages. IL-10 activates STAT3 signaling and IL-4 activates STAT6, both of which further downstream induce transcription of M2-specific genes such as arginase-1 and arginase-2, and inhibit NFκB signaling [28–31]. M2 macrophages show increased expression of immunosuppressive modulators including IL-10, scavenger receptor A, ornithine and arginase. In addition, they express different proangiogenic cytokines including vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and semaphorin 4D (see below). Besides their proangiogenic activity, M2 macrophages are potent inducers of lymphangiogenesis. Transcriptional profiling has revealed further significant differences in the transcriptome of M2 macrophages as compared to that of M1 macrophages, including expression of cyclooxygenase 1, mannose receptor 1, macrophage scavenger receptor 1 and the C-type lectin receptor Dectin-1 [32]. In general, M2 macrophages dampen inflammation and promote tissue remodeling and tumor progression [23].

As a note of caution, we need to keep in mind that classifying TAMs into M1 and M2 is a (useful) over-simplification, because macrophages are more plastic and less determined than Th1 and Th2 cells. Most likely TAMs rather form a continuum with partially overlapping functions than represent strictly binary M1 or M2 macrophages [7, 33]. This notion is supported by recent data demonstrating coexpression of M1 and M2 markers in subsets of TAMs in murine skin cancer [34]. Similarly, coexpression of CD163 (M2) and CXCL10 (M1) was observed in liver metastases of human colon cancer [35]. Interestingly, M1 and M2 macrophages exhibited different spatial distributions in a model of mammary carcinoma, where M1 macrophages resided more in normoxic tumor tissues, while M2 macrophages rather accumulated in hypoxic tumor regions [36]. To add further to the complexity of TAMs, certain macrophages, which resemble macrophages involved in tissue development during embryogenesis and in tissue-shaping during adulthood, coexist within the tumor microenvironment [21]. These "trophic" macrophages fail to fit into the immunological classification of M1 and M2 macrophages because they mainly develop in response to CSF-1 and show lower levels of expression of M1- and M2-related factors [37]. Altogether, TAMs represent different macrophage phenotypes; thus a dynamic model probably more accurately reflects their phenotype than the rather static M1/M2 classification. In any case, more work is needed to define better the specific fractional and functional contribution of different TAM phenotypes to tumor inflammation. However, the majority of TAMs isolated from established murine and human tumors exhibit immunosuppressive and tumor-fostering M2 properties, and thus promote rather than inhibit tumor progression [23].

This notion is supported by some clinical data indicating an adverse prognostic effect of macrophage infiltration in different cancers (Table 1; see also Supplementary Material Table 1 for detailed information). In breast cancer, uterine cancer, kidney cancer and melanoma the majority of published studies (three or more independent studies each) show a negative clinical impact of high TAM numbers, and in these cancers no data exist so far supporting a positive association between TAMs and prognosis.

Table 1 Systematic overview of studies correlating TAM infiltration with prognosis of different tumor entities. This overview includes the numbers of studies linking TAMs to bad, good, or no prognosis with the total number of patients included in these respective studies. Table references are as listed in the Supplementary Material

Tumor type	Studies indicating link to bad prognosis		Studies not indicating link to prognosis		Studies indicating link to good prognosis	
	References	No. of patients	References	No. of patients	References	No. of patients
Breast	8[1–8]	1702	2[1, 9]	198	–	
Pancreas	1[10]	76	–		–	
Kidney	3[11–13]	227	–		–	
Lung	5[14–18]	584	3[19–21]	462	4[15, 22–24]	383
Ewing sarcoma	1[25]	41	–		–	
Melanoma	5[26–30]	472	1[31]	47	–	
Liver	3[32–34]	313	–		2[35, 36]	316
Glioma	3[37–39]	149	2[40, 41]	104	–	
Prostate	3[42–44]	231	1[45]	92	2[46, 47]	185
Bladder	1[48]	63	–		–	
Uterus	4[49–52]	280	–		–	
Ovary	2[53, 54]	129	2[55, 56]	175	–	
Bulky ampullary	1[57]	100	–		–	
Esophageal squamous-cell	1[58]	137	–		–	
Colorectal	3[59–61]	468	–		3[62–64]	302
Gastric cancer	4[65–68]	343	2[66, 69]	106	2[70, 71]	162
Leiomyosarcomas, nongynecological	1[72]	73	–		–	
Leiomyosarcomas, gynecological	–		1[72]	76		
Nasopharyngeal	–		–		1[73]	60

In contrast, in lung cancer, more studies indicate no association (three studies) or a favorable association (four studies) between TAM numbers and prognosis than a negative prognostic impact. In other cancer types, including glioma, prostate cancer, gastric cancer, colorectal cancer and ovarian cancer, the numbers of studies indicating negative or no/positive correlations are almost equal. In some cancers only one or two studies have yet been reported. Hence, it is too early to draw conclusions about the prognostic impact of TAMs. Interestingly, in different histological subtypes of breast cancer (intraductal carcinoma vs. infiltrative lobular carcinoma) [38] and leiomyosarcoma (gynecological vs. non-gynecological) [39], TAMs have a negative or no prognostic impact, respectively (Supplementary Material Table 1). Thus, it cannot be excluded that interaction of TAMs with different types of cancer cells influences the tumor-promoting capacity of macrophages.

The spatial localization of TAMs also seems to matter, because in lung cancer TAM density in the surrounding stroma has been shown to have a negative association with prognosis, while the opposite is true when TAMs are present within tumor cell nests [40, 41]. Similarly, in melanoma [42] and uterine cancer [43] macrophages, which were located at the invasive front of tumors, have a negative impact on prognosis. Thus, macrophages at the invasive front might promote malignancy, while macrophages within tumors might be tumoricidal. Taken together, the picture is not yet crystal clear, and this might also be due to methodology. Possible reasons for incoherent data include small sample sizes, different markers used for macrophage identification and interobserver variability in immunohistochemical analysis. Clearly, in order to elucidate the prognostic impact of TAMs, larger studies are warranted, in which tissues are simultaneously stained, ideally on tissue microarrays. Also, it might be more informative to analyze phenotypes and the activation status of TAMs, rather than only determining their number, because function is most likely more important. In line with this, osteopontin-positive macrophages have a negative impact on prognosis in bulky ampullary cancer, which might be explained by the well-described promigratory action of this cytokine [44]. In another study, the presence of TAMs expressing high levels of thymidine phosphorylase, which promotes tumor growth and metastasis by enhancing angiogenesis, was independently associated with shorter survival, while thymidine phosphorylase expression by tumor cells was not significantly associated with prognosis [45]. Of note, TAMs isolated from patients with advanced clinical lung cancer produce higher levels of IL-10 when isolated and cultured in vitro than TAMs from patients with earlier disease stages [46].

However, the majority of published studies did not distinguish between different macrophage phenotypes, because CD68 expression was mainly used to identify TAMs. Few studies in humans have analyzed M1 and M2 macrophages, with $CD68^{+}CD163^{-}$ macrophages being considered M1-polarized, while M2-polarized macrophages are considered $CD68^{+}CD163^{+}$ [23]. Some of these analyses are in line with the concept that M2 macrophages foster tumor progression, whereas M1 macrophages inhibit tumor development. For instance, in intrahepatic cholangiocarcinomas and in uveal melanoma, high infiltration with $CD163^{+}$ M2 macrophages is correlated with shorter disease-free survival and overall survival, respectively, compared to low M2 infiltration [47, 48]. In contrast, the density of M1-polarized macrophages is positively associated with survival in lung cancer [49, 50]. Hence, therapeutic approaches aimed at skewing macrophage polarization towards an M1 phenotype might open up novel therapeutic avenues. In this respect, recent studies indicate that M2 macrophages can be induced to acquire an M1-like phenotype by inhibitors targeting cyclooxygenase 2 and placental growth factor (PlGF) [51, 52]. Moreover, $CD4^{+}$ Th1 cells are also capable of skewing macrophages from M2 to M1 polarization [27]. A recent study has shown that autocrine CXCL12 production by macrophages enhances their proangiogenic and immunosuppressive phenotype in vitro [53]. Interestingly, 60–90% of TAMs in primary metastatic melanoma coexpress CD68, CD163 and CXCL12; thus this mechanism might be of relevance in human cancer. Consequently, CXCL12 could be an additional target with the potential to decrease M2 properties of TAMs [53]. However, as the majority of human cancer studies did not distinguish between M1 and M2 (Supplementary Material Table 1), more work is needed to get a better view of the prognostic impact and target potential of M2-polarized macrophages. However, such correlative studies obviously need to be interpreted with caution, and more mechanistic studies are warranted to answer the question when and why TAMs show pro- and antitumoral activity.

Taken together, emerging data suggest an important role of TAMs in tumor biology. In the following section we describe how macrophages and closely related myeloid cell types interact with various cellular players present in tumors and vice versa (see Fig. 1 for an overview). For information about how other leukocyte populations interact with tumors, we refer the readers to recent comprehensive reviews on this topic [3, 4, 6].

Crosstalk between macrophages and different tumor components

Crosstalk between macrophages and tumor cells from cancer initiation until metastasis

There is a tight link between cancer initiation and inflammation, because an inflammatory microenvironment can

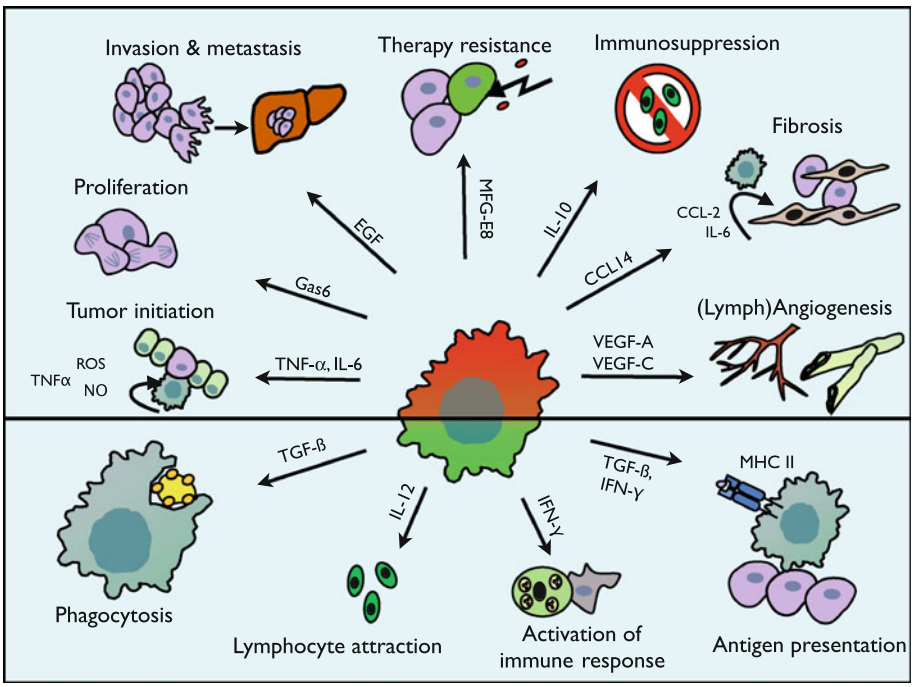

Fig. 1 Pleiotropic roles of macrophages in the tumor microenvironment. In the tumor microenvironment different types of "tumor-promoting" macrophages (*red*) can display protumorigenic roles during virtually every step of tumor development and progression (*top panel*). In contrast, "tumor-suppressive" macrophages (*green*) can fight tumor growth mainly by stimulating the immune system to kill cancer cells (*bottom panel*)

directly increase the mutation rate of cancer cells. Interestingly, during tumor initiation, macrophages obtain a more proinflammatory profile as opposed to the immunosuppressive M2 phenotype observed in established tumors (see previous section). These proinflammatory macrophages are characterized by strong activity of the NFκB pathway, which is induced by pathogen associated molecular patterns, by Toll-like receptor ligands and by cytokines including TNFα and IL-1β. As a result, they secrete abundant proinflammatory and protumoral cytokines such as IL-12, inducible nitric oxide synthase (iNOS), TNFα and IL-6 [28, 54, 55].

The importance of the NFκB pathway for tumor initiation is emphasized by data indicating reduced expression of several proinflammatory cytokines after ablation of IκB kinase (IKKβ) in myeloid cells. This dampened inflammatory reaction substantially reduced tumor incidence and progression in mouse models of intestinal cancer [56]. Furthermore, deletion of IKKβ in Kupffer cells (the macrophages of the liver) or inhibition of TNFα reduces hepatocellular carcinogenesis [57, 58]. The NFκB pathway also plays an important role in spontaneous mouse models of skin cancer, because tumorigenesis after application of carcinogens only occurs after additional induction of a pronounced inflammation by TNFα through tumor necrosis factor receptor 1 (TNFR1), a strong inducer of NFκB activity [59, 60]. Amongst other cell types, macrophages play a particularly important role in this NFκB-mediated inflammation, because they are induced to enter tumors by TNFα and at the same time secrete large amounts of this cytokine. TNFα can induce the Wnt/β-catenin signaling pathway in tumor cells, which is a strong promotor of tumorigenesis [61–63]. As TNFα is one of the main inducers of NFκB signaling, its secretion creates a feed-forward autocrine loop fueling further proinflammatory activity in the macrophages, but also activates other inflammatory cells present in the microenvironment of the developing tumor by acting in a paracrine manner [61]. Furthermore, TNFα promotes the formation of reactive oxygen species in macrophages, which directly induce DNA damage and genomic instability [25]. Reactive oxygen species and TNFα can cause inactivation of enzymes or regulators involved in DNA mismatch repair including p53 and poly-adenosine ribose polymerase [18, 25]. This suppression of DNA repair generates a promutagenic environment, which is further promoted by nitric oxide synthesized in macrophages by iNOS. Nitric oxide is a highly reactive substance that gives rise to intermediates directly causing mutations in epithelial cells [18].

Successful initiation of cancer depends on increased cell proliferation and reduced cell death, both of which are fostered by TAMs [7]. To achieve this, they produce a plethora of cytokines including IL-1, IL-6 and TNFα. After binding to their receptors at the tumor cell surface, these mediators activate the transcription factors NFκb, STAT3 and AP1. STAT3 elicits tumor cell proliferation by activating cyclin D1, cyclin D2, cyclin B and c-myc [64, 65]. NFκb and STAT3 promote cell survival by inducing expression of the antiapoptotic proteins Bcl-2 and Bcl-xl [65, 66]. The importance of IL-6 for tumor initiation has been underscored by the reduced risk of hepatocellular carcinoma in female mice, which produce lower levels of

IL-6 after treatment with a chemical carcinogen, whereas ablation of this growth factor leads to equal hepatocarcinogenesis in both genders [67]. Furthermore, macrophages can secrete heparin-binding EGF, which supports survival and proliferation of colon cancer cells via their Her1 receptor. Interestingly, in response, cancer cells produce GM-CSF, which acts on macrophages and induces them to further upregulate heparin-binding EGF expression [35]. Thus, macrophages are a rich source of cytokines directly promoting tumor initiation via autocrine and paracrine feed-back loops. Interestingly, recent data indicate a positive correlation between TAM density and the density of glioma-initiating cells in primary glioma [68]. Thus, TAMs might support cancer stem cells, which are regarded as key cellular components sustaining malignancy.

Interestingly, in established tumors, NFκB signaling in macrophages is suppressed due to constitutive expression of p50 homodimers. These homodimers are unable to induce transcription, but possess higher DNA-binding affinity than the bioactive p50/p65 heterodimer. Consequently, in the presence of p50 homodimers, macrophages express fewer NFκB target genes including IL-12, iNOS and TNFα, while M2-specific genes including arginase-1 and Fizz1 become upregulated [28, 69]. Therefore, TAMs in established human and murine tumors often acquire an M2 phenotype [70–73] (see above). This phenotypic M1 to M2 shift can be mimicked in vitro by blocking the NFκB pathway in macrophages by inhibition of IKKα. In addition to suppression of NFκB signaling in macrophages, cancer cells also secrete IL-4, IL-10 and IL-13, mediators capable of polarizing macrophages towards an M2 phenotype [4, 20, 74]. Thus, via different pathways, tumor cells can induce M2 polarization of macrophages, which in turn promote tumor progression.

In order to recruit more cancer-promoting macrophages, tumor cells produce different cytokines including CSF-1, monocyte chemotactic protein-1 (MCP-1/CCL2) and PlGF. The importance of CSF-1 has been amply demonstrated by a significant reduction in infiltrating macrophages after deletion of CSF-1 from transgene models of breast cancer, colon cancer and osteosarcoma, while their numbers were increased after overexpressing CSF-1 [62, 75, 76]. As a consequence, tumor progression was reduced or enhanced, respectively [62, 75, 76]. Consistent with these findings, therapeutic inhibition of CSF-1 by neutralizing antibodies or antisense strategies blocks tumor growth and metastasis in murine xenograft models [77, 78]. PlGF induces macrophage recruitment via VEGF receptor 1 (VEGFR-1). Consequently, inhibition and genetic ablation of PlGF inhibits tumor progression by reducing macrophage recruitment [14, 79, 80].

Macrophages promote additional hallmarks of malignancy including cancer cell migration, invasion and metastasis. In murine xenograft breast cancer models and spontaneous breast tumors, cancer cells produce CSF-1, thereby stimulating and attracting macrophages, which in response produce EGF. EGF subsequently activates tumor cells in a vicious circle to migrate. Interestingly, inhibition of either CSF-1 or EGF signaling is sufficient to impair migration and chemotaxis of both cell types, which underlines the central importance of this reciprocal paracrine interaction [81–84]. Of note, this crosstalk is further amplified in hypoxic conditions, because hypoxia activates hypoxia-inducible factor 2α (HIF-2α), which upregulates expression of EGFR in tumor cells and of CSFR in macrophages [85, 86]. In this way, via interaction with macrophages, tumor cells can better escape hostile hypoxic tumor environments. Interestingly, the presence of IL-4 is required, because in its absence TAMs are not able to induce invasion and migration of breast and pancreatic tumor cells, which strongly reduces the metastatic capacity of cancer cells [74, 84]. Besides IL-4 and EGF, other macrophage-derived mediators including Wnt5a and TNFα promote tumor cell invasiveness [7]. Interestingly, macrophages even physically promote extravasation of tumor cells by forming clusters on the abluminal side of blood vessels, through which tumor cells enter the circulation [82]. Of note, the transcriptome of these "tumor cell-bridging" macrophages closely resembles that of "trophic" macrophages, but has little similarity to M1 or M2 macrophages [7].

Crosstalk between macrophages and vascular endothelial cells

TAMs and closely related TEMs are involved in regulation and remodeling of blood vessels. Both TAMs and TEMs express higher levels of proangiogenic molecules than circulating monocytes [87, 88]. TAMs are required for the angiogenic switch and for vascular remodeling in spontaneous mammary tumors, because angiogenesis is impaired after inhibition of macrophage recruitment due to inactivation of CSF-1 [89]. Similarly, upon macrophage depletion by different approaches, such as clodronate liposomes, angiogenesis is reduced in different tumor models [7]. Conversely, CSF-1 overexpression, and subsequently enhanced TAM infiltration, substantially increases angiogenesis [89]. The proangiogenic function of M2-polarized TAMs is at least partly mediated by the transcription factor Fra-1, because its down-modulation in macrophages greatly decreases their ability to induce angiogenesis in experimental breast cancer [90]. The concept of potent induction of angiogenesis by TAMs is further substantiated by clinical data revealing a correlation between a high density of TAMs and increased microvessel density in different cancers, including lung cancer [91] and breast cancer [92].

TAMs are recruited specifically to hypoxic tumor regions, because important macrophage chemoattractors, including VEGF, endothelins and stromal cell-derived factor-1, are upregulated in hypoxia [93]. This process is further amplified in hypoxic conditions, where macrophages activate HIF-2α. HIF-2α then induces upregulation of M-CSFR and CXCR4, which potentiate chemotaxis of macrophages towards hypoxic tumor regions [86]. Once arrived, TAMs secrete VEGF, PlGF and other proangiogenic cytokines [94]. These potent mediators increase angiogenesis and at the same time, by acting on endothelial cells and pericytes, induce blood vessel abnormalities such as increased leakiness, multilayered endothelium and immaturity, together termed "vessel abnormalization". As the end result, vessel abnormalization leads to dysfunctional, hypoperfused vessels, which fail to adequately supply the tumor with oxygen and nutrients [95, 96]. Consequently, hypoxia increases, which leads to further fueling of macrophage infiltration. Therefore, vessel abnormalization increases even more, and as consequence, tumor cell intravasation through a leaky endothelial cell layer is enhanced [7, 97]. VEGF and PlGF not only act on endothelial cells, but can also stimulate tumor cell motility by activating VEGF receptors [14, 98]. These multitasking cytokines are additionally involved in TAM polarization towards an M2 phenotype [52, 79]. Consistently, in PlGF-deficient mice TAMs are skewed from the proangiogenic M2-like phenotype towards a more proinflammatory phenotype [52]. As a consequence, vessel abnormalization is decreased and tumor cells elicit less invasiveness and metastasis [52].

The TEM subset of monocytes predominantly resides close to tumor blood vessels, where they can potently induce angiogenesis [99]. This close association depends on endothelial secretion of angiopoietin-2 (Ang-2). Consequently, blockade of Ang-2 reduces tumor growth and angiogenesis partly by disrupting the close physical interaction of TEMs and endothelial cells [100]. Recent data indicate a similar localization of tissue-resident macrophages close to the tips of branching blood vessels, where they facilitate fusion of two adjacent vessel sprouts. Implications for tumor biology as well as molecular mechanisms are still not fully explored, but the Notch/Dll4 or Tie2/Ang-2 systems might mediate this interaction between macrophages and blood vessels [101, 102]. Altogether, TAMs represent potent inducers of angiogenesis and vessel abnormalization, and hence approaches aimed at inhibiting these important protumoral actions of TAMs may lead to novel cancer treatments.

Crosstalk between macrophages and lymphatic endothelial cells

Besides their role in angiogenesis, macrophages are involved in lymphangiogenesis during development and disease. Indeed, lymphatic vessel development was impaired in op/op mice exhibiting reduced macrophage numbers due to an inactivating mutation in the *Csf1* gene [75]. Macrophages are an important source of the lymphangiogenic cytokines VEGF-C and VEGF-D in different disease conditions including cancer [14, 103]. Consistent with this, depletion of macrophages strongly impairs lymphangiogenesis in different experimental cancer models, mainly because of reduced intratumoral levels of prolymphangiogenic cytokines. As a consequence, lymphatic metastasis is reduced [14, 104]. These findings may have implications for human cancer, because TAMs in primary human cutaneous squamous cell carcinomas are important producers of VEGF-C [105]. A novel concept of macrophage and lymphatic interaction has been found in the RipTag2 pancreatic tumor model and TRAMP-C1 prostate cancer model, in which BMDCs of the myelomonocytic lineage become integrated into tumor-associated lymphatic vessels. This effect is not based on cell fusion, but rather on phenotypical conversion of myeloid cells into lymphatic endothelial cells. Depletion of macrophages consequently reduces the lymphatic vessel density [106]. In line with the role of macrophages in lymphangiogenesis, TAM infiltration correlates with tumor lymphatic vessel density in lung cancer [107] and in pancreatic cancer [108].

Crosstalk between macrophages and fibroblasts

The development of cancer is often associated with an increase in fibroblast proliferation leading to extensive fibrosis. However, relatively little is known about the interaction of macrophages and fibroblasts. Recent data in a chemically induced skin cancer model indicate that $FSP1^+$ CAFs secrete MCP-1 (CCL2), IL-6 and TNFα, by which they recruit and polarize macrophages towards the M2 phenotype. Depletion of these CAFs consequently reduces macrophage infiltration and thereby inhibits tumor development [109]. These findings were further supported in experimental breast cancer, because recruitment of TAMs was enhanced after upregulation of CCL2 in CAFs, promoting tumor progression and metastasis [110]. The importance of fibroblasts for macrophage recruitment was also corroborated by in vitro data showing extensive CCL2-mediated infiltration of tumor-derived 3D fibroblast spheroids with monocytes. Normal fibroblasts fail to attract monocytes; hence this fibroblast–macrophage interaction appears tumor-specific [111]. Of note, CAFs in human cancer might show similar functions, because in pancreatic ductal adenocarcinoma and squamous cell carcinoma models, CAFs attract macrophages by NFκB-mediated upregulation of CXCL1, CXCL2 and CXCL5 [110]. Similarly, in comparison to fibroblasts present in healthy tissue,

CAFs in primary human prostate cancer tissue upregulate CCL14, which chemoattracts macrophages. Interestingly, in murine 4T1 breast tumors, CAFs can additionally act as immunomodulators, because inhibiting their activation shifts the immune microenvironment from Th2 towards Th1 polarization. Subsequently, tumor growth and metastasis are strongly reduced, which might be explained by decreased recruitment and M2 polarization of macrophages [112]. However, not all CAFs show proinflammatory functions; for instance CAFs in murine cervical cancer do not show upregulation of IL-1, IL-6, CXCL1 or CXCL-2 [110]. In addition to recruiting macrophages, CAFs can also support macrophage maturation. Indeed, coculture of CAFs with a monocytic cell line induces upregulation of the macrophage maturation marker F4/80 and induces morphological changes typical of mature macrophages. At the functional level, these mature macrophages secrete higher levels of proinflammatory and protumoral cytokines such as IL-1β and TNFα upon coculture with tumor cells when than immature monocytes [113]. In summary, the crosstalk between CAFs and macrophages plays an important role in tumor progression; hence it would be of interest to decipher further interactions at the molecular and functional levels.

Crosstalk between macrophages and other leukocytes

TAMs are essential players in the suppression of antitumoral immune responses. They express high levels of mediators interfering with T-cell activation and proliferation such as IL-10, TGFβ, prostaglandins and arginase-1 [73, 114]. This might be a reason for the rather weak antitumoral immune responses observed in most tumors. The immunosuppressive function of macrophages has already been extensively reviewed elsewhere [7, 19]. Interestingly, in hypoxic tumor regions, the immunesuppressive properties of macrophages are even more enhanced, because activation of HIF-1α leads to upregulation of arginase-1 and iNOS, both of which dampen T-cell function [115]. Indeed, macrophage-specific depletion of HIF-1α reduces breast cancer growth by activating the cytotoxic T-cell response, but without changing expression levels of the prototypic HIF-1α target gene VEGF [115]. Alternatively, in response to autocrine production of IL-10 and TNFα, macrophages upregulate the cosignaling molecule PD-L1 (also called B7-H1), which suppresses T-cell function by a yet-undiscovered mechanism. Therapeutic blockade of PD-L1 reduces tumor growth by enhancing intratumoral cytotoxic T-cell function [116]. Of note, in contrast, tumor-infiltrating T cells can polarize TAMs towards M2 macrophages, thereby creating another vicious circle promoting tumor development. Indeed, in a spontaneous breast cancer model, $CD4^+$ cells expressed IL-4, a strong inducer of M2 polarization of macrophages. Consequently, after therapeutic or genetic targeting of IL-4, the macrophage phenotype is skewed towards M1. Furthermore, IL-4 derived from tumor cells and from T cells induces high levels of cathepsin B and S protease activity in TAMs of murine breast and pancreatic tumors. As a consequence, tumor growth, angiogenesis and invasion are induced, because cathepsin B and S cleave extracellular matrix proteins, thereby liberating matrix-bound proangiogenic molecules [74, 84].

Macrophages not only interact with cytotoxic T cells, but they also cooperate in a complex manner with several other immune cell populations in tumors. Within the immunological tumor microenvironment, regulatory T cells (Tregs) play an important immunosuppressive role. Their importance has, for example, been proven in preclinical lymphoma models, where Treg depletion led to rapid tumor rejection by T cells and NK cells. Consistent with a protumoral role of Tregs, their density correlates with a poor prognosis in patients with different cancers, including hepatocellular cancer [117]. TAMs can augment the recruitment of Tregs by secreting CCL20, which chemoattracts them via chemokine receptor 6 (CCR6). Accordingly, tumor growth is reduced after macrophage depletion, because CCL20 levels are lowered and consequently Treg recruitment is reduced [117, 118]. Similarly, in colorectal cancer patients, macrophages produce high levels of CXCL11, which is another strong chemoattractant for Tregs. Tregs, besides their immunosuppressive action, produce IL-17 in the tumor microenvironment, which supports survival of colorectal cancer-initiating cells [119]. Interestingly, the crosstalk between TAMs and Tregs is bidirectional, because upon Treg depletion, TAM numbers decrease. Additionally, without the influence of Tregs, TAMs augment their proinflammatory properties by increasing MHC class II and immunoactivating chemokine expression (MIP-1β, MIP-2 and TNFα) [120]. However, similar to TAMs (see above), the role of Tregs may be different in different cancers. For example, in patients with gastric cancer, a high $CD68^+$/$FoxP3^+$ cell ratio (macrophage/Tregs) is associated with shorter survival, indicating that inhibition of Tregs might not be useful in some cancers [121].

Besides interacting with different T-cell subpopulations, macrophages also cooperate with B cells, and vice versa. For instance, B cells can skew the macrophage phenotype towards M2 polarization. This ability has been shown in the B16 melanoma model, because so-called B1 cells were able to drive macrophages to acquire an M2-biased phenotype mainly by secreting IL-10 [122].

Macrophages and cancer therapy resistance

Macrophages are implicated in resistance towards chemotherapy and biological therapies. Recent data indicate that breast cancer patients with a high number of TAMs and a low number of cytotoxic T cells within their tumor tissue have a poor response to neoadjuvant chemotherapy with taxanes, antimetabolites and anthracyclines. Interestingly, both in cancer patients and in mice, elevated CSF-1 levels and increased numbers of macrophages have been detected in tumor tissue after chemotherapy. Blockage of CSF-1 signaling or macrophage depletion enhances antitumor immunity and response to chemotherapy in murine cancer models, indicating functional involvement of macrophages in mediating resistance to chemotherapy [123]. Interestingly, TAMs are abundantly present in the bone marrow of patients with multiple myeloma, where they protect myeloma cells from chemotherapy-induced cell death. This protection depends on direct cell–cell contact and on ICAM-1, because it does not occur after physical separation of myeloma cells from TAMs or after antibody-mediated blockade of ICAM-1. TAMs achieve this protective effect partly by attenuating the activation and cleavage of caspase-dependent apoptotic signaling [124]. Interestingly, TAMs can render cancer stem cells, intrinsically relatively resistant to chemotherapy, even more resistant by producing milk-fat globule-epidermal growth factor VIII (MFG-E8) and IL-6. MFG-E8 is a potent activator of STAT3 signaling and of the hedgehog pathway, while IL-6 further fuels STAT3 activation. Both pathways have been shown to mediate the resistance of colorectal cancer stem cells by promoting their survival in the presence of cisplatin [125]. Moreover, in prostate cancer TAMs can confer resistance to androgen receptor antagonists. In this process, macrophages adhere to prostate cancer cells by VCAM-1 and subsequently produce IL-1β, which in turn blocks the function of nuclear receptor corepressors N-CoR. N-CoR normally associates with antiandrogens, and subsequently suppresses androgen-induced gene transcription. However, without binding to N-CoR, androgen receptor antagonists activate, instead of suppress, androgen-induced gene expression [126].

Via CSF-1, cells of the myeloid lineage are also recruited into tumors treated with VEGF-targeted antiangiogenic therapies, where they can directly confer resistance by producing alternative proangiogenic factors besides VEGF [15, 127]. Consequently, blockade of the CSF-1 pathway inhibits tumor angiogenesis and acts synergistically with anti-VEGFR-2-targeted therapy by reversing myeloid cell-mediated antiangiogenic therapy resistance [128]. The importance of pathways mediating myeloid cell recruitment in antiangiogenic escape is further supported by detection of increased levels of PlGF, stromal cell-derived factor-1 and MCP-3 in colorectal cancer patients treated with anti-VEGF antibodies and chemotherapy immediately before disease progression [129]. Overall, TAMs can facilitate a large variety of mechanisms to render tumors resistant to different therapeutic strategies [130]. Hence, therapeutic approaches aimed at inhibiting these TAM properties might be more efficient than current anticancer therapies.

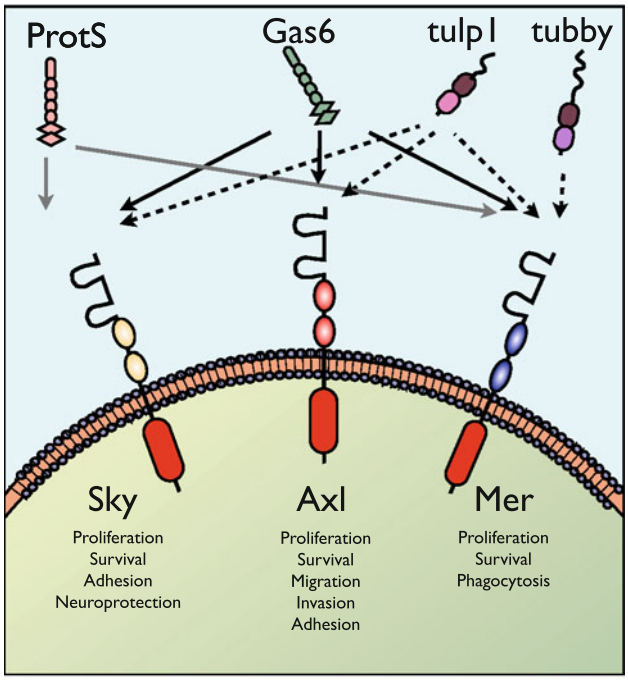

Fig. 2 Ligand–receptor specificity in TAMR. Gas6 binds to all three TAMR (Sky, Axl, Mer) with different affinities (Axl $\gg$ Mer $>$ Sky) and signals through them. Current knowledge indicates that protein S(*ProtS*) binds to Sky and Axl. Tubby-like protein 1 (*tulp1*) can bind and signal through all three TAMR, whereas tubby is only found to signal through Mer. Each receptor induces certain biological responses indicated below the respective receptor

In summary, TAMs interact with cancer cells and with different cellular components of the tumor microenvironment. This crosstalk can promote malignancy and therapy resistance via a plethora of complex mechanisms, but in principle macrophages can also show antitumoral activity. In experimental cancer models, some progress has been made recently in skewing the macrophage phenotype towards tumoricidal activity and in overcoming therapy resistance by targeting macrophages, but considerably more work is necessary to elucidate whether this approach has therapeutic potential in human cancer.

In the next section we introduce the Tyro3, Axl and Mer receptor (TAMR) family with their ligands, that are expressed by tumor cells and macrophages and have recently been shown to be involved in the tumor–macrophage crosstalk (Fig. 2). We also describe their role in (human) solid cancer and in hematological malignancies.

TAMR tyrosine kinase receptors and their ligands

Receptor tyrosine kinases (RTKs) are key players in cancer cell biology. They regulate cell survival, proliferation, migration and differentiation, cell cycle control and apoptosis [131]. The prototypical RTKs are activated by ligands such as growth factors, which induce receptor dimerization and subsequent autophosphorylation of tyrosine residues on the intracellular cytoplasmic domain with further downstream signaling [132]. Currently 58 RTKs, divided into 20 subfamilies, are known. The TAMR, named after Tyro3, Axl and Mer or their homologues, are present in chordates including urochordates and vertebrates [133, 134] (see below for alternative nomenclature). This RTK subfamily was identified only in 1991 and TAMR were initially considered as orphan receptors [135, 136]. Structurally, the TAMR family is characterized by an extracellular domain consisting of two immunoglobulin-like domains followed by two fibronectin type 3-like domains. These extracellular domains are followed by a transmembrane domain and a cytoplasmic tyrosine kinase domain [137, 138]. TAMR can be activated by (1) ligand-independent dimerization, (2) ligand-dependent dimerization, (3) heteromeric dimerization of two different TAMR, (4) heterotypic dimerization with a non-TAMR, and (5) trans-cellular binding of extracellular domains [139, 140].

Axl was initially identified as a transforming gene derived from chronic myeloid leukemia cells [136]. Overexpression of this gene in NIH 3T3 cells led to their transformation and the gene was therefore named *axl* from the Greek word "anexelekto", which means uncontrolled [136]. The oncogenic potential of Axl depends on its intracellular tyrosine kinase domain, because 33 amino acids of the intracellular domain are able to transform NIH 3T3 cells [141]. Axl is ubiquitously expressed and detectable in most organs as well as in different cell lines of mesenchymal, epithelial and hematopoietic origin. Axl expression becomes detectable in many tissues during embryonic development from day E12.5 onwards [142]. Due to independent cloning, Axl was also designated Ufo, Jkt11, Ark, Tyro7; however, Axl is the official NCBI designation. Axl promotes a large variety of biological functions including platelet aggregation [143, 144], regulation of proinflammatory cytokine production and control of the actin cytoskeleton [145]. Moreover, Axl mediates cell survival, proliferation and migration [139]. The important function of Axl in regulating survival was demonstrated in fibroblasts isolated from $Axl^{-/-}$ mice. These fibroblasts display enhanced serum deprivation-induced apoptosis when compared to fibroblasts derived from wild-type mice [146]. Axl controls survival mainly via the PI3K, AKT, and NFκB pathways, while Axl-induced proliferation depends mainly on ERK1/2 signaling [139, 140]. TAMR share structural features with cell adhesion proteins, in particular the ectodomain of Axl elicits adhesive properties. This "stickiness" can mediate cell–cell contact leading to aggregation of cells, which might facilitate metastasis. In line with this concept, Axl expression correlates with adherence of human lung cancer cell lines [147, 148].

Ample evidence in the literature points to an important functional role of Axl in tumor biology (Fig. 3). Activation of Axl induces proliferation [149, 150], survival [151–154], resistance against apoptosis [151, 155, 156], migration and invasiveness of cancer cells [149–154]. Furthermore, Axl mediates resistance towards chemo- and targeted therapy including anti-VEGF or anti-EGFR therapy in part by inducing secretion of proinflammatory and protumoral cytokines such as IL-6, TNFα and G-CSF in TAMs, where it is highly expressed [157]. Hence, treatment of breast cancer xenografts with anti-Axl antibodies inhibits the secretion of protumoral inflammatory cytokines and chemokines from TAMs, which have an inhibitory effect on tumor growth [157]. The precise mechanism involved in the inhibition of the production of inflammatory cytokines in TAMs by anti-Axl antibodies remains to be elucidated. Furthermore, recent data in thyroid cancer cell lines indicate constitutive Axl and Sky phosphorylation induced by autocrine production of Gas6. This autocrine loop, which is not present in normal thyroid cells, specifically mediates proliferation and apoptosis resistance of thyroid cancer cells in vitro and in vivo [151]. Altogether, Axl plays a prominent role in cancer biology by promoting malignancy at several levels.

In 1993 the Sky receptor was identified, which was also termed Tyro3, Brt (brain tyrosine kinase), Tif, Dtk, BYK and Etk-2 [158–160]. In a similar manner to Axl, Sky is also expressed in several embryonic tissues during development [161, 162], but shows a more restricted expression pattern in adulthood with the predominant expression in the brain [163–165], in hematopoietic cells [161], in pulmonary endothelial cells [166], in osteoclasts [167], and in the kidney, testis and ovary [163, 168]. In a similar manner to Axl and Mer, Sky is involved in platelet function, but single $Sky^{-/-}$ mice display mild platelet dysfunction without spontaneous bleeding. In contrast, double or triple TAMR-deficient mice suffer from pronounced bleeding diathesis [169]. Additionally, Sky plays an important role in osteoclastic bone resorption [167]. Recently, in exitotoxic brain injury, protein S has been shown to activate Sky leading to the suppression of proapoptotic Fas ligand production. Thus, by suppressing apoptosis, Sky acts in a neuroprotective manner [170]. Furthermore, together with Axl, Sky mediates survival and targeting of GnRH neurons to the ventral forebrain, which is important for reproductive function in female mice [152].

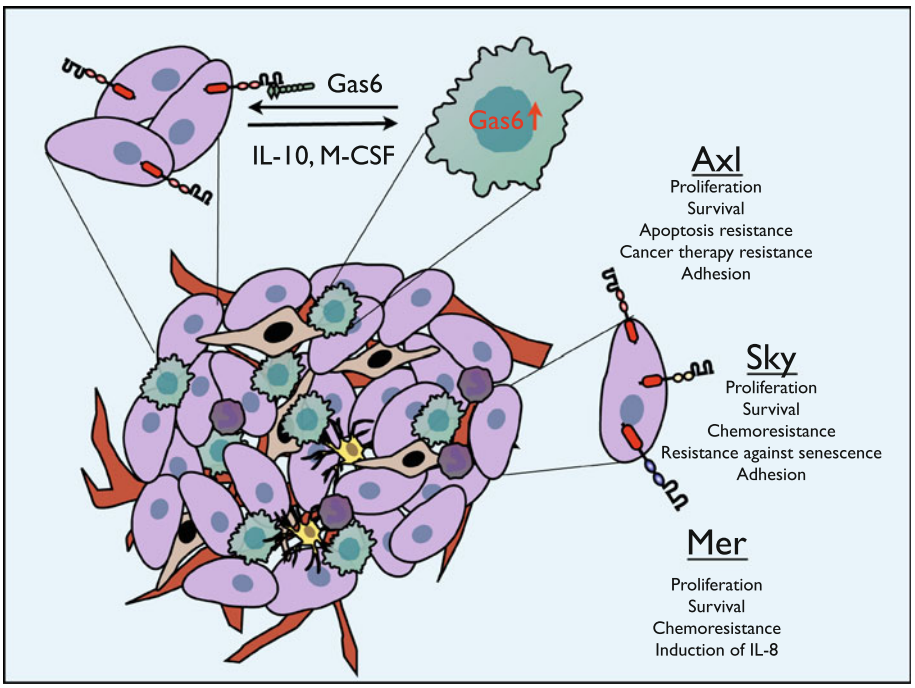

Fig. 3 Role of Gas6 and TAMR in tumor–macrophage interaction. Tumor cells stimulate macrophages to upregulate Gas6 by expressing IL-10 and M-CSF, thereby inducing a vicious circle, because Gas6 stimulates tumor cells to proliferate. Furthermore, all three TAMR can foster cancer by promoting different hallmarks of malignancy

Sky can transform cells in vitro, but its role in cancer is less well-defined than that of Axl (Fig. 3) [171, 172]. Sky can promote malignancy by inducing proliferation, which is at least partially mediated via PI3K signaling [171]. In malignant melanoma, activation of Sky induces the transcription factor microphthalmia-associated transcription factor, which strongly promotes malignancy in melanoma. Indeed, knock-down of Sky in melanoma cells suppresses proliferation and sensitizes them to chemotherapy. Conversely, Sky overexpression promotes cancer cell survival by overcoming senescence [173]. In addition, in a similar manner to Axl, Sky can also exert adhesive functions by homophilic interaction, which potentially enhances metastasis [174].

The Mer receptor was initially cloned as a human protooncogene from a leukemic cell line [175]. Sequence comparison indicates that this human kinase is 83% similar to the previously isolated chicken retroviral oncogene v-ryk (v-eyk) [175, 176]. Mer was named after its unique expression pattern in monocytes, epithelium and reproductive tissue. Mer was also designated mertk, eyk, nyk and rdy. Mer is expressed during most stages of embryonic development of the mouse [177]. Like the other members of the TAMR family, Mer is involved in platelet function [178], exerts mitogenic signals and has transforming ability [179]. Furthermore, Mer is crucially involved in apoptotic cell clearance by phagocytic cells including dendritic cells, macrophages, monocytes and retinal pigment epithelial (RPE) cells [180]. In order to mediate the cytoskeletal reorganization crucial for phagocytosis, Mer activates focal adhesion kinase via an alpha(v) beta(5) integrin-dependent pathway. Alternatively, after binding its ligand Gas6, Mer signaling leads to phosphorylation of Vav1 [181]. Both pathways finally activate Ras-related C3 botulinum toxin substrate (Rac), which induces cytoskeletal reorganization with subsequent phagocytosis [181]. In line with this mechanism, Mer-deficient mice exhibit delayed phagocytosis of apoptotic cells by macrophages. This defect leads to uncontrolled disposal of dying cells, thereby activating autoimmune responses, which are normally prevented due to "controlled" apoptotic cell clearance. As a consequence, intracellular antigens become exposed to the immune system, which fosters development of diseases such as lupus-like autoimmune disorders [182, 183]. Furthermore, clearance of degenerated photoreceptor fragments by RPE cells is impaired, leading to blindness of $Mer^{-/-}$ mice in adult life [184, 185]. Of note, a 91-kb deletion in exons 1–7 of the Mer gene was found to be present in 30% of patients with retinitis pigmentosa in an isolated population on the Faroe Islands. Interestingly, computed tomography revealed similar morphological changes such as abundant photoreceptor debris to those observed in $Mer^{-/-}$ mice. Thus Mer seems to exert similar functions in humans [186].

Mer is also involved in tumor biology, but data on this topic is still scarce (Fig. 3). Knocking down Mer in astrocytoma cell lines increases apoptosis, but the proapoptotic effect of knocking down Axl is more pronounced in comparison. In a similar manner to Axl, Mer signals through p-Akt and through p-Erk1/2, thereby enhancing survival and proliferation. Thus, inhibition of Mer cells leads to increased chemosensitivity of astrocytoma cells to temozolomide, carboplatin and vincristine [155]. Interestingly,

in prostate cancer cell lines, activation of Mer does not induce proliferation, but instead mediates differentiation of the cancer cells [187]. In addition, Mer can induce IL-8 secretion by tumor cells via Erk1/2-mediated signaling. IL-8 can foster angiogenesis and metastasis in murine prostate cancer. Therefore it is possible that Mer can enhance malignancy via this mechanism [187]. Interestingly, in a bioinformatic screening of public databases aimed at identifying differentially regulated genes in melanoma, Mer was among six genes found to be dysregulated in several independent studies [188]. This finding implies that Mer might play a more pronounced role in tumor biology than currently appreciated.

Initially, TAMR were considered to be orphan receptors until the vitamin K-dependent ligands Gas6 and protein S were discovered [189, 190]. Gas6 is a common ligand for all three TAMR with different affinities (Axl > Tyro3 > Mer), whereas protein S activates Tyro3 and Mer, but not Axl [191–194]. Gas6 is upregulated in NIH 3T3 fibroblasts under starvation conditions and protein S is a well-known negative regulator of coagulation [195–197]. Gas6 and protein S are structurally related secreted proteins sharing about 42% amino acid identity. They consist of a vitamin K-dependent post-translationally modified N-terminal gamma-carboxylated glutamic acid (Gla) domain, followed by four EGF-like domains and a C-terminal sex hormone binding globulin that consists of two laminin G-like domains [139]. Protein S exhibits a thrombin-sensitive cleavage site, which is not present in Gas6.

Gas6 shows pleiotropic functions in health and disease. Gas6-deficient mice are viable, fertile and born at a Mendelian frequency [144]. Gas6 induces cell proliferation, survival and migration [139]. Additionally, Gas6 plays a role in cell–cell adhesion, because Axl overexpression leads to aggregation of 32D cells only in the presence of Gas6. Interestingly, this aggregation does not induce Axl receptor downstream signaling but rather depends on extracellular calcium [153]. Gas6 and protein S play important roles in the immune system mainly by regulating phagocytosis and inflammatory reactions of antigen-presenting cells [180, 183, 198]. Gas6 amplifies platelet aggregation during thrombus formation, and as a consequence, $Gas6^{-/-}$ mice are protected against collagen/epinephrine-induced thromboembolism, but without suffering from spontaneous bleeding disorders [143, 144, 198]. Gas6 supports erythropoiesis by enhancing Epo receptor signaling [199]. Furthermore, it increases leukocyte extravasation by amplifying the response of endothelial cells in response to inflammatory stimuli [200], and induces plaque stabilization in atherosclerosis by enhancing plaque fibrosis [201]. Recent data indicate that Gas6 deficiency alleviates hepatic graft-versus-host disease in allogenic liver transplantation and that Gas6 is hepatoprotective against ischemia reperfusion injury, whereas Gas6 has been found to be upregulated in allograft rejection in murine kidney transplantation models and in human graft dysfunction [202–205].

Recently new light has been shed on the role of macrophage-derived Gas6 in experimental models of solid tumors, including colorectal cancer and breast cancer [206]. In this study, tumor cells did not express Gas6, while $CD45^{+}$ tumor-infiltrating leukocytes showed abundant expression of this protein. These leukocytes specifically upregulated Gas6 after entering the tumor, because they do not secrete Gas6 while circulating in the blood or while residing in the bone marrow. Further analysis revealed that TAMs are the main source of Gas6 within the tumor microenvironment. In contrast, tissue-resident macrophages isolated from lungs or from the peritoneum express much lower levels of Gas6 than TAMs. Thus crosstalk between tumors and macrophages leads to specific upregulation of Gas6. Interestingly, Gas6 production in macrophages can be induced by the cytokines IL-10 and M-CSF, which are also known to polarize macrophages more towards an M2-like phenotype [206].

Tumor growth was inhibited by 35–55% in mice with genetic deletions of Gas6 when compared to wild-type mice indicating that expression of Gas6 within the (host-derived) tumor microenvironment promotes tumor progression. This growth inhibition was due to decreased proliferation in the absence of Gas6, which is in line with published literature [207–209]. However, angiogenesis or tumor infiltration with inflammatory cells remained unchanged. Functionally, Gas6 is delivered into tumors by BMDCs, because the reduced tumor growth was abrogated in $Gas6^{-/-}$ mice transplanted with wild-type bone marrow prior to tumor implantation. Conversely, tumor growth reduction was phenocopied after transplantation of $Gas6^{-/-}$ bone marrow into wild-type mice. The importance of macrophage-derived Gas6 in promoting tumor cell proliferation has been further underscored by coculture experiments, in which $Gas6^{-/-}$ macrophages exhibited a significant reduction in their capacity to stimulate cancer cell proliferation when compared to wild-type macrophages [206]. Altogether, via the crosstalk with tumors, TAMs become educated to secrete Gas6 which then fuels proliferation of tumor cells and thereby promotes malignant progression. However, the relevance of this preclinical study for human disease still needs to be determined. The situation in human cancer might be different, because some studies in different primary cancer tissues have indicated that cancer cells express Gas6, which was not the case in the preclinical models. Furthermore, in lung cancer Gas6 is exclusively detected in TAMs, but its expression level as determined by immunohistochemistry is correlated with

Table 2 Overview of studies investigating the prognostic impact of Gas6 or Axl expression with clinical outcome for different cancers. Table references are as listed in the Supplementary Material

Cancer	Gas6			Axl		
	Reference	No. of patients	Prognostic impact	Reference	No. of patients	Prognostic impact
Gastric	[74]	33	Poorer outcome	[75]	97	No correlation
				[76]	96	Poorer outcome
Lung	[77]	63	Better outcome	[78]	58	Poorer outcome
Pancreatic				[79]	53	Poorer outcome
Breast	[80]	49	Better outcome	[82]	190	Poorer outcome
	[81]	74	No correlation			
Renal cell carcinoma	[83], protein (serum ELISA)	221	Better outcome	[83]	308	Poorer outcome
	[83], mRNA (tumor)	282	Poorer outcome			
Glioblastoma multiforme	[84]	76	Poorer outcome	[84]	76	Poorer outcome
Ovarian	[85]	90	Poorer outcome	[85]	90	No correlation
				[86]	297	Poorer outcome
Endometrial	[87]	60	No correlation	[87]	60	No correlation
Thyroid				[88]	112	No correlation
Bladder				[89]	65	Poorer outcome
Esophageal adenocarcinoma				[90]	92	Poorer outcome

prolonged survival after tumor resection [210] (see section below and Supplementary Material Table 2). It remains to be determined how these findings can be reconciled with the preclinical data.

The biological consequences of protein S binding to TAMR are much less well-described than those mediated by Gas6. Interestingly, protein S reaches plasma concentrations around 300 nM, while plasma Gas6 is present only in subnanomolar concentrations [139]. However, whether this difference might indicate that Gas6 acts mainly in an autocrine or paracrine manner over short distances, while protein S acts as an endocrine factor is currently unknown. Protein S can exert pro- and antiproliferative effects in vascular smooth muscle cells and astrocytes, respectively. Furthermore, protein S promotes phagocytic activity including bone-resorbing activity of osteoclasts and phagocytosis of photoreceptor fragments via Sky and Mer [139]. Additionally, protein S might be implicated in suppressing cell-mediated immune responses [211].

Recently, tubby and tubby-like protein 1 (Tulp1) have been identified as novel TAMR ligands (Fig. 2). They facilitate phagocytosis by RPE cells and by macrophages [212]. Tulp1 interacts with all three TAMR, whereas tubby exclusively binds to Mer. After binding to their receptors, both ligands serve as "eat-me" signals, thereby marking cells for phagocytosis. Mainly via Mer, phagocytes then bind to the C-terminal "prey-binding" domain of Tulp1 or tubby, which together with the N-terminal MerTK-bridging domain induces Mer receptor phosphorylation and subsequent phagocytic activity. Of note, tubby and Tulp1 are predominantly expressed intracellularly in photoreceptors and neural tissue, but also occur as soluble "eat-me" signals, whose role outside their tissues of origin still needs to be determined [213].

The roles of TAMR and of their ligands in solid tumors and in leukemia are discussed in the following sections.

TAMR, Gas6 and protein S in human cancer

Ample preclinical evidence indicates prominent involvement of Gas6 and TAMR in the pathobiology of cancer (see previous section). Therefore, it is not unexpected that different TAMR and Gas6 are overexpressed in different human tumor cell lines as well as in primary cancer tissues [139, 140]. Several studies link expression levels of Gas6 and Axl to prognosis of cancer patients, while only scarce data exist on the prognostic impact of Sky, Mer and protein S (Table 2; for more detailed information see Supplementary Material Table 2).

High Axl expression levels as determined by quantitative PCR and/or immunohistochemistry have a negative prognostic impact in the majority of cancers, while in some neoplasms, including thyroid and uterine cancer, Axl levels have no prognostic impact. In ovarian and gastric cancer, conflicting evidence about the prognostic impact of Axl exists (Table 2). Of note, no study has thus far found a positive prognostic impact of Axl expression. Thus, although more work is necessary to fully elucidate the prognostic impact of Axl, this receptor represents a promising novel target for cancer treatment based on functional and clinical correlative data. Interestingly, R428, a small-molecule Axl inhibitor, blocked metastasis in different preclinical cancer models, but surprisingly treatment did not influence primary tumor growth. In any case, it will be worthwhile to evaluate the therapeutic efficacy of R428 in the clinic [214].

The picture concerning the prognostic implications of Gas6 expression levels in cancer patients is considerably more heterogeneous compared with that of Axl, because positive, negative and absence of correlation with clinical outcome have been reported (Table 2). Interestingly, one study in renal cancer found prolonged survival of patients expressing high levels of Gas6 mRNA in their tumor tissue, while the opposite was true for high serum Gas6 levels [215]. This divergence might reflect differences in the function of Gas6 depending on its location. However, as mentioned above, in general caution is warranted when interpreting descriptive data generated with human tumor tissues using diverse methodology (Supplementary Material Table 2). Overall, large studies on uniform sample types, ideally on tissue microarrays, are warranted to more accurately determine the prognostic impact of Gas6 and Axl in different malignancies. Additionally, it would be of special interest to analyze the impact of Sky, Mer and protein S in more detail, because almost no data exist on their roles in human cancer (Supplementary Material Table 2).

TAMR, Gas6 and protein S in leukemia

Gas6 and TAMR are present in normal and malignant hematopoiesis as well as in different populations of tumor-infiltrating leukocytes [138, 206, 216]. Axl is currently the most well-studied member of this ligand–receptor family in hematology. Axl is expressed in most hematopoietic lineages with predominant expression in myeloid precursors [138, 206, 216]. In addition, different myeloid and lymphoid leukemia cell lines exhibit Axl on their surface [149, 216, 217]. Axl has also been detected in primary leukemia cells isolated from AML patients and, interestingly, became upregulated upon treatment with chemotherapy, which indicates a link to drug resistance [218, 219]. Further data supporting a role for Axl in chemoresistance in AML cells comes from a small clinical study indicating a worse

prognosis for patients with detectable Axl expression ($n = 19$) as compared to those who did not express Axl [219]. However, as a note of caution, the sample size in this study was small, patients were treated with different chemotherapeutic regimens and the link to prognosis was shown only by multivariate analysis, and not by univariate analysis. In this multivariate analysis, several important risk factors including cytogenetics were not analyzed [219]. Thus these data need to be corroborated in larger, well-defined patient cohorts. In addition to its potential role in AML, Axl was upregulated upon development of imatinib resistance in a CML cell line [220], and has very recently been linked to resistance to nilotinib [221]. Axl also plays a role in B-cell chronic lymphocytic leukemia, because it is constitutively activated in these leukemia cells and acts as a docking site for nonreceptor kinases [222]. Hence, Axl plays a role in myeloid and lymphoid leukemia, but further research is necessary to better define its functional and prognostic implications.

Only scarce data exist about the role of Mer in leukemia. Mer is abundantly expressed in pediatric B-cell and T-cell acute lymphoblastic leukemia (ALL), whereas healthy B and T cells are negative for Mer expression [138, 223, 224]. The Gas6–Mer axis has been shown to mediate homing and survival of B-cell ALL cells in the bone marrow niche, because Mer-expressing leukemia cells migrated towards Gas6, which is secreted by bone marrow stromal cells. Furthermore, Gas6–Mer interaction mediates survival and prevents chemotherapy-mediated apoptosis of B-cell ALL cells [225]. Almost nothing is known about the impact of Tyro3 in leukemia besides its expression in the chronic myeloid leukemia cell line K562 [226].

Summary

Crosstalk between tumors and macrophages is emerging as one of the key mechanisms in the promotion of all the essential hallmarks of malignancy. However, in principle macrophages can also support the host in suppressing cancer growth. This "ying and yang" of TAMs is reflected in heterogeneous clinical datasets reporting both positive and negative impacts of macrophage density on clinical outcome. Thus, it is of high relevance to improve our understanding of the essential cues driving macrophages into a protumoral or an antitumoral mode of action. Amongst other molecular mechanisms, the importance of the Gas6–TAMR axis in fostering a protumoral action of TAMs has recently been recognized. Disrupting this axis, for example by small molecule Axl inhibitors, might therefore open up novel avenues in cancer treatment.

Acknowledgments S.L. is funded by the Max-Eder group leader program of the Deutsche Krebshilfe, by the Hamburger Krebsgesellschaft and by the Roggenbuck Stiftung. A.S. receives funding from the Roggenbuck Stiftung.

References

1. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100(1):57–70 S0092-8674(00)81683-9[pii]
2. Allen M, Louise Jones J (2011) Jekyll and Hyde: the role of the microenvironment on the progression of cancer. J Pathol 223(2): 162–176. doi:10.1002/path.2803
3. Joyce JA, Pollard JW (2009) Microenvironmental regulation of metastasis. Nat Rev Cancer 9(4):239–252. doi:10.1038/nrc2618
4. Mantovani A, Allavena P, Sica A, Balkwill F (2008) Cancer-related inflammation. Nature 454(7203):436–444. doi:10.1038/nature07205
5. Tlsty TD, Coussens LM (2006) Tumor stroma and regulation of cancer development. Annu Rev Pathol 1:119–150. doi:10.1146/annurev.pathol.1.110304.100224
6. Grivennikov SI, Greten FR, Karin M (2010) Immunity, inflammation, and cancer. Cell 140(6):883–899. doi:10.1016/j.cell.2010.01.025
7. Qian BZ, Pollard JW (2010) Macrophage diversity enhances tumor progression and metastasis. Cell 141(1):39–51. doi:10.1016/j.cell.2010.03.014
8. Balkwill F, Charles KA, Mantovani A (2005) Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell 7(3):211–217. doi:10.1016/j.ccr.2005.02.013
9. DeClerck YA, Mercurio AM, Stack MS, Chapman HA, Zutter MM, Muschel RJ, Raz A, Matrisian LM, Sloane BF, Noel A, Hendrix MJ, Coussens L, Padarathsingh M (2004) Proteases, extracellular matrix, and cancer: a workshop of the path B study section. Am J Pathol 164(4):1131–1139. doi:10.1016/S0002-9440(10)63200-2
10. Carmeliet P, Jain RK (2011) Molecular mechanisms and clinical applications of angiogenesis. Nature 473(7347):298–307. doi:10.1038/nature10144
11. Folkman J (1971) Tumor angiogenesis: therapeutic implications. N Engl J Med 285(21):1182–1186. doi:10.1056/NEJM197111182852108
12. Kerbel RS (2008) Tumor angiogenesis. N Engl J Med 358(19):2039–2049. doi:10.1056/NEJMra0706596
13. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, Worthen GS, Albelda SM (2009) Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. Cancer Cell 16(3):183–194. doi:10.1016/j.ccr.2009.06.017
14. Fischer C, Jonckx B, Mazzone M, Zacchigna S, Loges S, Pattarini L, Chorianopoulos E, Liesenborghs L, Koch M, De Mol M, Autiero M, Wyns S, Plaisance S, Moons L, van Rooijen N, Giacca M, Stassen JM, Dewerchin M, Collen D, Carmeliet P (2007) Anti-PlGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. Cell 131(3): 463–475
15. Shiai F, Wu X, Malik AK, Zhong C, Baldwin ME, Schanz S, Fuh G, Gerber HP, Ferrara N (2007) Tumor refractoriness to anti-VEGF treatment is mediated by CD11b+ Gr1+ myeloid cells. Nat Biotechnol 25(8):911–920. doi:10.1038/nbt1323
16. Bissell MJ, Hines WC (2011) Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression. Nat Med 17(3):320–329. doi:10.1038/nm.2328

17. Tiwari M (2010) From tumor immunology to cancer immunotherapy: miles to go. J Cancer Res Ther 6(4):427–431. doi:10.4103/0973-1482.77071
18. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A (2009) Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis 30(7):1073–1081. doi:10.1093/carcin/bgp127
19. de Visser KE, Eichten A, Coussens LM (2006) Paradoxical roles of the immune system during cancer development. Nat Rev Cancer 6(1):24–37. doi:10.1038/nrc1782
20. Mantovani A, Sica A (2010) Macrophages, innate immunity and cancer: balance, tolerance, and diversity. Curr Opin Immunol 22(2):231–237. doi:10.1016/j.coi.2010.01.009
21. Pollard JW (2009) Trophic macrophages in development and disease. Nat Rev Immunol 9(4):259–270. doi:10.1038/nri2528
22. Condeelis J, Pollard JW (2006) Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell 124(2):263–266. doi:10.1016/j.cell.2006.01.007
23. Biswas SK, Mantovani A (2010) Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nat Immunol 11(10):889–896. doi:10.1038/ni.1937
24. Gordon S, Taylor PR (2005) Monocyte and macrophage heterogeneity. Nat Rev Immunol 5(12):953–964. doi:10.1038/nri1733
25. Hussain SP, Hofseth LJ, Harris CC (2003) Radical causes of cancer. Nat Rev Cancer 3(4):276–285. doi:10.1038/nrc1046
26. Jeannin P, Duluc D, Delneste Y (2011) IL-6 and leukemia-inhibitory factor are involved in the generation of tumor-associated macrophage: regulation by IFN-gamma. Immunotherapy 3(4 Suppl):23–26. doi:10.2217/imt.11.30
27. Heusinkveld M, van Steenwijk PJ, Goedemans R, Ramwadhdoebe TH, Gorter A, Welters MJ, van Hall T, van der Burg SH (2011) M2 macrophages induced by prostaglandin E2 and IL-6 from cervical carcinoma are switched to activated M1 macrophages by CD4+ Th1 cells. J Immunol 187(3):1157–1165. doi:10.4049/jimmunol.1100889
28. Hagemann T, Biswas SK, Lawrence T, Sica A, Lewis CE (2009) Regulation of macrophage function in tumors: the multifaceted role of NF-kappaB. Blood 113(14):3139–3146. doi:10.1182/blood-2008-12-172825
29. Sica A, Bronte V (2007) Altered macrophage differentiation and immune dysfunction in tumor development. J Clin Invest 117(5):1155–1166. doi:10.1172/JCI31422
30. Jiang H, Harris MB, Rothman P (2000) IL-4/IL-13 signaling beyond JAK/STAT. J Allergy Clin Immunol 105(6 Pt 1):1063–1070 S0091674900452255[pii]
31. Donnelly RP, Dickensheets H, Finbloom DS (1999) The interleukin-10 signal transduction pathway and regulation of gene expression in mononuclear phagocytes. J Interferon Cytokine Res 19(6):563–573. doi:10.1089/107999099313695
32. Martinez FO, Gordon S, Locati M, Mantovani A (2006) Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. J Immunol 177(10):7303–7311 177/10/7303[pii]
33. Mosser DM, Edwards JP (2008) Exploring the full spectrum of macrophage activation. Nat Rev Immunol 8(12):958–969. doi:10.1038/nri2448
34. Pettersen JS, Fuentes-Duculan J, Suarez-Farinas M, Pierson KC, Pitts-Kiefer A, Fan L, Belkin DA, Wang CQ, Bhuvanendran S, Johnson-Huang LM, Bluth MJ, Krueger JG, Lowes MA, Carucci JA (2011) Tumor-associated macrophages in the cutaneous SCC microenvironment are heterogeneously activated. J Invest Dermatol 131(6):1322–1330. doi:10.103/jid.2011.9
35. Rigo A, Gottardi M, Zamo A, Mauri P, Bonifacio M, Krampera M, Damiani E, Pizzolo G, Vinante F (2010) Macrophages may promote cancer growth via a GM-CSF/HB-EGF paracrine loop that is enhanced by CXCL12. Mol Cancer 9:273. doi:10.1186/1476-4598-9-273
36. Movahedi K, Laoui D, Gysemans C, Baeten M, Stange G, Van den Bossche J, Mack M, Pipeleers D, In't Veld P, De Baetselier P, Van Ginderachter JA (2010) Different tumor microenvironments contain functionally distinct subsets of macrophages derived from Ly6C(high) monocytes. Cancer Res 70(14):5728–5739. doi:10.1158/0008-5472.CAN-09-4672
37. Hamilton JA (2008) Colony-stimulating factors in inflammation and autoimmunity. Nat Rev Immunol 8(7):533–544. doi:10.1038/nri2356
38. Bolat F, Kayaselcuk F, Nursal TZ, Yagmurdur MC, Bal N, Demirhan B (2006) Microvessel density, VEGF expression, and tumor-associated macrophages in breast tumors: correlations with prognostic parameters. J Exp Clin Cancer Res 25(3):365–372
39. Lee CH, Espinosa I, Vrijaldenhoven S, Subramanian S, Montgomery KD, Zhu S, Marinelli RJ, Peterse JL, Poulin N, Nielsen TO, West RB, Gilks CB, van de Rijn M (2008) Prognostic significance of macrophage infiltration in leiomyosarcomas. Clin Cancer Res 14(5):1423–1430. doi:10.1158/1078-0432.CCR-07-1712
40. Ohno S, Inagawa H, Dhar DK, Fujii T, Ueda S, Tachibana M, Ohno Y, Suzuki N, Inoue M, Soma G, Nagasue N (2005) Role of tumor-associated macrophages (TAM) in advanced gastric carcinoma: the impact on FasL-mediated counterattack. Anticancer Res 25(1B):463–470
41. Ohno S, Inagawa H, Dhar DK, Fujii T, Ueda S, Tachibana M, Suzuki N, Inoue M, Soma G, Nagasue N (2003) The degree of macrophage infiltration into the cancer cell nest is a significant predictor of survival in gastric cancer patients. Anticancer Res 23(6D):5015–5022
42. Jensen TO, Schmidt H, Moller HJ, Hoyer M, Maniecki MB, Sjoegren P, Christensen IJ, Steiniche T (2009) Macrophage markers in serum and tumor have prognostic impact in American Joint Committee on Cancer stage I/II melanoma. J Clin Oncol 27(20):3330–3337. doi:10.1200/JCO.2008.19.9919
43. Soeda S, Nakamura N, Ozeki T, Nishiyama H, Hojo H, Yamada H, Abe M, Sato A (2008) Tumor-associated macrophages correlate with vascular space invasion and myometrial invasion in endometrial carcinoma. Gynecol Oncol 109(1):122–128. doi:10.1016/j.ygyno.2007.12.033
44. Hsu HP, Shan YS, Lai MD, Lin PW (2010) Osteopontin-positive infiltrating tumor-associated macrophages in bulky ampullary cancer predict survival. Cancer Biol Ther 10(2):144–154
45. Tanaka Y, Kobayashi H, Suzuki M, Kanayama N, Terao T (2002) Thymidine phosphorylase expression in tumor-infiltrating macrophages may be correlated with poor prognosis in uterine endometrial cancer. Hum Pathol 33(11):1105–1113. doi:10.1053/hupa.2002.129203S0046817702001636
46. Wang R, Lu M, Zhang J, Chen S, Luo X, Qin Y, Chen H (2011) Increased IL-10 mRNA expression in tumor-associated macrophage correlated with late stage of lung cancer. J Exp Clin Cancer Res 30:62. doi:10.1186/1756-9966-30-62
47. Bronkhorst IH, Ly LV, Jordanova ES, Vrolijk J, Versluis M, Luyten GP, Jager MJ (2011) Detection of M2-macrophages in uveal melanoma and relation with survival. Invest Ophthalmol Vis Sci 52(2):643–650. doi:10.1167/iovs.10-5979
48. Hasita H, Komohara Y, Okabe H, Masuda T, Ohnishi K, Lei XF, Beppu T, Baba H, Takeya M (2010) Significance of alternatively activated macrophages in patients with intrahepatic cholangiocarcinoma. Cancer Sci 101(8):1913–1919. doi:10.1111/j.1349-7006.2010.01614.x
49. Ma J, Liu L, Che G, Yu N, Dai F, You Z (2010) The M1 form of tumor-associated macrophages in non-small cell lung cancer is

positively associated with survival time. BMC Cancer 10:112. doi:10.1186/1471-2407-10-112
50. Ohri CM, Shikotra A, Green RH, Waller DA, Bradding P (2009) Macrophages within NSCLC tumour islets are predominantly of a cytotoxic M1 phenotype associated with extended survival. Eur Respir J 33(1):118–126. doi:10.1183/09031936.00065708
51. Markosyan N, Chen E, Ndong V, Yao Y, Sterner CJ, Chodosh LA, Lawson JA, Fitzgerald GA, Smyth EM (2011) Deletion of cyclooxygenase 2 in mouse mammary epithelial cells delays breast cancer onset through augmentation of type 1 immune responses in tumors. Carcinogenesis 32(10):1441–1449. doi:10.1093/carcin/bgr134
52. Rolny C, Mazzone M, Tugues S, Laoui D, Johansson I, Coulon C, Squadrito ML, Segura I, Li X, Knevels E, Costa S, Vinckier S, Dresselaer T, Akerud P, De Mol M, Salomaki H, Phillipson M, Wyns S, Larsson E, Buysschaert I, Botling J, Himmelreich U, Van Ginderachter JA, De Palma M, Dewerchin M, Claesson-Welsh L, Carmeliet P (2011) HRG inhibits tumor growth and metastasis by inducing macrophage polarization and vessel normalization through downregulation of PlGF. Cancer Cell 19(1):31–44. doi:10.1016/j.ccr.2010.11.009
53. Sanchez-Martin L, Estecha A, Samaniego R, Sanchez-Ramon S, Vega MA, Sanchez-Mateos P (2011) The chemokine CXCL12 regulates monocyte-macrophage differentiation and RUNX3 expression. Blood 117(1):88–97. doi:10.1182/blood-2009-12-258186
54. Deng L, Zhou JF, Sellers RS, Li JF, Nguyen AV, Wang Y, Orlofsky A, Liu Q, Hume DA, Pollard JW, Augenlicht L, Lin EY (2010) A novel mouse model of inflammatory bowel disease links mammalian target of rapamycin-dependent hyperproliferation of colonic epithelium to inflammation-associated tumorigenesis. Am J Pathol 176(2):952–967. doi:10.2353/ajpath.2010.090622
55. Karin M, Greten FR (2005) NF-kappaB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol 5(10):749–759. doi:10.1038/nri1703
56. Greten FR, Eckmann L, Greten TF, Park JM, Li ZW, Egan LJ, Kagnoff MF, Karin M (2004) IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. Cell 118(3):285–296. doi:10.1016/j.cell.2004.07.013S0092867404006713
57. Maeda S, Kamata H, Luo JL, Leffert H, Karin M (2005) IKKbeta couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. Cell 121(7):977–990. doi:10.1016/j.cell.2005.04.014
58. Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, Gutkovich-Pyest E, Urieli-Shoval S, Galun E, Ben-Neriah Y (2004) NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature 431(7007):461–466. doi:10.1038/nature02924
59. Arnott CH, Scott KA, Moore RJ, Robinson SC, Thompson RG, Balkwill FR (2004) Expression of both TNF-alpha receptor subtypes is essential for optimal skin tumour development. Oncogene 23(10):1902–1910. doi:10.1038/sj.onc.12073171207317
60. Moore RJ, Owens DM, Stamp G, Arnott C, Burke F, East N, Holdsworth H, Turner L, Rollins B, Pasparakis M, Kollias G, Balkwill F (1999) Mice deficient in tumor necrosis factor-alpha are resistant to skin carcinogenesis. Nat Med 5(7):828–831. doi:10.1038/10552
61. Balkwill F (2009) Tumour necrosis factor and cancer. Nat Rev Cancer 9(5):361–371. doi:10.1038/nrc2628
62. Oguma K, Oshima H, Aoki M, Uchio R, Naka K, Nakamura S, Hirao A, Saya H, Taketo MM, Oshima M (2008) Activated macrophages promote Wnt signalling through tumour necrosis factor-alpha in gastric tumour cells. EMBO J 27(12):1671–1681. doi:10.1038/emboj.2008.105
63. Popivanova BK, Kitamura K, Wu Y, Kondo T, Kagaya T, Kaneko S, Oshima M, Fujii C, Mukaida N (2008) Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis. J Clin Invest 118(2):560–570. doi:10.1172/JCI32453
64. Bollrath J, Phesse TJ, von Burstin VA, Putoczki T, Bennecke M, Bateman T, Nebelsiek T, Lundgren-May T, Canli O, Schwitalla S, Matthews V, Schmid RM, Kirchner T, Arkan MC, Ernst M, Greten FR (2009) gp130-mediated Stat3 activation in enterocytes regulates cell survival and cell-cycle progression during colitis-associated tumorigenesis. Cancer Cell 15(2):91–102. doi:10.1016/j.ccr.2009.01.002
65. Yu H, Kortylewski M, Pardoll D (2007) Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment. Nat Rev Immunol 7(1):41–51. doi:10.1038/nri1995
66. Karin M, Lawrence T, Nizet V (2006) Innate immunity gone awry: linking microbial infections to chronic inflammation and cancer. Cell 124(4):823–835. doi:10.1016/j.cell.2006.02.016
67. Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, Karin M (2007) Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science 317(5834):121–124. doi:10.1126/science.1140485
68. Yi L, Xiao H, Xu M, Ye X, Hu J, Li F, Li M, Luo C, Yu S, Bian X, Feng H (2011) Glioma-initiating cells: a predominant role in microglia/macrophages tropism to glioma. J Neuroimmunol 232(1–2):75–82. doi:10.1016/j.jneuroim.2010.10.011
69. 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 Immunol 23(11):549–555 S1471490602023025[pii]
70. Biswas SK, Gangi L, Paul S, Schioppa T, Saccani A, Sironi M, Bottazzi B, Doni A, Vincenzo B, Pasqualini F, Vago L, Nebuloni M, Mantovani A, Sica A (2006) A distinct and unique transcriptional program expressed by tumor-associated macrophages (defective NF-kappaB and enhanced IRF-3/STAT1 activation). Blood 107(5):2112–2122. doi:10.1182/blood-2005-01-0428
71. Porta C, Rimoldi M, Raes G, Brys L, Ghezzi P, Di Liberto D, Dieli F, Ghisletti S, Natoli G, De Baetselier P, Mantovani A, Sica A (2009) Tolerance and M2 (alternative) macrophage polarization are related processes orchestrated by p50 nuclear factor kappaB. Proc Natl Acad Sci U S A 106(35):14978–14983. doi:10.1073/pnas.0809784106
72. Saccani A, Schioppa T, Porta C, Biswas SK, Nebuloni M, Vago L, Bottazzi B, Colombo MP, Mantovani A, Sica A (2006) p50 nuclear factor-kappaB overexpression in tumor-associated macrophages inhibits M1 inflammatory responses and antitumor resistance. Cancer Res 66(23):11432–11440. doi:10.1158/0008-5472.CAN-06-1867
73. Sica A, Saccani A, Bottazzi B, Polentarutti N, Vecchi A, van Damme J, Mantovani A (2000) Autocrine production of IL-10 mediates defective IL-12 production and NF-kappa B activation in tumor-associated macrophages. J Immunol 164(2):762–767 ji_v164n2p762[pii]
74. Gocheva V, Wang HW, Gadea BB, Shree T, Hunter KE, Garfall AL, Berman T, Joyce JA (2010) IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion. Genes Dev 24(3):241–255. doi:10.1101/gad.1874010
75. Kubota Y, Takubo K, Shimizu T, Ohno H, Kishi K, Shibuya M, Saya H, Suda T (2009) M-CSF inhibition selectively targets pathological angiogenesis and lymphangiogenesis. J Exp Med 206(5):1089–1102. doi:10.1084/jem.20081605
76. Lin EY, Nguyen AV, Russell RG, Pollard JW (2001) Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. J Exp Med 193(6):727–740

77. Abraham D, Zins K, Sioud M, Lucas T, Schafer R, Stanley ER, Aharinejad S (2010) Stromal cell-derived CSF-1 blockade prolongs xenograft survival of CSF-1-negative neuroblastoma. Int J Cancer 126(6):1339–1352. doi:10.1002/ijc.24859
78. Aharinejad S, Sioud M, Lucas T, Abraham D (2009) Targeting stromal-cancer cell interactions with siRNAs. Methods Mol Biol 487:243–266
79. Loges S, Schmidt T, Carmeliet P (2009) "Antimyeloangiogenic" therapy for cancer by inhibiting PlGF. Clin Cancer Res 15(11):3648–3653. doi:10.1158/1078-0432.CCR-08-2276
80. Van de Veire S, Stalmans I, Heindryckx F, Oura H, Tijeras-Raballand A, Schmidt T, Loges S, Albrecht I, Jonckx B, Vinckier S, Van Steenkiste C, Tugues S, Rolny C, De Mol M, Dettori D, Hainaud P, Coenegrachts L, Contreres JO, Van Bergen T, Cuervo H, Xiao WH, Le Henaff C, Buysschaert I, Kharabi Masouleh B, Geerts A, Schomber T, Bonnin P, Lambert V, Haustraete J, Zacchigna S, Rakic JM, Jimenez W, Noel A, Giacca M, Colle I, Foidart JM, Tobelem G, Morales-Ruiz M, Vilar J, Maxwell P, Vinores SA, Carmeliet G, Dewerchin M, Claesson-Welsh L, Dupuy E, Van Vlierberghe H, Christofori G, Mazzone M, Detmar M, Collen D, Carmeliet P (2010) Further pharmacological and genetic evidence for the efficacy of PlGF inhibition in cancer and eye disease. Cell 141(1):178–190. doi:10.1016/j.cell.2010.02.039
81. Wyckoff J, Wang W, Lin EY, Wang Y, Pixley F, Stanley ER, Graf T, Pollard JW, Segall J, Condeelis J (2004) A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. Cancer Res 64(19):7022–7029. doi:10.1158/0008-5472.CAN-04-1449
82. Wyckoff JB, Wang Y, Lin EY, Li JF, Goswami S, Stanley ER, Segall JE, Pollard JW, Condeelis J (2007) Direct visualization of macrophage-assisted tumor cell intravasation in mammary tumors. Cancer Res 67(6):2649–2656. doi:10.1158/0008-5472.CAN-06-1823
83. Green CE, Liu T, Montel V, Lester RD, Subramaniam S, Gonias SL, Klemke RL (2009) Chemoattractant signaling between tumor cells and macrophages regulates cancer cell migration, metastasis and neovascularization. PLoS One 4(8):e6713. doi:10.1371/journal.pone.0006713
84. DeNardo DG, Barreto JB, Andreu P, Vasquez L, Tawfik D, Kolhatkar N, Coussens LM (2009) CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. Cancer Cell 16(2):91–102. doi:10.1016/j.ccr.2009.06.018
85. Franovic A, Gunaratnam L, Smith K, Robert I, Patten D, Lee S (2007) Translational up-regulation of the EGFR by tumor hypoxia provides a nonmutational explanation for its overexpression in human cancer. Proc Natl Acad Sci U S A 104(32):13092–13097. doi:10.1073/pnas.0702387104
86. Imtiyaz HZ, Williams EP, Hickey MM, Patel SA, Durham AC, Yuan LJ, Hammond R, Gimotty PA, Keith B, Simon MC (2010) Hypoxia-inducible factor 2alpha regulates macrophage function in mouse models of acute and tumor inflammation. J Clin Invest 120(8):2699–2714. doi:10.1172/JCI39506
87. Ojalvo LS, Whittaker CA, Condeelis JS, Pollard JW (2010) Gene expression analysis of macrophages that facilitate tumor invasion supports a role for Wnt-signaling in mediating their activity in primary mammary tumors. J Immunol 184(2):702–712. doi:10.4049/jimmunol.0902360
88. Pucci F, Venneri MA, Biziato D, Nonis A, Moi D, Sica A, Di Serio C, Naldini L, De Palma M (2009) A distinguishing gene signature shared by tumor-infiltrating Tie2-expressing monocytes, blood "resident" monocytes, and embryonic macrophages suggests common functions and developmental relationships. Blood 114(4):901–914. doi:10.1182/blood-2009-01-200931
89. Lin EY, Li JF, Gnatovskiy L, Deng Y, Zhu L, Grzesik DA, Qian H, Xue XN, Pollard JW (2006) Macrophages regulate the angiogenic switch in a mouse model of breast cancer. Cancer Res 66(23):11238–11246. doi:10.1158/0008-5472.CAN-06-1278
90. Luo YP, Zhou H, Krueger J, Kaplan C, Liao D, Markowitz D, Liu C, Chen T, Chuang TH, Xiang R, Reisfeld RA (2010) The role of proto-oncogene Fra-1 in remodeling the tumor microenvironment in support of breast tumor cell invasion and progression. Oncogene 29(5):662–673. doi:10.1038/onc.2009.308
91. Takanami I, Takeuchi K, Kodaira S (1999) Tumor-associated macrophage infiltration in pulmonary adenocarcinoma: association with angiogenesis and poor prognosis. Oncology 57(2):138–142
92. Murri AM, Hilmy M, Bell J, Wilson C, McNicol AM, Lannigan A, Doughty JC, McMillan DC (2008) The relationship between the systemic inflammatory response, tumour proliferative activity, T-lymphocytic and macrophage infiltration, microvessel density and survival in patients with primary operable breast cancer. Br J Cancer 99(7):1013–1019. doi:10.1038/sj.bjc.6604667
93. Fraisl P, Mazzone M, Schmidt T, Carmeliet P (2009) Regulation of angiogenesis by oxygen and metabolism. Dev Cell 16(2):167–179. doi:10.1016/j.devcel.2009.01.003
94. Murdoch C, Muthana M, Coffelt SB, Lewis CE (2008) The role of myeloid cells in the promotion of tumour angiogenesis. Nat Rev Cancer 8(8):618–631
95. Carmeliet P, Jain RK (2011) Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. Nat Rev Drug Discov 10(6):417–427. doi:10.1038/nrd3455
96. Jain RK (2005) Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science 307(5706):58–62
97. De Bock K, Cauwenberghs S, Carmeliet P (2011) Vessel abnormalization: another hallmark of cancer? Molecular mechanisms and therapeutic implications. Curr Opin Genet Dev 21(1):73–79. doi:10.1016/j.gde.2010.10.008
98. Du R, Lu KV, Petritsch C, Liu P, Ganss R, Passegue E, Song H, Vandenberg S, Johnson RS, Werb Z, Bergers G (2008) HIF1alpha induces the recruitment of bone marrow-derived vascular modulatory cells to regulate tumor angiogenesis and invasion. Cancer Cell 13(3):206–220. doi:10.1016/j.ccr.2008.01.034
99. De Palma M, Venneri MA, Galli R, Sergi Sergi L, Politi LS, Sampaolesi M, Naldini L (2005) Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. Cancer Cell 8(3):211–226
100. Mazzieri R, Pucci F, Moi D, Zonari E, Ranghetti A, Berti A, Politi LS, Gentner B, Brown JL, Naldini L, De Palma M (2011) Targeting the ANG2/TIE2 axis inhibits tumor growth and metastasis by impairing angiogenesis and disabling rebounds of proangiogenic myeloid cells. Cancer Cell 19(4):512–526. doi:10.1016/j.ccr.2011.02.005
101. Fantin A, Vieira JM, Gestri G, Denti L, Schwarz Q, Prykhozhij S, Peri F, Wilson SW, Ruhrberg C (2010) Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. Blood 116(5):829–840. doi:10.1182/blood-2009-12-257832
102. Schmidt T, Carmeliet P (2010) Blood-vessel formation: bridges that guide and unite. Nature 465(7299):697–699. doi:10.1038/465697a
103. Baluk P, Tammela T, Ator E, Lyubynska N, Achen MG, Hicklin DJ, Jeltsch M, Petrova TV, Pytowski B, Stacker SA, Yla-Herttuala S, Jackson DG, Alitalo K, McDonald DM (2005) Pathogenesis of persistent lymphatic vessel hyperplasia in

chronic airway inflammation. J Clin Invest 115(2):247–257. doi:10.1172/JCI22037
104. Jeon BH, Jang C, Han J, Kataru RP, Piao L, Jung K, Cha HJ, Schwendener RA, Jang KY, Kim KS, Alitalo K, Koh GY (2008) Profound but dysfunctional lymphangiogenesis via vascular endothelial growth factor ligands from CD11b+ macrophages in advanced ovarian cancer. Cancer Res 68(4):1100–1109. doi:10.1158/0008-5472.CAN-07-2572
105. Moussai D, Mitsui H, Pettersen JS, Pierson KC, Shah KR, Suarez-Farinas M, Cardinale IR, Bluth MJ, Krueger JG, Carucci JA (2011) The human cutaneous squamous cell carcinoma microenvironment is characterized by increased lymphatic density and enhanced expression of macrophage-derived VEGF-C. J Invest Dermatol 131(1):229–236. doi:10.1038/jid.2010.266
106. Zumsteg A, Baeriswyl V, Imaizumi N, Schwendener R, Ruegg C, Christofori G (2009) Myeloid cells contribute to tumor lymphangiogenesis. PLoS One 4(9):e7067. doi:10.1371/journal.pone.0007067
107. Zhang BC, Gao J, Wang J, Rao ZG, Wang BC, Gao JF (2010) Tumor-associated macrophages infiltration is associated with peritumoral lymphangiogenesis and poor prognosis in lung adenocarcinoma. Med Oncol. doi:10.1007/s12032-010-9638-5
108. Kurahara H, Shinchi H, Mataki Y, Maemura K, Noma H, Kubo F, Sakoda M, Ueno S, Natsugoe S, Takao S (2011) Significance of M2-polarized tumor-associated macrophage in pancreatic cancer. J Surg Res 167(2):e211–e219. doi:10.1016/j.jss.2009.05.026
109. Zhang J, Chen L, Xiao M, Wang C, Qin Z (2010) FSP1+ fibroblasts promote skin carcinogenesis by maintaining MCP-1-mediated macrophage infiltration and chronic inflammation. Am J Pathol 178(1):382–390. doi:10.1016/j.ajpath.2010.11.017
110. Erez N, Truitt M, Olson P, Arron ST, Hanahan D (2010) Cancer-associated fibroblasts are activated in incipient neoplasia to orchestrate tumor-promoting inflammation in an NF-kappaB-dependent manner. Cancer Cell 17(2):135–147. doi:10.1016/j.ccr.2009.12.041
111. Silzle T, Kreutz M, Dobler MA, Brockhoff G, Knuechel R, Kunz-Schughart LA (2003) Tumor-associated fibroblasts recruit blood monocytes into tumor tissue. Eur J Immunol 33(5):1311–1320. doi:10.1002/eji.200323057
112. Liao D, Luo Y, Markowitz D, Xiang R, Reisfeld RA (2009) Cancer associated fibroblasts promote tumor growth and metastasis by modulating the tumor immune microenvironment in a 4T1 murine breast cancer model. PLoS One 4(11):e7965. doi:10.1371/journal.pone.0007965
113. Rama D, Esendagli G, Guc D (2011) Expression of chemokine-like receptor 1 (CMKLR1) on J744A.1 macrophages co-cultured with fibroblast and/or tumor cells: modeling the influence of microenvironment. Cell Immunol 271(1):134–140. doi:10.1016/j.cellimm.2011.06.016
114. Sinha P, Clements VK, Ostrand-Rosenberg S (2005) Interleukin-13-regulated M2 macrophages in combination with myeloid suppressor cells block immune surveillance against metastasis. Cancer Res 65(24):11743–11751. doi:10.1158/0008-5472.CAN-05-0045
115. Doedens AL, Stockmann C, Rubinstein MP, Liao D, Zhang N, DeNardo DG, Coussens LM, Karin M, Goldrath AW, Johnson RS (2010) Macrophage expression of hypoxia-inducible factor-1 alpha suppresses T-cell function and promotes tumor progression. Cancer Res 70(19):7465–7475. doi:10.1158/0008-5472.CAN-10-1439
116. Kuang DM, Zhao Q, Peng C, Xu J, Zhang JP, Wu C, Zheng L (2009) Activated monocytes in peritumoral stroma of hepatocellular carcinoma foster immune privilege and disease progression through PD-L1. J Exp Med 206(6):1327–1337. doi:10.1084/jem.20082173
117. Zhou J, Ding T, Pan W, Zhu LY, Li L, Zheng L (2009) Increased intratumoral regulatory T cells are related to intratumoral macrophages and poor prognosis in hepatocellular carcinoma patients. Int J Cancer 125(7):1640–1648. doi:10.1002/ijc.24556
118. Liu J, Zhang N, Li Q, Zhang W, Ke F, Leng Q, Wang H, Chen J (2011) Tumor-associated macrophages recruit CCR6+ regulatory T cells and promote the development of colorectal cancer via enhancing CCL20 production in mice. PLoS One 6(4):e19495. doi:10.1371/journal.pone
119. Yang S, Wang B, Guan C, Wu B, Cai C, Wang M, Zhang B, Liu T, Yang P (2011) Foxp3+ IL-17+ T cells promote development of cancer-initiating cells in colorectal cancer. J Leukoc Biol 89(1):85–91. doi:10.1189/jlb.0910506
120. Galani IE, Wendel M, Stojanovic A, Jesiak M, Muller MM, Schellack C, Suri-Payer E, Cerwenka A (2010) Regulatory T cells control macrophage accumulation and activation in lymphoma. Int J Cancer 127(5):1131–1140. doi:10.1002/ijc.25132
121. Haas M, Dimmler A, Hohenberger W, Grabenbauer GG, Niedobitek G, Distel LV (2009) Stromal regulatory T-cells are associated with a favourable prognosis in gastric cancer of the cardia. BMC Gastroenterol 9:65. doi:10.1186/1471-230X-9-65
122. Wong SC, Puaux AL, Chittezhath M, Shalova I, Kajiji TS, Wang X, Abastado JP, Lam KP, Biswas SK (2010) Macrophage polarization to a unique phenotype driven by B cells. Eur J Immunol 40(8):2296–2307. doi:10.1002/eji.200940288
123. DeNardo DG, Brennan DJ, Rexhapaj E, Ruffell B, Shiao SL, Madden SF, Gallagher WM, Wadhwani N, Keil SD, Junaid SA, Rugo HS, Hwang ES, Jirström K, West BL, Coussens LM (2011) Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. Cancer Discovery 1(1):54–67
124. Zheng Y, Cai Z, Wang S, Zhang X, Qian J, Hong S, Li H, Wang M, Yang J, Yi Q (2009) Macrophages are an abundant component of myeloma microenvironment and protect myeloma cells from chemotherapy drug-induced apoptosis. Blood 114(17):3625–3628. doi:10.1182/blood-2009-05-220285
125. Jinushi M, Chiba S, Yoshiyama H, Masutomi K, Kinoshita I, Dosaka-Akita H, Yagita H, Takaoka A, Tahara H (2011) Tumor-associated macrophages regulate tumorigenicity and anticancer drug responses of cancer stem/initiating cells. Proc Natl Acad Sci U S A 108(30):12425–12430. doi:10.1073/pnas.1106645108
126. Zhu P, Baek SH, Bourk EM, Ohgi KA, Garcia-Bassets I, Sanjo H, Akira S, Kotol PF, Glass CK, Rosenfeld MG, Rose DW (2006) Macrophage/cancer cell interactions mediate hormone resistance by a nuclear receptor derepression pathway. Cell 124(3):615–629. doi:10.1016/j.cell.2005.12.032
127. Shiai F, Wu X, Zhong C, Yu L, Liang XH, Yao J, Blanchard D, Bais C, Peale FV, van Bruggen N, Ho C, Ross J, Tan M, Carano RA, Meng YG, Ferrara N (2007) Bv8 regulates myeloid-cell-dependent tumour angiogenesis. Nature 450(7171):825–831. doi:10.1038/nature06348
128. Priceman SJ, Sung JL, Shaposhnik Z, Burton JB, Torres-Collado AX, Moughon DL, Johnson M, Lusis AJ, Cohen DA, Iruela-Arispe ML, Wu L (2011) Targeting distinct tumor-infiltrating myeloid cells by inhibiting CSF-1 receptor: combating tumor evasion of antiangiogenic therapy. Blood 115(7):1461–1471. doi:10.1182/blood-2009-08-237412
129. Kopetz S, Hoff PM, Morris JS, Wolff RA, Eng C, Glover KY, Adinin R, Overman MJ, Valero V, Wen S, Lieu C, Yan S, Tran HT, Ellis LM, Abbruzzese JL, Heymach JV (2010) Phase II trial of infusional fluorouracil, irinotecan, and bevacizumab for metastatic colorectal cancer: efficacy and circulating angiogenic

biomarkers associated with therapeutic resistance. J Clin Oncol 28(3):453–459. doi:10.1200/JCO.2009.24.8252
130. Loges S, Schmidt T, Carmeliet P (2010) Mechanisms of resistance to anti-angiogenic therapy and development of third-generation anti-angiogenic drug candidates. Genes Cancer 1(1):12–25. doi:10.1177/1947601909356574
131. Lemmon MA, Schlessinger J (2010) Cell signaling by receptor tyrosine kinases. Cell 141(7):1117–1134. doi:10.1016/j.cell.2010.06.011
132. Schlessinger J (2000) Cell signaling by receptor tyrosine kinases. Cell 103(2):211–225 S0092-8674(00)00114-8[pii]
133. Lapraz F, Rottinger E, Duboc V, Range R, Duloquin L, Walton K, Wu SY, Bradham C, Loza MA, Hibino T, Wilson K, Poustka A, McClay D, Angerer L, Gache C, Lepage T (2006) RTK and TGF-beta signaling pathways genes in the sea urchin genome. Dev Biol 300(1):132–152. doi:10.1016/j.ydbio.2006.08.048
134. Satou Y, Imai KS, Levine M, Kohara Y, Rokhsar D, Satoh N (2003) A genomewide survey of developmentally relevant genes in Ciona intestinalis. I. Genes for bHLH transcription factors. Dev Genes Evol 213(5-6):213–221. doi:10.1007/s00427-003-0319-7
135. Lai C, Lemke G (1991) An extended family of protein-tyrosine kinase genes differentially expressed in the vertebrate nervous system. Neuron 6(5):691–704 0896-6273(91)90167-X[pii]
136. O'Bryan JP, Frye RA, Cogswell PC, Neubauer A, Kitch B, Prokop C, Espinosa R 3rd, Le Beau MM, Earp HS, Liu ET (1991) axl, a transforming gene isolated from primary human myeloid leukemia cells, encodes a novel receptor tyrosine kinase. Mol Cell Biol 11(10):5016–5031
137. Sasaki T, Knyazev PG, Clout NJ, Cheburkin Y, Gohring W, Ullrich A, Timpl R, Hohenester E (2006) Structural basis for Gas6-Axl signalling. EMBO J 25(1):80–87. doi:10.1038/sj.emboj.7600912
138. Lemke G, Rothlin CV (2008) Immunobiology of the TAM receptors. Nat Rev Immunol 8(5):327–336. doi:10.1038/nri2303
139. Hafizi S, Dahlback B (2006) Gas6 and protein S. Vitamin K-dependent ligands for the Axl receptor tyrosine kinase subfamily. FEBS J 273(23):5231–5244. doi:10.1111/j.1742-4658.2006.05529.x
140. Linger RM, Keating AK, Earp HS, Graham DK (2011) Taking aim at Mer and Axl receptor tyrosine kinases as novel therapeutic targets in solid tumors. Expert Opin Ther Targets 14(10):1073–1090. doi:10.1517/14728222.2010.515980
141. Zhang QK, Boast S, de los Santos K, Begemann M, Goff SP (1996) Transforming activity of retroviral genomes encoding Gag-Axl fusion proteins. J Virol 70(11):8089–8097
142. Faust M, Ebensperger C, Schulz AS, Schleithoff L, Hameister H, Bartram CR, Janssen JW (1992) The murine ufo receptor: molecular cloning, chromosomal localization and in situ expression analysis. Oncogene 7(7):1287–1293
143. Angelillo-Scherrer A, Burnier L, Flores N, Savi P, DeMol M, Schaeffer P, Herbert JM, Lemke G, Goff SP, Matsushima GK, Earp HS, Vesin C, Hoylaerts MF, Plaisance S, Collen D, Conway EM, Wehrle-Haller B, Carmeliet P (2005) Role of Gas6 receptors in platelet signaling during thrombus stabilization and implications for antithrombotic therapy. J Clin Invest 115(2):237–246. doi:10.1172/JCI22079
144. Angelillo-Scherrer A, de Frutos P, Aparicio C, Melis E, Savi P, Lupu F, Arnout J, Dewerchin M, Hoylaerts M, Herbert J, Collen D, Dahlback B, Carmeliet P (2001) Deficiency or inhibition of Gas6 causes platelet dysfunction and protects mice against thrombosis. Nat Med 7(2):215–221. doi:10.1038/84667
145. Linger RM, Keating AK, Earp HS, Graham DK (2008) TAM receptor tyrosine kinases: biologic functions, signaling, and potential therapeutic targeting in human cancer. Adv Cancer Res 100:35–83. doi:10.1016/S0065-230X(08)00002-X
146. Bellosta P, Zhang Q, Goff SP, Basilico C (1997) Signaling through the ARK tyrosine kinase receptor protects from apoptosis in the absence of growth stimulation. Oncogene 15(20):2387–2397. doi:10.1038/sj.onc.1201419
147. Bellosta P, Costa M, Lin DA, Basilico C (1995) The receptor tyrosine kinase ARK mediates cell aggregation by homophilic binding. Mol Cell Biol 15(2):614–625
148. Wimmel A, Glitz D, Kraus A, Roeder J, Schuermann M (2001) Axl receptor tyrosine kinase expression in human lung cancer cell lines correlates with cellular adhesion. Eur J Cancer 37(17):2264–2274 S0959-8049(01)00271-4[pii]
149. Neubauer A, Burchert A, Maiwald C, Gruss HJ, Serke S, Huhn D, Wittig B, Liu E (1997) Recent progress on the role of Axl, a receptor tyrosine kinase, in malignant transformation of myeloid leukemias. Leuk Lymphoma 25(1–2):91–96. doi:10.3109/10428199709042499
150. Ou WB, Corson JM, Flynn DL, Lu WP, Wise SC, Bueno R, Sugarbaker DJ, Fletcher JA (2011) AXL regulates mesothelioma proliferation and invasiveness. Oncogene 30(14):1643–1652. doi:10.1038/onc.2010.555
151. Avilla E, Guarino V, Visciano C, Liotti F, Svelto M, Krishnamoorthy G, Franco R, Melillo RM (2011) Activation of TYRO3/AXL tyrosine kinase receptors in thyroid cancer. Cancer Res 71(5):1792–1804. doi:10.1158/0008-5472.CAN-10-2186
152. Pierce A, Bliesner B, Xu M, Nielsen-Preiss S, Lemke G, Tobet S, Wierman ME (2008) Axl and Tyro3 modulate female reproduction by influencing gonadotropin-releasing hormone neuron survival and migration. Mol Endocrinol 22(11):2481–2495. doi:10.1210/me.2008-0169
153. Scutera S, Fraone T, Musso T, Cappello P, Rossi S, Pierobon D, Orinska Z, Paus R, Bulfone-Paus S, Giovarelli M (2009) Survival and migration of human dendritic cells are regulated by an IFN-alpha-inducible Axl/Gas6 pathway. J Immunol 183(5):3004–3013. doi:10.4049/jimmunol.0804384
154. Song X, Wang H, Logsdon CD, Rashid A, Fleming JB, Abbruzzese JL, Gomez HF, Evans DB (2011) Overexpression of receptor tyrosine kinase Axl promotes tumor cell invasion and survival in pancreatic ductal adenocarcinoma. Cancer 117(4):734–743. doi:10.1002/cncr.25483
155. Keating AK, Kim GK, Jones AE, Donson AM, Ware K, Mulcahy JM, Salzberg DB, Foreman NK, Liang X, Thorburn A, Graham DK (2010) Inhibition of Mer and Axl receptor tyrosine kinases in astrocytoma cells leads to increased apoptosis and improved chemosensitivity. Mol Cancer Ther 9(5):1298–1307. doi:10.1158/1535-7163.MCT-09-0707
156. Papadakis ES, Cichon MA, Vyas JJ, Patel N, Ghali L, Cerio R, Storey A, O'Toole EA (2010) Axl promotes cutaneous squamous cell carcinoma survival through negative regulation of pro-apoptotic Bcl-2 family members. J Invest Dermatol 131(2):509–517. doi:10.1038/jid.2010.326
157. Ye X, Li Y, Stawicki S, Couto S, Eastham-Anderson J, Kallop D, Weimer R, Wu Y, Pei L (2010) An anti-Axl monoclonal antibody attenuates xenograft tumor growth and enhances the effect of multiple anticancer therapies. Oncogene 29(38):5254–5264
158. Polvi A, Armstrong E, Lai C, Lemke G, Huebner K, Spritz RA, Guida LC, Nicholls RD, Alitalo K (1993) The human TYRO3 gene and pseudogene are located in chromosome 15q14-q25. Gene 134(2):289–293
159. Ohashi K, Mizuno K, Kuma K, Miyata T, Nakamura T (1994) Cloning of the cDNA for a novel receptor tyrosine kinase, Sky, predominantly expressed in brain. Oncogene 9(3):699–705
160. Fujimoto J, Yamamoto T (1994) Brt, a mouse gene encoding a novel receptor-type protein-tyrosine kinase, is preferentially expressed in the brain. Oncogene 9(3):693–698

161. Crosier PS, Freeman SA, Orlic D, Bodine DM, Crosier KE (1996) The Dtk receptor tyrosine kinase, which binds protein S, is expressed during hematopoiesis. Exp Hematol 24(2):318–323
162. Jansa Perez M, Walshe JA, Crosier KE, Crosier PS (1996) Expression of the DTK receptor tyrosine kinase during zebrafish development. Int J Dev Biol Suppl 1:101S–102S
163. Biscardi JS, Denhez F, Buehler GF, Chesnutt DA, Baragona SC, O'Bryan JP, Der CJ, Fiordalisi JJ, Fults DW, Maness PF (1996) Rek, a gene expressed in retina and brain, encodes a receptor tyrosine kinase of the Axl/Tyro3 family. J Biol Chem 271(46): 29049–29059
164. Mark MR, Scadden DT, Wang Z, Gu Q, Goddard A, Godowski PJ (1994) rse, a novel receptor-type tyrosine kinase with homology to Axl/Ufo, is expressed at high levels in the brain. J Biol Chem 269(14):10720–10728
165. Schulz NT, Paulhiac CI, Lee L, Zhou R (1995) Isolation and expression analysis of tyro3, a murine growth factor receptor tyrosine kinase preferentially expressed in adult brain. Brain Res Mol Brain Res 28(2):273–280 0169328X94002162[pii]
166. Healy AM, Schwartz JJ, Zhu X, Herrick BE, Varnum B, Farber HW (2001) Gas 6 promotes Axl-mediated survival in pulmonary endothelial cells. Am J Physiol Lung Cell Mol Physiol 280(6):L1273–L1281
167. Nakamura YS, Hakeda Y, Takakura N, Kameda T, Hamaguchi I, Miyamoto T, Kakudo S, Nakano T, Kumegawa M, Suda T (1998) Tyro 3 receptor tyrosine kinase and its ligand, Gas6, stimulate the function of osteoclasts. Stem Cells 16(3):229–238. doi:10.1002/stem.160229
168. Dai W, Pan H, Hassanain H, Gupta SL, Murphy MJ Jr (1994) Molecular cloning of a novel receptor tyrosine kinase, tif, highly expressed in human ovary and testis. Oncogene 9(3):975–979
169. Wang H, Chen S, Chen Y, Wu H, Tang H, Xiong W, Ma J, Ge Y, Lu Q, Han D (2007) The role of Tyro 3 subfamily receptors in the regulation of hemostasis and megakaryocytopoiesis. Haematologica 92(5):643–650
170. Zhong Z, Wang Y, Guo H, Sagare A, Fernandez JA, Bell RD, Barrett TM, Griffin JH, Freeman RS, Zlokovic BV (2010) Protein S protects neurons from excitotoxic injury by activating the TAM receptor Tyro3-phosphatidylinositol 3-kinase-Akt pathway through its sex hormone-binding globulin-like region. J Neurosci 30(46):15521–15534. doi:10.1523/JNEUROSCI.4437-10.2010
171. Lan Z, Wu H, Li W, Wu S, Lu L, Xu M, Dai W (2000) Transforming activity of receptor tyrosine kinase tyro3 is mediated, at least in part, by the PI3 kinase-signaling pathway. Blood 95(2):633–638
172. Taylor IC, Roy S, Varmus HE (1995) Overexpression of the Sky receptor tyrosine kinase at the cell surface or in the cytoplasm results in ligand-independent activation. Oncogene 11(12): 2619–2626
173. Zhu S, Wurdak H, Wang Y, Galkin A, Tao H, Li J, Lyssiotis CA, Yan F, Tu BP, Miraglia L, Walker J, Sun F, Orth A, Schultz PG, Wu X (2009) A genomic screen identifies TYRO3 as a MITF regulator in melanoma. Proc Natl Acad Sci U S A 106(40):17025–17030. doi:10.1073/pnas.0909292106
174. Heiring C, Dahlback B, Muller YA (2004) Ligand recognition and homophilic interactions in Tyro3: structural insights into the Axl/Tyro3 receptor tyrosine kinase family. J Biol Chem 279(8):6952–6958. doi:10.1074/jbc.M311750200
175. Graham DK, Dawson TL, Mullaney DL, Snodgrass HR, Earp HS (1994) Cloning and mRNA expression analysis of a novel human protooncogene, c-mer. Cell Growth Differ 5(6): 647–657
176. Jia R, Hanafusa H (1994) The proto-oncogene of v-eyk (v-ryk) is a novel receptor-type protein tyrosine kinase with extracellular Ig/GN-III domains. J Biol Chem 269(3):1839–1844
177. Graham DK, Bowman GW, Dawson TL, Stanford WL, Earp HS, Snodgrass HR (1995) Cloning and developmental expression analysis of the murine c-mer tyrosine kinase. Oncogene 10(12):2349–2359
178. Chen C, Li Q, Darrow AL, Wang Y, Derian CK, Yang J, de Garavilla L, Andrade-Gordon P, Damiano BP (2004) Mer receptor tyrosine kinase signaling participates in platelet function. Arterioscler Thromb Vasc Biol 24(6):1118–1123. doi: 10.1161/01.ATV.0000130662.30537.0801
179. Ling L, Kung HJ (1995) Mitogenic signals and transforming potential of Nyk, a newly identified neural cell adhesion molecule-related receptor tyrosine kinase. Mol Cell Biol 15(12): 6582–6592
180. Lemke G, Lu Q (2003) Macrophage regulation by Tyro 3 family receptors. Curr Opin Immunol 15(1):31–36 S095279150200016X[pii]
181. Binder MD, Kilpatrick TJ (2009) TAM receptor signalling and demyelination. Neurosignals 17(4):277–287. doi:10.1159/000231894
182. Cohen PL, Caricchio R, Abraham V, Camenisch TD, Jennette JC, Roubey RA, Earp HS, Matsushima G, Reap EA (2002) Delayed apoptotic cell clearance and lupus-like autoimmunity in mice lacking the c-mer membrane tyrosine kinase. J Exp Med 196(1):135–140
183. Rothlin CV, Lemke G (2010) TAM receptor signaling and autoimmune disease. Curr Opin Immunol 22(6):740–746. doi: 10.1016/j.coi.2010.10.001
184. Scott RS, McMahon EJ, Pop SM, Reap EA, Caricchio R, Cohen PL, Earp HS, Matsushima GK (2001) Phagocytosis and clearance of apoptotic cells is mediated by MER. Nature 411(6834):207–211. doi:10.1038/35075603
185. Todt JC, Hu B, Curtis JL (2004) The receptor tyrosine kinase MerTK activates phospholipase C gamma2 during recognition of apoptotic thymocytes by murine macrophages. J Leukoc Biol 75(4):705–713. doi:10.1189/jlb.0903439
186. Ostergaard E, Duno M, Batbayli M, Vilhelmsen K, Rosenberg T (2011) A novel MERTK deletion is a common founder mutation in the Faroe Islands and is responsible for a high proportion of retinitis pigmentosa cases. Mol Vis 17:1485–1492
187. Wu YM, Robinson DR, Kung HJ (2004) Signal pathways in up-regulation of chemokines by tyrosine kinase MER/NYK in prostate cancer cells. Cancer Res 64(20):7311–7320. doi: 10.1158/0008-5472.CAN-04-0972
188. Gyorffy B, Lage H (2007) A web-based data warehouse on gene expression in human malignant melanoma. J Invest Dermatol 127(2):394–399. doi:10.1038/sj.jid.5700543
189. Stitt TN, Conn G, Gore M, Lai C, Bruno J, Radziejewski C, Mattsson K, Fisher J, Gies DR, Jones PF et al (1995) The anticoagulation factor protein S and its relative, Gas6, are ligands for the Tyro 3/Axl family of receptor tyrosine kinases. Cell 80(4):661–670 0092-8674(95)90520-0[pii]
190. Varnum BC, Young C, Elliott G, Garcia A, Bartley TD, Fridell YW, Hunt RW, Trail G, Clogston C, Toso RJ et al (1995) Axl receptor tyrosine kinase stimulated by the vitamin K-dependent protein encoded by growth-arrest-specific gene 6. Nature 373(6515):623–626. doi:10.1038/373623a0
191. Nagata K, Ohashi K, Nakano T, Arita H, Zong C, Hanafusa H, Mizuno K (1996) Identification of the product of growth arrest-specific gene 6 as a common ligand for Axl, Sky, and Mer receptor tyrosine kinases. J Biol Chem 271(47):30022–30027
192. Godowski PJ, Mark MR, Chen J, Sadick MD, Raab H, Hammonds RG (1995) Reevaluation of the roles of protein S and Gas6 as ligands for the receptor tyrosine kinase Rse/Tyro 3. Cell 82(3):355–358 0092-8674(95)90424-7[pii]
193. Mark MR, Chen J, Hammonds RG, Sadick M, Godowsk PJ (1996) Characterization of Gas6, a member of the superfamily

of G domain-containing proteins, as a ligand for Rse and Axl. J Biol Chem 271(16):9785–9789
194. Prasad D, Rothlin CV, Burrola P, Burstyn-Cohen T, Lu Q, Garcia de Frutos P, Lemke G (2006) TAM receptor function in the retinal pigment epithelium. Mol Cell Neurosci 33(1):96–108. doi:10.1016/j.mcn.2006.06.011
195. Schneider C, King RM, Philipson L (1988) Genes specifically expressed at growth arrest of mammalian cells. Cell 54(6): 787–793 S0092-8674(88)91065-3[pii]
196. Dahlback B (1991) Protein S and C4b-binding protein: components involved in the regulation of the protein C anticoagulant system. Thromb Haemost 66(1):49–61
197. Manfioletti G, Brancolini C, Avanzi G, Schneider C (1993) The protein encoded by a growth arrest-specific gene (gas6) is a new member of the vitamin K-dependent proteins related to protein S, a negative coregulator in the blood coagulation cascade. Mol Cell Biol 13(8):4976–4985
198. Sather S, Kenyon KD, Lefkowitz JB, Liang X, Varnum BC, Henson PM, Graham DK (2007) A soluble form of the Mer receptor tyrosine kinase inhibits macrophage clearance of apoptotic cells and platelet aggregation. Blood 109(3):1026–1033. doi:10.1182/blood-2006-05-021634
199. Angelillo-Scherrer A, Burnier L, Lambrechts D, Fish RJ, Tjwa M, Plaisance S, Sugamele R, DeMol M, Martinez-Soria E, Maxwell PH, Lemke G, Goff SP, Matsushima GK, Earp HS, Chanson M, Collen D, Izui S, Schapira M, Conway EM, Carmeliet P (2008) Role of Gas6 in erythropoiesis and anemia in mice. J Clin Invest 118(2):583–596. doi:10.1172/JCI30375
200. Tjwa M, Bellido-Martin L, Lin Y, Lutgens E, Plaisance S, Bono F, Delesque-Touchard N, Herve C, Moura R, Billiau AD, Aparicio C, Levi M, Daemen M, Dewerchin M, Lupu F, Arnout J, Herbert JM, Waer M, Garcia de Frutos P, Dahlback B, Carmeliet P, Hoylaerts MF, Moons L (2008) Gas6 promotes inflammation by enhancing interactions between endothelial cells, platelets, and leukocytes. Blood 111(8):4096–4105. doi:10.1182/blood-2007-05-089565
201. Lutgens E, Tjwa M, Garcia de Frutos P, Wijnands E, Beckers L, Dahlback B, Daemen MJ, Carmeliet P, Moons L (2008) Genetic loss of Gas6 induces plaque stability in experimental atherosclerosis. J Pathol 216(1):55–63. doi:10.1002/path.2381
202. Burnier L, Saller F, Kadi L, Brisset AC, Sugamele R, Baudino L, Bono F, Herbert JM, Carmeliet P, Schapira M, Izui S, Angelillo-Scherrer A (2011) Gas6 deficiency in recipient mice of allogeneic transplantation alleviates hepatic graft-versus-host disease. Blood 115(16):3390–3397. doi:10.1182/blood-2009-02-206920
203. Llacuna L, Barcena C, Bellido-Martin L, Fernandez L, Stefanovic M, Mari M, Garcia-Ruiz C, Fernandez-Checa JC, Garcia de Frutos P, Morales A (2010) Growth arrest-specific protein 6 is hepatoprotective against murine ischemia/reperfusion injury. Hepatology 52(4):1371–1379. doi:10.1002/hep.23833
204. Yin JL, Pilmore HL, Yan YQ, McCaughan GW, Bishop GA, Hambly BD, Eris JM (2002) Expression of growth arrest-specific gene 6 and its receptors in a rat model of chronic renal transplant rejection. Transplantation 73(4):657–660
205. Yin JL, Hambly BD, Bao SS, Painter D, Bishop GA, Eris JM (2003) Expression of growth arrest-specific gene 6 and its receptors in dysfunctional human renal allografts. Transpl Int 16(9):681–688. doi:10.1007/s00147-003-0593-3
206. Loges S, Schmidt T, Tjwa M, van Geyte K, Lievens D, Lutgens E, Vanhoutte D, Borgel D, Plaisance S, Hoylaerts M, Luttun A, Dewerchin M, Jonckx B, Carmeliet P (2010) Malignant cells fuel tumor growth by educating infiltrating leukocytes to produce the mitogen Gas6. Blood 115(11):2264–2273. doi:10.1182/blood-2009-06-228684
207. Goruppi S, Chiaruttini C, Ruaro ME, Varnum B, Schneider C (2001) Gas6 induces growth, beta-catenin stabilization, and T-cell factor transcriptional activation in contact-inhibited C57 mammary cells. Mol Cell Biol 21(3):902–915. doi:10.1128/MCB.21.3.902-915.2001
208. Sainaghi PP, Castello L, Bergamasco L, Galletti M, Bellosta P, Avanzi GC (2005) Gas6 induces proliferation in prostate carcinoma cell lines expressing the Axl receptor. J Cell Physiol 204(1):36–44. doi:10.1002/jcp.20265
209. van Ginkel PR, Gee RL, Shearer RL, Subramanian L, Walker TM, Albert DM, Meisner LF, Varnum BC, Polans AS (2004) Expression of the receptor tyrosine kinase Axl promotes ocular melanoma cell survival. Cancer Res 64(1):128–134
210. Koessler W, Fiebeler A, Willms A, Elaidi T, Klosterhalfen B, Klinge U (2011) Formation of translational risk score based on correlation coefficients as an alternative to Cox regression models for predicting outcome in patients with NSCLC. Theor Biol Med Model 8(1):28. doi:10.1186/1742-4682-8-28
211. Smiley ST, Boyer SN, Heeb MJ, Griffin JH, Grusby MJ (1997) Protein S is inducible by interleukin 4 in T cells and inhibits lymphoid cell procoagulant activity. Proc Natl Acad Sci U S A 94(21):11484–11489
212. Caberoy NB, Zhou Y, Li W (2010) Tubby and tubby-like protein 1 are new MerTK ligands for phagocytosis. EMBO J 29(23):3898–3910. doi:10.1038/emboj.2010.265
213. Li W (2011) Eat-me signals: keys to molecular phagocyte biology and "appetite" control. J Cell Physiol. doi:10.1002/jcp.22815
214. Holland SJ, Pan A, Franci C, Hu Y, Chang B, Li W, Duan M, Torneros A, Yu J, Heckrodt TJ, Zhang J, Ding P, Apatira A, Chua J, Brandt R, Pine P, Goff D, Singh R, Payan DG, Hitoshi Y (2010) R428, a selective small molecule inhibitor of Axl kinase, blocks tumor spread and prolongs survival in models of metastatic breast cancer. Cancer Res 70(4):1544–1554. doi:10.1158/0008-5472.CAN-09-2997
215. Gustafsson A, Martuszewska D, Johansson M, Ekman C, Hafizi S, Ljungberg B, Dahlback B (2009) Differential expression of Axl and Gas6 in renal cell carcinoma reflecting tumor advancement and survival. Clin Cancer Res 15(14):4742–4749. doi:10.1158/1078-0432.CCR-08-2514
216. Neubauer A, Fiebeler A, Graham DK, O'Bryan JP, Schmidt CA, Barckow P, Serke S, Siegert W, Snodgrass HR, Huhn D et al (1994) Expression of axl, a transforming receptor tyrosine kinase, in normal and malignant hematopoiesis. Blood 84(6): 1931–1941
217. Challier C, Uphoff CC, Janssen JW, Drexler HG (1996) Differential expression of the ufo/axl oncogene in human leukemia-lymphoma cell lines. Leukemia 10(5):781–787
218. Hong CC, Lay JD, Huang JS, Cheng AL, Tang JL, Lin MT, Lai GM, Chuang SE (2008) Receptor tyrosine kinase AXL is induced by chemotherapy drugs and overexpression of AXL confers drug resistance in acute myeloid leukemia. Cancer Lett 268(2):314–324. doi:10.1016/j.canlet.2008.04.017
219. Rochlitz C, Lohri A, Bacchi M, Schmidt M, Nagel S, Fopp M, Fey MF, Herrmann R, Neubauer A (1999) Axl expression is associated with adverse prognosis and with expression of Bcl-2 and CD34 in de novo acute myeloid leukemia (AML): results from a multicenter trial of the Swiss Group for Clinical Cancer Research (SAKK). Leukemia 13(9):1352–1358
220. Grosso S, Puissant A, Dufies M, Colosetti P, Jacquel A, Lebrigand K, Barbry P, Deckert M, Cassuto JP, Mari B, Auberger P (2009) Gene expression profiling of imatinib and PD166326-resistant CML cell lines identifies Fyn as a gene associated with resistance to BCR-ABL inhibitors. Mol Cancer Ther 8(7): 1924–1933. doi:10.1158/1535-7163.MCT-09-0168

221. Gioia R, Leroy C, Drullion C, Lagarde V, Etienne G, Dulucq S, Lippert E, Roche S, Mahon FX, Pasquet JM (2011) Quantitative phosphoproteomics revealed interplay between Syk and Lyn in the resistance to nilotinib in chronic myeloid leukemia cells. Blood 118(8):2211–2221. doi:10.1182/blood-2010-10-313692
222. Ghosh AK, Secreto C, Boysen J, Sassoon T, Shanafelt TD, Mukhopadhyay D, Kay NE (2011) The novel receptor tyrosine kinase Axl is constitutively active in B-cell chronic lymphocytic leukemia and acts as a docking site of nonreceptor kinases: implications for therapy. Blood 117(6):1928–1937. doi:10.1182/blood-2010-09-305649
223. Graham DK, Salzberg DB, Kurtzberg J, Sather S, Matsushima GK, Keating AK, Liang X, Lovell MA, Williams SA, Dawson TL, Schell MJ, Anwar AA, Snodgrass HR, Earp HS (2006) Ectopic expression of the proto-oncogene Mer in pediatric T-cell acute lymphoblastic leukemia. Clin Cancer Res 12(9):2662–2669. doi:10.1158/1078-0432.CCR-05-2208
224. Linger RM, DeRyckere D, Brandao L, Sawczyn KK, Jacobsen KM, Liang X, Keating AK, Graham DK (2009) Mer receptor tyrosine kinase is a novel therapeutic target in pediatric B-cell acute lymphoblastic leukemia. Blood 114(13):2678–2687. doi:10.1182/blood-2009-03-209247
225. Shiozawa Y, Pedersen EA, Taichman RS (2010) GAS6/Mer axis regulates the homing and survival of the E2A/PBX1-positive B-cell precursor acute lymphoblastic leukemia in the bone marrow niche. Exp Hematol 38(2):132–140. doi:10.1016/j.exphem.2009.11.002
226. Dai W, Pan HQ, Ouyang B, Greenberg JM, Means RT Jr, Li B, Cardie J (1996) Expression of receptor protein tyrosine kinase tif is regulated during leukemia cell differentiation. Leukemia 10(6):978–983