From DEPARTMENT OF ONCOLOGY-PATHOLOGY
Karolinska Institutet, Stockholm, Sweden

Exploring mechanisms regulating the heterogeneity of Tumor-Associated Macrophages

Majken Wallerius

Karolinska Institutet

Stockholm 2018

Published by Karolinska Institutet.
Printed by E-Print AB 2018

ISBN 978-91-7831-011-1

Exploring mechanisms regulating the heterogeneity of Tumor-Associated Macrophages

THESIS FOR DOCTORAL DEGREE (Ph.D.)

Cancer Center Karolinska (CCK) Lecture Hall, R8:00, Karolinska University Hospital, Stockholm

Friday, May 25th, 2018 at 09.15

By

Majken Wallerius

Principal Supervisor:
Associate professor Charlotte Rolny
Karolinska Institutet
Department of Oncology-Pathology

Co-supervisor(s):
Associate professor Andreas Lundqvist
Karolinska Institutet
Department of Oncology-Pathology

Professor Arne Östman
Karolinska Institutet
Department of Oncology-Pathology

Opponent:
Professor Jo Van Ginderachter
Vrije Universiteit Brussel
Department of Bio-engineering Sciences
Division of Cellular and Molecular Immunology, Myeloid Cell Immunology

Examination Board:
Associate professor Anna-Karin Olsson
Uppsala University
Department of Medical Biochemistry and Microbiology

Associate professor Kaisa Lehti
Karolinska Institutet
Department of Microbiology, Tumor and Cell Biology

Professor Teresa Frisan
Umeå University
Department of Molecular Biology

ABSTRACT

Accumulation of macrophages in the tumor microenvironment is associated to poor prognoses in most human cancers. Tumor-associated macrophages (TAMs) represent a heterogeneous and plastic population of cells that contribute to tumor growth, metastatic dissemination, angiogenesis, and immune suppression. Both recruitment of monocytes from the blood and *in situ* proliferation contribute to the accumulation of TAMs. During the course of tumor progression, the phenotype of the infiltrating TAMs is changing by influences from other cells, extracellular signal molecules and availability of oxygen and nutrients. Therapeutic targeting of TAMs as a monotherapy has limited success, however, experimental studies show promising results when selectively depleting specific subsets of TAMs or changing the function of the TAM population. Yet, the mechanisms whereby macrophage phenotypes are regulated during tumor growth are still largely unknown.

In this thesis, we investigated mechanisms underlying the dynamic changes in the TAM population observed during tumor growth. In our first study, overexpression of semaphorin 3A induced the proliferation of anti-tumoral macrophages and at the same time reduced the proliferation of pro-tumoral TAMs resulting in accumulation and activation of $CD8^+$ T-cells and NK-cells and restricted tumor growth. In study II, we identified translational regulation of gene expression as an important mechanism regulating the TAM phenotype during tumor growth. By selective inhibition of translational activation, pro-tumoral macrophages were skewed towards an anti-tumoral phenotype. In the third study, we demonstrated a functional difference between macrophages of different ontogeny in a mouse model of glioblastoma. M2-polarized microglia, but not bone-marrow derived macrophages induced the expression of platelet-derived growth factor receptor B (PDGFRB) in glioma cells, enhancing their migratory capacity. In study IV, we showed that Zoledronic acid in combination with interleukin-2 induced the expression of interferon-γ by monocytes leading to an up-regulation of TNF-related apoptosis-inducing ligand (TRAIL) on NK-cells, inducing their cytotoxicity against tumor cells.

In summary, we describe several mechanisms whereby the TAM phenotype may be regulated i.e translational control of gene expression, regulation of proliferation and ontogeny.

LIST OF SCIENTIFIC PAPERS

I. Wallerius M*, Wallmann T*, Bartish M, Östling J, Mezheyeuski A, Tobin NP, Nygren E, Pangigadde P, Pellegrini P, Squadrito ML, Pontén F, Hartman J, Bergh J, De Milito A1, De Palma M, Östman A, Andersson J, Rolny C. **Guidance Molecule SEMA3A Restricts Tumor Growth by Differentially Regulating the Proliferation of Tumor-Associated Macrophages**
Cancer Res. 2016 Jun 1;76(11):3166-78

II. Bartish M*, Wallerius M*, Wallmann T, Masvidal L, Liu H, Joly AL, van Hoef V, Bergh J, Hartman J, Andersson J, Rolny C#, Larsson O#.
RNA Translational Control of the Tumor-Associated Macrophage Phenotype
Manuscript

III. Wallmann T, Zhang XM, Wallerius M, Bolin S, Joly AL, Sobocki C, Leiss L, Jiang Y, Bergh J, Andersson J, Holland E, Enger P, Swartling F, Uhrbom L, Miletic H, Harris R, Rolny, C.
Pro-Angiogenic microglia-induced expression of platelet-derived growth factor receptor beta in glioma cells promotes their migratory capacity
Submitted

IV. Sarhan D, D'Arcy P, Wennerberg E, Lidén M, Hu J, Winqvist O, Rolny C, Lundqvist A.
Activated monocytes augment TRAIL-mediated cytotoxicity by human NK cells through release of IFN-γ
Eur J Immunol. 2013 Jan;43(1):249-57

ADDITIONAL PUBLICATIONS
Not included in this thesis

Sarhan D, Leijonhufvud C, Murray S, Witt K, Seitz C, Wallerius M, Xie H, Ullén A, Hamenberg U, Lidbrink E, Rolny C, Andersson J, Lundqvist A.
Zoledronic acid inhibits NFAT and IL-2 signaling pathways in regulatory T cells and diminishes their suppressive function in patients with metastatic cancer
Oncoimmunology. 2017 Jun 14;6(8):e1338238

Christoffersson G, Vågesjö E, Vandooren J, Lidén M, Massena S, Reinert RB, Brissova M, Powers AC, Opdenakker G, Phillipson M.
VEGF-A recruits a proangiogenic MMP-9-delivering neutrophil subset that induces angiogenesis in transplanted hypoxic tissue
Blood. 2012 Nov 29;120(23)4653-62

CONTENTS

LIST OF ABBREVIATIONS

4E-BP	4E binding protein
bFGF	basic fibroblast growth factor
BM	Bone marrow
BMDM	Bone marrow-derived macrophage
CBR	Carbonyl reductas
CCL	Chemokine (C-C motif) ligand
CCR	Chemokine (C-C motif) receptor
CNS	Central nervous system
CSF	Colony stimulating factor
CTL	Cytotoxic T lymphocytes
CX3CR1	CX3C chemokine receptor 1
CXCL	Chemokine (C-X-C motif) ligands
EGF	Epidermal growth factor
eIF	Eukaryotic initiation factor
FcγR	Fcγ receptor
GBM	Glioblastoma multiforme
GO	Gene-ontology
HIF	Hypoxia-inducible factor
hnRNP	Heterogeneous nuclear ribonucleoprotein
IFN	Interferon
IL	Interleukin
IPA	Ingenuity Pathway Analysis
IRF	Interferon regulatory factor
IκBα	NF-κB inhibitor NFKBIA
LPS	Lipopolysaccharides
MAPK	Mitogen-activated protein kinase
MDSC	Myeloid-derived suppressor cells
MEF	Mouse embryonic fibroblasts
Met-tRNAi	Methionyl initiator transfer RNA
MHC	Major histocompatibility complex
MMP	Matrix metalloproteinases
MMTV	Mouse Mammary Tumor Virus
MNK	MAPK interacting protein kinase

MRC	Mannose receptor C-type
mRNA	Messenger RNA
NK	Natural killer
NP1	Neuropilin 1
PBMC	Peripheral blood mononuclear cell
PD-1	Programmed cell death-1
PD-L1	Programmed death - ligand 1
PDAC	Pancreatic ductal adenocarcinoma
PDGF	Platelet-derived growth factor
PDGFR	Platelet-derived growth factor receptor
PHD	Prolylhydroxylase domain
PIC	Pre-initiation complex
PyMT	Polyoma middle T antigen
ROS	Reactive oxygen speices
SEMA	Semaphorin
shRNA	Short hairpin RNA
TAK	Transforming growth factor beta-activated kinase
TAM	Tumor-associated macrophage
TAMM	Tumor-associated macrophages and microglia
TEM	Tie2-expressing monocyte
TGF	Transforming growth factor
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TRAIL	TNF-related apoptosis-inducing ligand
uPA	Urokinasetype plasminogen activator
VEGF	Vascular endothelial growth factor
ZA	Zoledronic acid

1 GENERAL BACKGROUND

1.1 CANCER

Cancer is one of the leading causes of morbidity and mortality worldwide, and accounted for 8.8 million deaths in 2015 (3). The disease is characterized by abnormal cell growth, ability to invade surrounding tissues and spread to distant sites. The transformation of normal cells to cancer cells is a multistep process where the cells acquire capabilities to proliferate, survive and disseminate. In the review "Hallmarks of cancer: The next generation" Hanahan and Weinberg propose 10 hallmarks that drive tumorigenesis (**Figure 1**). Importantly, those hallmarks do not only involve the tumor-cells themselves, but also tumor-associated stromal cells, including immune cells, as active participants in tumorigenesis (2).

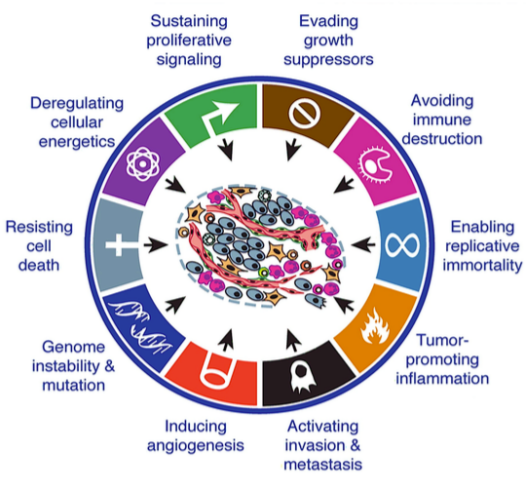

Figure 1. Hallmarks of cancer. Adopted and modified from (2).

1.2 TUMOR MICROENVIRONMENT

The development and progression of tumors is supported by a heterogeneous population of stromal cells and secreted factors. The tumor's ability to avoid immune destruction, induce angiogenesis and promote inflammation are all hallmarks of tumor development.

Tumor angiogenesis

Growing tumors induce angiogenesis for an adequate blood supply to satisfy their demand of oxygen and nutrients, as well as removing carbon dioxide and metabolic waste. The "angiogenic switch", where the local balance of pro-angiogenic and anti-angiogenic factors

tilts towards a pro-angiogenic outcome, typically occurs when tumors reach the size of $1mm^3$ (4) and involves infiltration of myeloid cells (5, 6). At this point, new blood vessels develop from the pre-existing vasculature. Environmental stress, such as hypoxia, glucose deprivation or formation of reactive oxygen species (ROS) induces the production of pro-angiogenic factors, in particular vascular endothelial growth factor (VEGF)-A. This factor is secreted from both the tumor cells themselves and from tumor-infiltrating inflammatory cells (7). In addition, activation of oncogenes or the loss of the function of tumor suppressor genes can also induce the production of pro-angiogenic factors (8). The overexpression of pro-angiogenic factors creates an abnormal vasculature that displays features of discontinuous endothelial cell lining, defective basement membrane and pericyte coverage. The disorganized vascular network has increased vessel leakage and poor perfusion resulting in poor oxygenation of the tumor (7). "Normalization" of the abnormal structure and function of tumor vasculature to make it more efficient for oxygen and drug delivery therefore represents an attractive anti-cancer strategy (9).

Tumor immunity

It is thought that the initial immune response to an early neoplasm mirrors the response to acute tissue injury where inflammatory cells respond to disruption of tissue homeostasis. Locally secreted soluble factors recruit additional inflammatory cells from the circulation. If the immune cells do not eradicate the early neoplasm, the local microenvironment will be altered, and a state of chronic inflammation that fosters cancer development is established in the tissue (10). However, following tumor initiation, the pro-inflammatory milieu is progressively reversed to silence the immunity against cancer cells. Tumors typically recruit a set of immune regulatory and suppressive cells including $CD4^+$ regulatory T cells, myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs). The recruited immune cells, together with the tumor cells themselves, secret anti-inflammatory factors including transforming growth factor (TGF)-β, and interleukin (IL)-10 that negatively regulate the activity of cytotoxic cells i.e. natural killer (NK) cells and $CD8^+$ T cells, thereby supporting tumor growth.

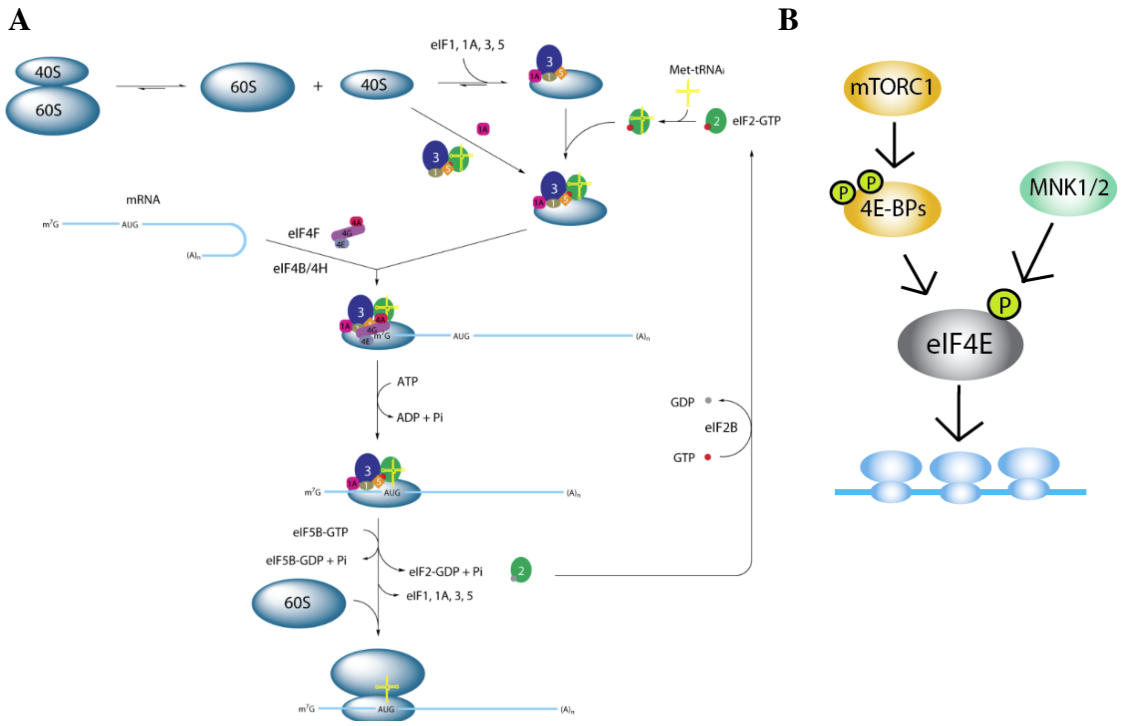

Figure 2. Initiation and regulation of mRNA translation. **(A)** Translational initiation is a multistep pathway where the two subunits of the ribosome assemble near the 5' end of the mRNA. Several eIFs are involved in the initiation process and much of the regulation is controlled via the activity of eIF4E. **(B)** eIF4E is regulated by two distinct pathways; activation of the mTOR pathway induces the release of eIF4E from 4E-BPs making it available for initiating translation. In the second pathway, the kinases MNK1 and MNK2 are activated in response to mitogens and phosphorylate eIF4E to increase its activity.

1.3 mRNA ABUNDANCE AND RNA TRANSLATIONAL REGULATION

Cell phenotypes largely arise via distinct patterns of gene expression. Measurements of the abundance of messenger RNA (mRNA) is often used to describe gene expression, however, the direct correlation to protein levels are often poor (11). The final expression of the protein is regulated at several post-transcriptional steps including splicing, export of mRNAs to the cytosol, regulation of the translation of mRNA, and post-translational regulations such as protein degradation. The relative contribution of transcriptional, translational and post-translational regulation remains unknown and will probably depend on the time from stimulation as well as the cell-type and its context. Dynamic regulation of mRNA translation is central for the immune system (12), cell proliferation (13), and diseases including fibrosis (14) and cancer (13). Under these contexts, mRNA translation is selectively modulated to alter the synthesis of subsets of proteins, despite of constant mRNA levels.

Translation can be separated into four steps: initiation, elongation, termination and ribosome recycling, where most of the regulation occurs at the initiation step (15). Translational initiation is a multistep pathway where the 80S ribosome is assembled at the start codon near the 5'end of the mRNA (**Figure 2A**). The small 40S ribosomal subunit loaded with the anticodon of methionyl initiator transfer RNA (Met-tRNAi) and eukaryotic initiation factors (eIFs) including eIF1, 1A, 2, 3 and 5 form the pre-initiation complex (PIC) (16). PIC is recruited to the m^7G cap structures at the mRNA by the cap-binding factor eIF4E and its partners, eIF4G and eIF4A, in the eIF4F complex. The large (60S) ribosome subunit joins to form an 80S initiation complex and a protein can be synthesized (16).

The rate limiting step in initiation of translation involves recruitment of PIC to the 5'cap structure of the mRNA (16). eIF4E directs the ribosomes to the cap structure and is therefore essential in translational regulation. eIF4E activity can be regulated by two distinct mechanisms i.e via mTOR signaling or direct phosphorylation by mitogen-activated protein kinase (MAPK) interacting protein kinase (MNK) 1 and 2 (**Figure 2B**). The first pathway involves mTOR phosphorylation of 4E binding proteins (4E-BPs) leading to their dissociation from eIF4E enabling assembly of the eIF4F complex. The mTOR pathway may be activated by several factors including growth factors and hormones but can also be inhibited by hypoxia (17). In the second mechanism, eIF4E activity is modulated via direct phosphorylation of Ser209. MNK1 and MNK2 are the sole known kinases to phosphorylate eIF4E in mice (18). MNK2 accounts for constitutive phosphorylation, and MNK1 is regulated by signaling cascades of the MAPKs p38 and ERK in response to mitogens (19). In addition to regulation of eIF4E, translation may also be regulated by the activity of other eIFs and other RNA-binding proteins (12).

2 TUMOR-ASSOCIATED MACROPHAGES

Macrophages are found in all tissues and populate the microenvironment of most cancers (20). For instance, in breast cancer, macrophages can represent more than 50% of the tumor mass (21). Cells from the monocyte-macrophage lineage are highly plastic, and even though macrophages from non-pathological tissues possess anti-tumoral activities, most TAMs lack these properties. Furthermore, an increased density of macrophages correlates with poor prognoses in most human cancers (22). TAMs contribute to a local immune-suppression, facilitate angiogenesis, invasion, and metastasis by direct interactions and by

supply of bioactive molecules to the tumor microenvironment (23). Although the infiltration of macrophages is well established as a contributor to tumor development, the mechanisms for the dynamic change of the population during tumor growth is not well understood.

2.1 MACROPHAGE ORIGIN AND MAINTENANCE DURING HOMEOSTASIS

During development, first the yolk sac, and later hematopoiesis from the fetal liver gives rise to macrophages. After birth, the hematopoiesis from the liver is replaced by bone marrow (BM) hematopoiesis that together with local proliferation of tissue resident macrophages maintain homeostasis during adulthood. Although all tissues are populated by fetal macrophages at birth, each organ dictates the degree to which circulating monocytes replace resident macrophages under homeostasis (24). The degree of replacement spans from no replacement to that all macrophages are replaced a few months after birth. For example, in the brain, all macrophages, called microglia, are derived from yolk sack progenitors (25) and the maintenance during homeostasis is thought to occur through prolonged cellular longevity and local proliferation (26). In contrast, organs such as the intestine and the dermis have a fast replacement of the embryonic-derived macrophages by recruited BM-derived monocytes that are differentiated into macrophages within the tissue (27, 28).

2.2 TAM PHENOTYPES

M1- and M2 macrophages

TAMs consist of a heterogeneous population of macrophages that can generally be classified into two extremes: anti-tumoral M1-macrophages and pro-tumoral M2-macrophages. M1-macrophages, also called classically activated macrophages, are pro-inflammatory and anti-tumoral. Macrophages exposed to interferon (IFN)-γ, for instance from T_H1 T-cells (29), or lipopolysaccharides (LPS) typically become M1-like (30). On the other hand, macrophages exposed to the T_H2 cytokines IL-4 (31) and IL-13 (32) become M2- or alternatively activated macrophages that are anti-inflammatory, pro-angiogenic and pro-tumoral. Other factors such as B cell-mediated activation of Fcγ receptors (FcγR) expressed on TAMs (33) and hypoxia (34) also skews or fine-tunes the M2-macrophage phenotype.

Of note, the classification of macrophages into M1- and M2-phenotypes is a simplification of the *in vivo* situation. The microenvironment is often complex and numerous cytokines,

growth factors and physiological conditions interact with the macrophages to define their final differentiated state. The scientific community have attempted to agree on a unified definition and nomenclature around macrophage subtypes and most researchers agree to that one need to investigate the phenotype of macrophages in several ways such as cytokine gene expression, surface markers and function. For instance, Murray *et al* (30) describe a set of standards including three principles to describe macrophage activation; these are the source of the macrophage, definition of the activators, and a consensus collection of markers (30). Typical markers for murine M1-macrophages include the expression of pro-inflammatory cytokines and chemokines including tumor necrosis factor (TNF)-α, IL-12a, IFN-γ and chemokine (C-X-C motif) ligands (CXCL) 9 and 10 (30) and cell surface markers such as $CD11c^{+}$mannose receptor C-type (MRC) $1^{low/-}$ (35) or high levels of major histocompatibility complex (MHC) class II (36). M2-macrophages typically have high cell surface expression of the scavenger receptor MRC1 (35), and low levels of MHC class II (36), and express anti-inflammatory cytokines such as IL-10 (30).

TAM phenotypes during tumor growth

During the course of tumor growth, the tumor microenvironment is dynamically changing in regard to the composition of cells, extracellular signal molecules and availability of oxygen and nutrients, factors that are all contributing to the phenotype of TAMs (**Figure 4**). In fact, single cell sequencing analyses from our lab (unpublished data) showed that based on transcription only, the TAMs can be divided into nine different subgroups that are dynamically changed during tumor growth (**Figure 3**). Knowing that the transcriptional pattern is only a part of what defines the phenotype, we believe that these 9 subgroups are only a fraction of the existing subtypes.

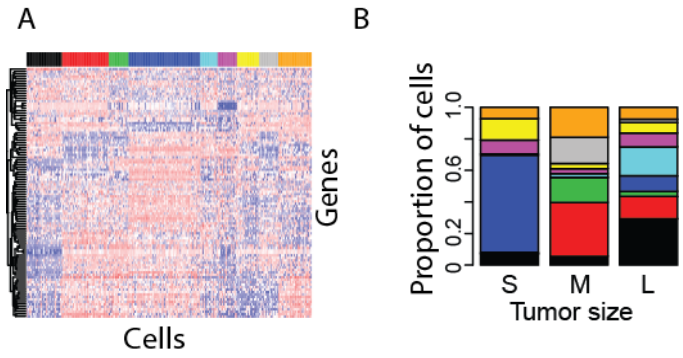

Figure 3. Single cell RNAseq reveals dynamic changes in macrophage subsets during tumor growth. **(A)** Genes with the most variable expression across single macrophages isolated from small (S), medium (M) or large (L) tumors were used to identify 9 macrophage subsets (indicated by colors). **(B)** The proportion of macrophages from each subset varied depending on the size of the tumor (colors correspond to subsets in **(A)**).

During tumor initiation, inflammatory macrophages contribute to chronic inflammation by the production of cytotoxic molecules such as reactive oxygen intermediates that create tissue and DNA damage contributing transformation of cells (37). Further, production of inflammatory cytokines like TNF-α and IL-1 provide the transformed cells with pro-survival signals supporting tumorigenesis (38).

During the course of tumor progression, TAMs are accumulating in tumors by recruitment and/or *in situ* proliferation (described below) (39). The accumulated TAMs are subjected to differential challenges depending on their location within the tumor, and their phenotype will differ accordingly. For instance, in endometrial cancer, the accumulation of TAMs in different histological areas (i.e areas with high density of cancer cells, necrotic areas and the stroma) correlates to differential outcome in the patients. Hence, accumulation of TAMs in cancer cell nests or in close contact with cancer cells correlates to a high relapse-free survival rate after surgery, whereas accumulation of TAMs in necrotic areas correlates to disease progression and finally infiltration of TAMs in the stroma correlates to lymph node metastasis (40). In sum, these data indicate that the function of TAMs may be influenced by the particular microenvironment in which they reside. In corroboration, TAMs are often found in poorly vascularized areas of tumors and hypoxia is suggested to influence the phenotype of TAMs (**Figure 4**). Hypoxia induces an upregulation of transcription factors including hypoxia-inducible factors (HIFs) 1 and 2 (41, 42) that regulate the expression of a broad array of genes. *In vitro*, human monocyte-derived macrophages subjected to hypoxia display an up-regulation of a wide panel of pro-tumoral genes such as VEGF-A, TNF-α and Angiopoietin-2, which supports tumor growth (43). By using prolylhydroxylase domain (PHD) 2-haplodeficient mice that have better vascularized tumors, Laoui *et al* studied the effect of hypoxia on TAMs in mouse lung carcinoma. Interestingly, the relative abundance of MHC class II^{low} (M2-like) and MHC class II^{high} (M1-like) macrophages do not change with reduced tumor hypoxia. Instead, the expression of hypoxia-sensitive genes and the angiogenic activity of MHC class II^{low} TAMs were lowered suggesting that hypoxia does not drive the differentiation of TAMs but rather fine-tunes the M2-like TAM population (44). However, in glioma, hypoxia is found to promote the recruitment of macrophages as well as polarize them toward an M2-phenotype (45). Hence, hypoxia in different tumor types may dictate the TAM phenotype differently.

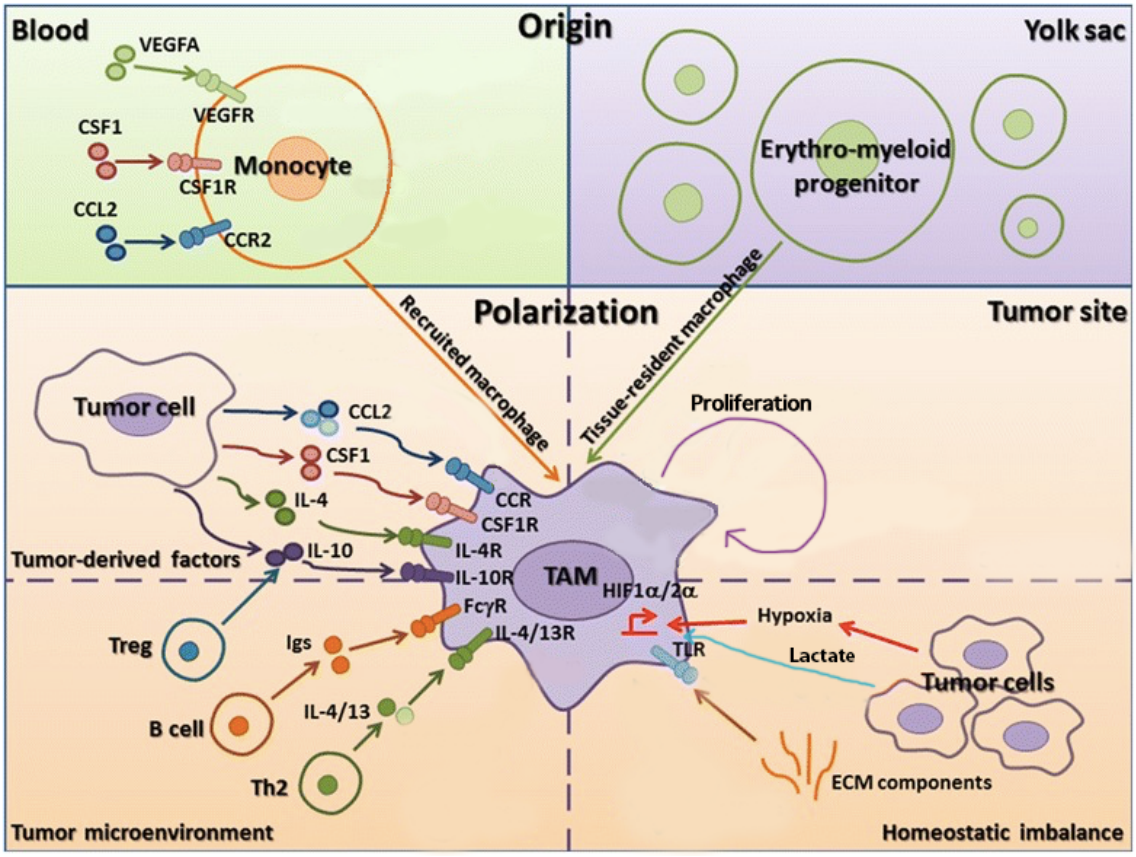

Figure 4. TAM accumulation and polarization. TAMs accumulate at the tumor site via recruitment and/or proliferation. Their phenotype is dependent on signals they receive from the local microenvironment they reside in. Figure is adapted and modified from (1)

In addition to being subjected to hypoxia, macrophages also receive signals from various cells in tumor (**Figure 4**). For example, Colegio and co-authors identify lactic acid as a link of communication between tumor cells and TAMs. Lactic acid induces the expression of the M2-markers *vegf* and *arginase1* in TAMs via the same mechanism as hypoxia, i.e. stabilization of HIF-1α (46). Another example is IL-4, one of the classical drivers of M2-polarization, which is secreted by both tumor cells and T_H2 polarized $CD4^+$ cells (31, 47). In pancreatic tumors, IL-4 is mainly secreted by tumor cells and induce the activity of cathepsin protease in TAMs, promoting pancreatic tumor growth, angiogenesis, and invasion *in vivo* (47). In concordance, in a spontaneous mouse model of mammary adenocarcinoma, where expression of the oncoprotein, polyoma middle T (PyMT) antigen from mouse polyoma virus, is under the control of the mouse mammary tumor virus (MMTV) promoter, IL-4 secreted by $CD4^+$ T effector lymphocytes skews TAMs towards an M2-phenotype and thereby promotes carcinoma invasion (31). Additionally, B cells can influence the activity of TAMs. In fact, macrophages deficient in FcγR display an up-regulation of classical M1-genes whereas M2-genes are downregulated, and co-

transplantation of squamous cell carcinoma PDSC5 cells with BM-derived FcRγ$^{-/-}$ macrophages does not only fail to promote tumor development, but also impedes tumor growth (33).

TAM recruitment during tumor progression

During tumor development, stromal and tumor cells produce monocyte chemoattractants such as chemokine (C-C motif) ligand (CCL) 2 and colony stimulating factor (CSF)-1, that recruit monocytes derived from the BM from the bloodstream to the peripheral tissue where they differentiate into macrophages (20).

Murine monocytes are typically divided into two subsets based on their expression of Ly6C. Ly6C^{low} monocytes (also called "resident" or "non-classical") have a low expression of the chemokine (C-C motif) receptor (CCR) 2, and a high expression of CX3C chemokine receptor 1 (CX3CR1) (48). These cells have reparative properties and patrol the vasculature. In fact, Ly6C^{low} monocytes are shown to maintain the integrity of the vasculature by cleaning damaged endothelial cells (49). In contrast, "Classical" or "Inflammatory" Ly6C^{high} monocytes that are CCR2^{high}CX3CR1^{low} migrate to extravascular tissues and are recruited to sites of inflammation (48). Once in the tissue, Ly6C^{high} monocytes can differentiate to Ly6C^{low} cells and take on many of their characteristics. For instance, in myocardial infection, Ly6C^{high} monocytes infiltrate the infarcted myocardium to participate in inflammation. However, later during the reparative phase of the myocardial infection, Ly6C^{high} monocytes are the pre-cursors for F4/80^{high}Ly6C^{low} macrophages that contribute to collagen deposition and scar formation (50). In cancer, in a model of mammary adenocarcinoma, Ly6C^{high} and Ly6C^{low} monocytes in the blood were selectively labeled with fluorescent latex beads and the authors could conclude that Ly6C^{high} monocytes are recruited to the tumor where they differentiate to Ly6C^{low} TAMs, including both M1-like MHC class II^{high} and M2-like MHC class II^{low} TAMs (51).

In situ proliferation of TAMs

Until recently, it was believed that the majority of macrophages involved in inflammation, infection or during tumor progression arise from recruited BM-derived monocytes. However, current evidence implies that *in situ* proliferation of macrophages is of great importance during inflammatory conditions. For example, in obesity, an important mechanism whereby macrophages accumulate within the visceral adipose tissue is CCL2 driven *in situ* proliferation (52). Collected evidence suggests that both freshly recruited BM-derived macrophage (BMDM) (53) and tissue-resident macrophages of embryonic

origin (39) are capable of undergoing local proliferation under certain inflammatory conditions. For example, in atherosclerosis, BMDM proliferation rather than recruitment is a key event for the renewal of macrophages (53).

Examples of selective proliferation of specific macrophage phenotypes that dictate the overall composition of the macrophage population are emerging in the literature. For instance, in a model of rodent filarial nematode *Litomosoides sigmodontis*, the expansion of anti-inflammatory macrophages is mediated by an overall T_H2-inflammatory response and dependent on IL-4 increasing the local proliferation of M2-macrophages (54). In the field of cancer, a recent publication by Zhu *et al* investigate the origin of the expanded TAM population in pancreatic ductal adenocarcinoma (PDAC) models. By creating CD45.1/CD45.2 chimeric mice by surgical parabiosis or BM transplants, the authors find that both blood monocytes and tissue-resident macrophages contribute to the expanded macrophage population during tumor progression. In fact, tissue-resident macrophages of embryonic origin are expanding by *in situ* proliferation during tumor progression and serve as a source of TAMs. Interestingly, TAMs derived from different origins demonstrate distinct phenotypes and divergent functionality. Donor-derived monocytes seem to mainly contribute to the expansion of MHC class II^{high} M1-like TAMs whereas the host derived macrophages preferentially, but not exclusively, are MHC class II^{low} macrophages that are more potent drivers of PDAC progression than their monocyte-derived counterparts (39). Another study that investigates the selective proliferation of specific macrophage subtypes comes from Franklin *et al* (55). Interestingly, in concordance to the study from Zhu *et al*, Franklin and co-authors identify *in situ* proliferation as a mechanism of TAM accumulation during tumor growth. However, in contrast to what was found in PDAC models, the proliferating macrophages in the MMTV-PyMT model are derived from recruited CCR2 expressing monocytes (55).

2.3 TAM FUNCTIONS

Angiogenesis

In response to hypoxia exposure and other microenvironmental signals, TAMs release a number of potent pro-angiogenic cytokines and growth factors (22) such as VEGF, TNF-α, IL-8 and basic fibroblast growth factor (bFGF). Additionally, macrophages can produce angiogenesis-modulating enzymes and inhibitors, which regulate the digestion of the extracellular matrix, including matrix metalloproteinases (MMPs), plasmin, urokinasetype plasminogen activator (uPA) and the uPA receptor (56). TAMs therefore play an important

part in regulating angiogenesis (57) and are often found to surround the blood vessels (22). The many pro-angiogenic functions of TAMs may help explain reported correlations between increased numbers of TAMs and high vessel density and poor prognoses of many tumor types (57).

Experimental evidence for the role of TAMs in tumor angiogenesis is reported by Lin and colleagues using the MMTV-PyMT spontaneous mouse model of mammary adenocarcinoma (6). In CSF-1-null mutant mice, where the infiltration of macrophages to the tumor is inhibited, the development of the vasculature network is impaired. Tumors also display depleted VEGF in the stromal cells suggesting that this is a significant reason of impaired angiogenesis. Restoration of macrophage numbers in the tumors of CSF-1-null mutant mice by transgenic expression of CSF-1, specifically in the mammary epithelium, results in increased vessel density (6). Interestingly, De Palma identified a subset of monocytes that express the Tie2 receptor, Tie2-expressing monocytes (TEMs), that are highly pro-angiogenic and are located in proximity of tumor vessels. In fact, in the absence of TEMs, angiogenesis is severely hampered (58).

Invasion and metastasis

In addition to induced vessel density, high numbers of TAMs also correlate with increased metastatic dissemination to distant sites in many tumor types (22). In animal studies, tumor progression is inhibited and the number of metastasis is decreased when inhibiting the infiltration of macrophages into tumors by neutralizing antibodies or genetic alterations (22, 59). In PyMT-induced mammary tumors, macrophages are found in areas of basement membrane breakdown and invasion during the development of early-stage lesions (60). This finding led to the model that macrophages promote migration of tumor cells out from the primary tumor through holes in the basement membranes, creating an invasive microenvironment that gives tumor cells access to the vasculature, and thereby, increases their metastatic capacity (21). Indeed, experimental studies demonstrate that TAMs contribute to induced vascular abnormalization, creating leaky vessels that contributes to enhanced tumor cell intravasation. For instance, VEGF-A signaling from perivascular TEMs causes local loss of vascular junctions, transient vascular permeability, and tumor cell intravasation (61) Thus, macrophage depletion or M2-macrophage polarization towards an M1 angiostatic phenotype normalizes the vessel wall and increases pericyte coverage leading to prevention of tumor cell intravasation and metastatic dissemination (59).

Several studies indicate that the intravasation of tumor cells into the circulation requires a close interaction with TAMs. Goswami *et al* (62) report that the production of CSF-1 by tumor cells stimulate macrophages to migrate and to produce epidermal growth factor (EGF), which in turn activates migration of the tumor cells. Blockade of EGF or CSF-1 signaling results in inhibited migration of both macrophages and tumor cells (62). Further, the communication between these cells is also demonstrated by *in vivo* multiphoton microscopy, where Wyckoff *et al* (63) observe an increased number of metastasis when mammary tumor cells are located close to TAMs.

In glioma, microglia induce the migration and invasive properties of glioma cells. *In vitro*, glioma cell exposure to microglia increase their migratory capacity threefold (64). Several microglia-derived factors, including TGF-β (65), IL-6 (66), and EGF (67) are identified as promoters of glioma cell invasion.

Evidence suggests that macrophages also play an important role during the seeding of extravasated tumor cells at distant sites. Clinical observations correlate the number of macrophages associated with metastasis in the lymph nodes with poor survival (68). In addition, depletion of macrophages in the peritoneum reduce the ability of carcinoma cells injected to the circulation to seed and grow in the lung (69).

Immune suppression

In acute inflammation, pro-inflammatory macrophages stimulate the cytotoxic activity of T-cells and NK-cells. In the tumor microenvironment, TAMs normally lack these activities (57). TAMs express cell surface receptors, and secrete cytokines, chemokines and enzymes important for the suppression of effector cells and recruitment and activation of T regulatory cells in the tumor microenvironment. For instance, the immune suppressive programmed death-ligand 1 (PD-L1) is reported to be expressed by TAMs in high grade serous ovarian carcinoma (70). In addition, TAMs also express programmed cell death-1 (PD-1), and the expression is negatively correlated with phagocytic potency against tumor cells (71). Immunosuppressive factors secreted by TAMs include CCL22, IL-10, and TGF-β. CCL22 is reported to promote recruitment of T regulatory cells into cancer tissue (72). These cells suppress immune surveillance through multiple mechanisms including suppression of anti-tumoral cytotoxic $CD8^+$ T cell and/or NK-cell activity.

Data from our lab show that skewing the balance from an anti- to a pro-inflammatory phenotype of TAMs induce the accumulation and activation of $CD8^+$ T-cells and NK-cells that in turn restricts tumor growth (36).

3 TRANSLATIONAL REGULATION IN THE IMMUNE SYSTEM WITH A FOCUS ON MACROPHAGES

It is essential for cells from the immune system to respond specific and rapid to changes in their environment, and the mechanisms controlling their activity need to be tightly regulated. Regulation of translation of already existing mRNAs allows a rapid change in protein abundance. An important mechanism for translational control in the immune system is regulation of the activity of eIF4E (**Figure 2B**). For example, the mTOR pathway regulates translation of mRNAs encoding the transcription factors interferon regulatory factor (IRF) 7 (73), GATA-3 (74), and the cytokine IL-4 (75), that all play central roles in immunology. The second mechanism whereby eIF4E activity can be modulated is via phosphorylation by MNK1 and MNK2. This pathway regulates the translation of mRNA transcripts encoding IRF8 (76), the NF-κB inhibitor NFKBIA (IκBα) (77) and the chemokine CCL5 (78), all controlling the activity of transcription factors regulating immunological functions.

Mouse embryonic fibroblasts (MEFs) lacking either the translational repressors 4E-BP1/2 that binds eIF4E or the ability to phosphorylate eIF4E show enhanced resistance to viral infections due to translational control of key immune regulators involved in the production of type-1 IFNs (IFN-α and IFN-β). Activation of the NF-κB pathway, with downstream targets including TNF and IFN-β1, is a central pathway in innate immunity. As a regulatory mechanism, NF-κB activation does not only result in activation of a pro-inflammatory program, but also in a negative feedback loop to resolve inflammation, including increased expression of the inhibitor IκBα. By using MEFs lacking functional eIF4e phosphorylation Herdy *et al* show that the observed increased resistance to viral infections is due to translational control of IκBα via the MNK-eIF4e pathway (77). MEFs lacking functional eIF4e phosphorylation display a lower abundance of IκBα and thereby enhanced activity of the transcription factor NF-κB, which promote the production of IFN-β (77). In MEFs lacking 4E-BPs, translational control of the master regulator of IFN type 1, IRF7, accounts for the increased viral resistance. MEFs missing 4E-BPs, display a 12-fold upregulation of

IRF7 compared to wild type MEFs, leading to enhanced type-I IFN production and subsequent enhanced resistance to viral infections (73).

A few studies investigate specific translational control in macrophages during inflammation and increased translation of selected cytokine-, chemokine- and transcription factor-mRNAs is observed after toll-like receptor (TLR) stimulation. To study translation in an early phase of macrophage response, Schott *et al* use mouse macrophages stimulated with LPS, that activates the TLR4 receptor and induces numerous inflammatory pathways in macrophages including activation of the NF-κB pathway. In concordance with data from viral infected MEFs, LPS stimulation induce translational activation of many feedback inhibitors of the inflammatory response including NF-κB inhibitors (79). The notch–RBP-J pathway is shown to up-regulate the translation of IRF8 that induce downstream M1-associated genes through augmenting TLR4 activated MAPK-MNK1-eIF4E signaling and thereby regulate the polarization of M1-macrophages (76). Additionally, LPS dependent TLR4 activation abrogates translational repression by heterogeneous nuclear ribonucleoprotein (hnRNP) on transforming growth factor beta-activated kinase (TAK) 1, and thereby allows TAK1 to boost macrophage inflammatory response (80). Another example of a cytokine that can be regulated via translational control in macrophages is TNF. A RNA binding protein named TIA-1 binds the 3'UTR of the TNF mRNA transcript and thereby inhibits its translation (81).

In human macrophages, Su *et al* demonstrate that IFN-γ selectively modulate the macrophage translatome to promote inflammation. Genome-wide analysis of translational regulation by IFN-γ after TLR2 stimulation demonstrated significant changes in translational efficiency in almost 1,000 genes, of which 396 were affected greater than twofold. The changes are bidirectional; IFN-γ increases and decreases the translational efficiency of a similar numbers of genes. Ingenuity pathway analysis (IPA) of canonical pathways display significant translational regulation of well-known IFN-γ–mediated pathways important in immune responses such as antigen presentation (82).

For cells in the tumor microenvironment, the impact of translational control has not been well studied. However, a recently published paper demonstrates the importance of translational regulation in immune cells in the tumor microenvironment in a mouse mammary carcinoma model (83). By inoculating wild type tumor cells into a mouse where the phosphorylation site S209 of eIF4E is mutated, they demonstrate that the development

of lung metastasis is dependent on phosphorylation of eIF4E in cells from the tumor microenvironment. In fact, phosphorylation of eIF4E regulates the accumulation and survival of intratumoral pro-metastatic neutrophils that promotes pulmonary metastases (83).

4 THERAPEUTIC IMPLICATIONS

The establishment of the contribution of macrophages to tumor progression, and the fact that macrophages do not harbor malignant mutations and thus are much less likely to develop drug resistance, makes them a good target for therapeutic agents (84). Inhibition of monocyte recruitment into the tumor, eradication of macrophages already in the tumor, neutralization of key molecules that TAMs release (22) and preventing and/or re-orienting M2-like TAMs in favor of a more anti-tumoral phenotype (59) are some strategies for therapeutic targeting of TAMs.

The CSF-1/CSF-1R pathway plays a crucial role in recruiting macrophages to the tumor site that are involved in the angiogenic switch and metastatic dissemination (60, 84). Several strategies to block this pathway the with neutralizing antibodies or small molecules have been developed. For instance, in humans, depletion of TAMs by administration of the monoclonal antibody RG7155, which targets the CSF-1R, improve clinical outcome in patients with diffuse giant sarcoma (85). In a mouse model of proneural glioblastoma multiforme (GBM), CSF-1R inhibition blocks tumor growth, regresses established GBMs and dramatically increases survival (86). Interestingly, glioma-secreted factors (including IFN-γ and GM-CSF) facilitated survival of a subset of TAMs with decreased expression of M2-genes, a signature associated with enhanced survival in patients with proneural GBM (86). In concordance, in lung and breast carcinoma, Van Overmeire and colleagues show that following CSF-1R blockade, Ly6C^{high} monocytes preferably differentiated to a MCH class II^{high} M1-like phenotype, thereby promoting a shift towards a predominant M1-like TAM accumulation in tumors (87).

However, the currently available literature on TAM depletion/modulation via CSF-1/CSF-1R pathway blockade suggests that monotherapy will not be sufficient for cancer therapy. Today, the therapeutic benefits have as its best only resulted in a delay of tumor growth. Therefore, various combination partners for macrophage depleting agents are currently

under investigation. In pre-clinical models, well-established treatments such as chemotherapeutic agents (88) and irradiation (89) but also targeted therapies (90), anti-angiogenic therapies (91), adoptive T cell transfer (92) and immune checkpoint inhibitors (93) have been evaluated as possible combination partners. For example, administration of PLX3397 (a small molecule that targets the CSF-1R) to the spontaneous mouse mammary carcinoma model MMTV-PyMT, does not have an effect as a monotherapy. Nonetheless, in combination with the chemotherapeutic agent paclitaxel, PLX3397 treatment results in an efficient anti-tumoral response associated with an increased accumulation of cytotoxic T lymphocytes (CTLs) (88).

Several experimental studies demonstrate the possibility to skew the phenotype or selectively change the composition of TAMs of different subtypes. For instance, antibody targeting of the pattern recognition scavenger receptor MARCO, re-programs a subset of immunosuppressive TAMs via FcγRIIb to become of a more pro-inflammatory phenotype (94). Moreover, Rolny *et al* show that re-education of TAMs towards an anti-tumoral phenotype efficiently hampered mammary tumor growth by sustaining the activity of CTLs and NK-cells (59). Similarly, changing the ratio of M1- and M2-macrophages in the tumor by inducing differential proliferation with semaphorin (SEMA) 3A is shown to give similar outcome (36).

In conclusion, current immunotherapies towards the macrophage/monocyte linages show limited success and modulating the function of the existing pool of TAMs is suggested to be a more successful strategy than depleting the whole population. However, in order to do this, a more detailed insight into how TAMs are regulated is needed to identify new opportunities for therapeutic intervention.

5 AIMS OF THE THESIS

The overall aim of this thesis is to increase the knowledge about how the composition and function of macrophages within the tumor is dynamically changing during tumor growth. Understanding the mechanisms that dictate the dynamic change will help us develop tools and find targets to re-educate or change the composition of TAMs to favor the anti-tumoral phenotype and create a pro-inflammatory microenvironment to inhibit tumor growth.

Specific aims of the studies included in this thesis:

Study I: To explore the effect of SEMA3A on accumulation and activity of tumor-associated immune cells in mouse and human breast cancer.

Study II: To investigate of role of mRNA translation in regulating the TAM phenotype during tumor growth.

Study III: To understand the role of tumor-associated macrophages and microglia (TAMMs) in platelet-derived growth factor (PDGF) B driven glioma.

Study IV: To increase the knowledge about how Zoledronic acid (ZA) affects the activity of NK-cells, and how monocytes are involved in the observed effects.

6 RESULTS AND DISCUSSION

6.1 SUMMARY OF THE MAIN FINDINGS

In this thesis, we have used several different strategies to study the heterogeneity and functions of macrophages within tumors and mechanisms that regulate the composition of the TAM population during tumor growth.

Related to the overall aim of the thesis, the first study, **Study I**, showed that SEMA3A overexpression in tumors induced the proliferation of M1-macrophages, and at the same time reduced the proliferation of pro-tumoral M2 macrophages. The subsequent changed composition of TAMs increased the recruitment and activation of cytotoxic immune cells that restricted tumor growth. Hence, we could show that differential regulation of proliferation of different TAM subsets regulated tumor growth.

In **Study II**, we identified translational regulation of gene expression as an important mechanism that regulate the phenotype of TAMs during tumor growth. By performing gene-ontology (GO) to study the enrichment of cellular functions among genes regulated via translation in macrophages, we also gained further evidence that regulation of *in situ* proliferation of macrophages is of importance for TAMs during tumor growth. We could further identify a possible target for therapeutic interventions as M2-macrophages were identified to have more phosphorylation of eIF4E compared to M1-macrophages. Blocking MNK1/2 (by using cercosporamide) that specifically phosphorylates eIF4E resulted in re-programing of M2-BMDMs towards an M1-phenotype with acquired ability to activate $CD8^+$ T-cells. Further, inhibition of MNK1/2 resulted in hampered proliferation of M2-macrophages but not M1-macrophages.

The possibility of studying the ontogeny of macrophages has developed during the last decades and in addition to bone marrow chimeras, there are today several genetic mouse models that can be used to trace the origin of specific macrophages. However, the understanding of how influences from origin and environment are integrated to define functional capacities is far from understood. In central nervous system (CNS) tumors, two distinct populations of macrophages are observed. During normal physiological conditions, only microglia, the tissue resident macrophages of the CNS are observed inside the blood brain barrier. However, during many neuropathological conditions, the blood brain barrier is impaired, resulting in an infiltration of monocytes from the periphery. Increased

evidence indicates that microglia and monocyte-derived myeloid cells play distinct roles during neuro-inflammatory conditions (95). However, the knowledge about how the two types of macrophages differ in their function in brain tumors is still not well known. In **Study III**, we identified a distinct functional difference between microglia and BMDMs. M2-polarized microglia but not M2-BMDMs induced PDGF receptor (PDGFR) B expression in glioma cells and thereby stimulated their migratory capacity.

In our fourth study, we moved from studying the differentiated macrophages within the tumor to instead study the precursor in peripheral blood, the monocyte, and highlighted the importance of monocytes in regulating other cells in the innate tumor immunity. In **Study IV,** we showed that ZA + IL-2 induced the expression of IFN-γ in monocytes derived from human peripheral blood mononuclear cells (PBMCs). In co-cultures with NK-cells, the increased IFN-γ production upregulated TNF-related apoptosis-inducing ligand (TRAIL) on the NK-cells and induced their cytotoxicity against tumor cells.

6.2 STUDY I

Guidance Molecule SEMA3A Restricts Tumor Growth by Differentially Regulating the Proliferation of Tumor-Associated Macrophages

SEMA3A is a secreted protein that was first described as an axon guidance factor but has more recently been shown to be involved in several physiological and pathological processes such as migration of myeloid cells, angiogenesis and tumor growth (96-99). SEMA3A binds to its co-receptor neuropilin 1 (NP1) that associates with the Plexin A family (Plexin A1-4) of receptors to transfer intracellular signals (100). In human cancer, SEMA3A is downregulated in several types of cancers (101-104), among them breast cancer (105). In concordance, we found that SEMA3A protein was decreased from grade I to grade III breast cancer.

In mice models, the role of SEMA3A and its co-receptor NP1 in tumor progression and immunity is somewhat controversial. Studies demonstrate that SEMA3A inhibits tumor growth (97, 98) and recruits a population of circulating $NP1^+$ monocytes that induce tumor vessel normalization (97), whereas others report that loss of NP1 in macrophages hinders their entry into hypoxic areas and thereby restores anti-tumor immunity and reduces angiogenesis (99). In our study, we overexpressed SEMA3A by lentiviral mediated gene-transfer in the orthotropic mouse mammary carcinoma model 4T1. The 4T1 model is a

model resembling late stage breast cancer and efficiently metastasizes to lung, liver, brain and bone of syngeneic mice. During tumor growth, 4T1 tumors progressively accumulate $CD45^+$ haematopoietic cells consisting predominantly of $CD11b^+$ myeloid cells (106). Following orthotropic cell injection, SEMA3A overexpressing tumors grew significantly slower and displayed an increased infiltration of macrophages and cytotoxic lymphocytes compared to control tumors.

We used several different strategies to evaluate the phenotype of the accumulated TAM population. On flow cytometry, we classified $CD11b^+Ly6G^-$ cells into M1- and M2-like macrophages based on their expression of Ly6C and MHC class II, where $Ly6C^{low}$MHC class II^{low} cells were classified as M2-like, and $Ly6C^{low}$MHC class II^{high} cells were classified as M1-like. In addition, we used a set of known M1- and M2-associated cell surface markers and studied how these differed in the $CD11b^+F480^+$ population in SEMA3A overexpressing and control tumors. Both flow cytometry gating strategies displayed an increased accumulation of M1-like macrophages in SEMA3A overexpressing tumors. This finding was also verified by qPCR for a broad range of M1- and M2-associated cytokines and chemokines on $CD11b^+F480^+$ macrophages sorted from the tumors.

Previous studies have shown that SEMA3A/NP1 signaling effects the migration of myeloid cells and we hypothesized that increased recruitment accounted for the accumulation of M1-macrophages in SEMA3A overexpressing tumors. However, we could not identify increased migration of BMDMs in response to SEMA3A. Further, mice with SEMA3A overexpressing tumors did not have a significant increase in the frequency of monocytes in the blood nor in the tumor. In addition, SEMA3A also failed to induce a direct change of the phenotype of BMDMs. Recent data indicate that local proliferation of macrophages with a specific phenotype can dictate the overall composition of the macrophage pool (39, 54). Interestingly, both *ex vivo* and *in vivo*, we identified a mechanism whereby SEMA3A selectively increased the proliferation of M1-BMDMs and TAMs and reduced the proliferation of M2-BMDMs and TAMs. The mechanism was shown to be dependent on NP1 and mediated via signaling pathways regulating Akt and MAPK phosphorylation. Hence, our studies indicated that SEMA3A regulates TAM proliferation differentially rather than migration as reported previously by others (99). In a study by Casazza and colleagues (99), SEMA3A/NP1 signaling is described to guide macrophages to hypoxic areas in the tumor where they contribute to angiogenesis and tumor growth. Importantly, data from study II in this thesis, showed that macrophage proliferation was decreased as

tumors were growing and when they reached a size of 2g (~2000mm^3) there was no longer a difference between M1- and M2-TAM proliferation. Hence, one important discrepancy that at least in part could explain the differential findings of Casazzas and our studies, is that they performed most of their studies in tumors larger than the tumors we have studied. Furthermore, in many of the experiments they use macrophages depleted in NP1, rather than overexpression of SEMA3A as we do, and it is therefore possible that the observed effect is not solely dependent on SEMA3A signaling but also signaling from other factors that share NP1 as a co-receptor, such as VEGF-A. In addition, we showed that in BMDMs, NP1 repression mimics the effect of SEMA3A pre-treatment on phosphorylation of Akt and MAPK following CSF-1 stimulation. M2-BMDMs displayed decreased CSF-1 mediated phosphorylation of Akt and MAPK when pre-treated with SEMA3A or repressed in NP1 compared to control M2-BMDMs. In M1-BMDMs on the other hand, CSF-1 mediated phosphorylation of Akt and MAPK was induced upon SEMA3A pre-treatment or NP1 inhibition. We therefore speculate that NP1 knock-down in macrophages can induce differential outcome depending on the TAM phenotype, however, further studies need to elucidate this. Further, the observation that SEMA3A had opposing effects on the proliferation of M1- and M2-TAMs and BMDMs may depend on the differential expression of the Plexin A family that mediate downstream signaling of the SEMA3A-NP1 complex.

TAMs were not the only tumor-associated immune cells that were affected by SEMA3A. In human breast cancer, SEMA3A levels correlated to macrophage, CD8$^+$ T-cell and NK-cell markers. In concordance, SEMA3A overexpressing tumors displayed increased infiltration and activation in CD8$^+$ T-cells and NK-cells. However, SEMA3A did not have any direct effect on these cell types that hardly express the NP1 co-receptor, instead the increased accumulation and activation was a result of the changes in the TAM population. By performing several depletion studies we could conclude that the reduced tumor growth mediated by SEMA3A was dependent on both macrophages and cytotoxic lymphocytes. In mice depleted in macrophages by a CSF-1 blocking antibody (clone 5A1), SEMA3A failed to reduce the tumor growth and to increase the infiltration of cytotoxic lymphocytes. Additionally, SEMA3A also lost its effect on tumor growth in mice depleted in CD8$^+$ T-cells or NK-cells.

In summary, we identified a mechanism whereby SEMA3A increased the accumulation of M1-like TAMs by selectively inducing their proliferation, and at the same time reducing

the proliferation of M2-like TAMs. The increased proportion of M1/M2 TAMs resulted in an induced pro-inflammatory tumor microenvironment and subsequent increased infiltration and activation of cytotoxic lymphocytes that inhibited tumor growth (**Figure 5**). Depletion of all macrophages in our model did not inhibit tumor growth and we therefore provided results strengthening the theory that skewing the TAM phenotype, rather than depleting the whole population, serves as a preferable strategy in targeting TAMs in cancer. Only a few previous studies have shown the importance of *in situ* proliferation of macrophages in dictating the overall composition of the macrophage pool. We believe that identifying differential regulation of proliferation as a mechanism that contributes to the composition of TAMs widens the knowledge about how the TAM composition can be regulated. By elucidating mechanisms, we create new windows for therapies targeting the tightly regulated balance of TAMs.

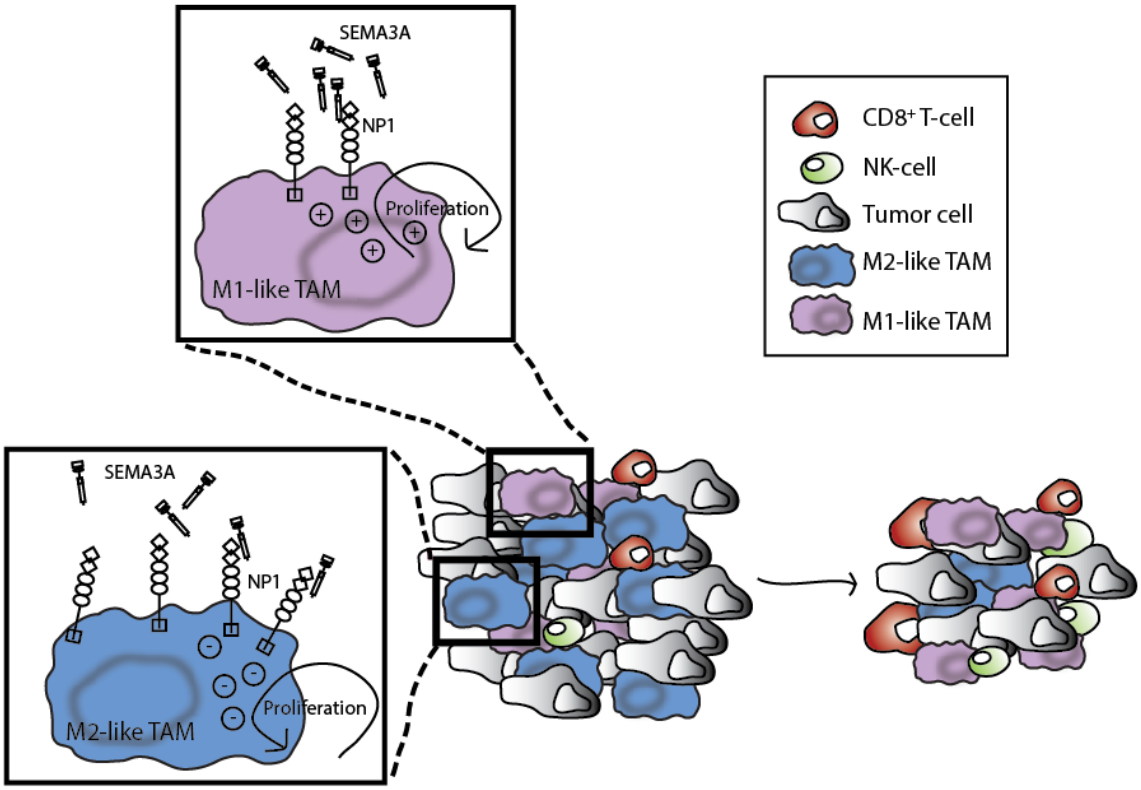

Figure 5. Summary of Study I. SEMA3A induces the proliferation of M1-like TAMs and reduces the proliferation of M2-like TAMs in an NP1 dependent manner resulting in accumulation and activation of $CD8^+$ T-cells and NK-cells and restricted tumor growth.

6.3 STUDY II

RNA Translational Control of the Tumor-Associated Macrophage Phenotype

During tumor growth, the function of TAMs is dramatically altered by mechanisms that are not fully understood. It is well known that the proteome in TAMs, including surface markers and secreted proteins, is dynamically changing with tumor growth; from having a pro-inflammatory function at the initiation step of tumor growth to being more immune suppressive when the tumor is growing (37). However, how different mechanisms contribute to regulate gene expression in TAMs is not fully understood.

To study mechanisms involved in changing the TAM phenotypes during tumor growth, we needed a model where there was a correlation between how the TAM population was composed to an easily defined property such as tumor size or stage of malignancy. To this purpose, we decided to use a cell-line derived from a tumor that arose in the mammary carcinoma model MMTV-PyMT. The MMTV-PyMT tumor model is a highly metastatic model that progresses through stages that resembles the development of human breast cancer (107). In the spontaneous MMTV-PyMT model, mice develop multiple tumors that at a given time point can be in different stages of tumor progression. In our study, such model would have been difficult to work with due to the heterogeneity of the tumors. Neither tumor size nor stage of malignancy would be a good property to correlate to TAM composition because the immune cell infiltration would be effected by other tumors developing in the same mouse. Instead, we decided to perform orthotropic injections into the mammary fat pad with a cell-line derived from this transgenic model. Doing this, we probably lost some of the similarities to human breast cancer progression when using cells that were derived from a late stage tumor and thereby already at the stage of injection had acquired mutations associated to late carcinoma. On the other hand, it gave us a more homogeneous model that allowed us to study single tumors at distinct sizes that were not influenced by other tumors developing in the same mouse. Because of the injection of fully transformed tumor cells, we chose to study the tumor growth and have tumor weight as the measurement we correlated to the composition of the TAM population, and not the tumor stage. We used two different gating strategies for flow cytometry analyses to verify that the tumor weight correlated to the shift in M1-like and M2-like TAMs during tumor growth.

Given that the overall function of TAMs in a tumor is rapidly changing in response to environmental changes, we hypothesized that translational control of existing mRNAs that allow for rapid changes in cellular concentrations of the encoded proteins, could be a

contributing mechanism to the regulation of macrophage gene expression and phenotype during tumor growth. To prove this hypothesis, macrophages were flow sorted from tumors of different sizes and subjected to simultaneous polysome and cytosolic RNA isolation, RNA extraction, and RNA sequencing. By using anota analyses, an algorithm designed to account for changes in translational efficiency that take polysomal bound RNA, total RNA and tumor weight into account, we found that during tumor growth, the gene expression in macrophages was regulated by translation to a higher degree than transcription. In fact, almost 1000 genes were regulated by translation while only 100 genes were regulated by mRNA abundance. This might reflect that TAMs need to adapt fast to microenvironmental changes. These results also highlight the importance of using several read-outs such as the transcriptional profile, protein expression, and cellular function, when defining the present state of a macrophage.

GO analyses that show enrichment of cellular functions among genes regulated via translation, identified several clusters that correlated to transition from a pro- towards an anti-inflammatory signature of TAMs. In the clusters of cell cycle and proliferation, we found a translational upregulation of genes associated to increased proliferation and repressed apoptosis with increased tumor weight. Interestingly, during the past few years, we and others, have identified proliferation as an important mechanism regulating the composition of macrophages in inflammation (36, 39, 54). In the first study of this thesis, we identified *in situ* proliferation as a mechanism increasing the pool of anti-inflammatory macrophages in the tumor microenvironment. The results of the present study strengthen our previous data, and we can now speculate that proliferation is a general mechanism contributing to the composition of macrophages with different functions within the tumor. However, the role of proliferation is probably tissue and context dependent and there are studies showing that *in situ* proliferation of differentiated macrophages is not a mechanism contributing to the composition of TAMs (51).

Another cluster that appeared in the GO included genes involved in metabolic processes. During tumor progression, it is an advantage for cells in the tumor microenvironment to be able to adopt their metabolism to the current circumstances. Tumor associated cells will experience tough physiological changes in pH, oxygen and nutrient availability and need to cope with these stressful conditions. It is established that the two extremes of M1- and M2-macrophages have different metabolic profiles (108). The energy metabolism in M1 macrophages is characterized by aerobic glycolysis, converting glucose into lactate,

involving processes that contribute to the pro-inflammatory function of these cells, such as production of nitric oxide and ROS. M2-macrophages on the other hand, mainly use fatty acids and glutamine to fuel the tricarboxylic acid (TCA) cycle and produce ATP through oxidative phosphorylation (108). In the top of our data set over genes regulated by translation, was the family of carbonyl reductases (CBRs). CBRs are NADPH-dependent cytosolic enzymes that for instance catalyze the reduction of endogenous prostaglandins and steroids (109). In addition, we found genes that are involved in oxidative metabolism involving amide biosynthesis, peptide biosynthesis and fatty acid biosynthesis. Interestingly, also genes involved in glutathione metabolism were regulated. Glutathione maintain redox homeostasis by acting as a reducing agent protecting cells against ROS (110). However, the role of the identified genes in TAMs is not known and needs further investigations.

From the list of genes generated with anota analyses, we picked three genes that were significantly regulated at the level of mRNA translation but not under transcriptional regulation in macrophages during tumor growth. By flow cytometry and western blot analyses we could verify that these genes were higher expressed in M2- compared to M1-macrophages both in the TAM population and after polarization of BMDMs *ex vivo*. By looking at translational regulation, we have identified genes that have previously not been described as typical M1- or M2-penotype genes but in fact are differently expressed in the two phenotypes. However, if the genes have a significance in driving the functional phenotype remains to be elucidated. In addition to the genes mentioned here, we have a long list of possible candidates to go through.

Moreover polysome-profiling of M1- or M2-BMDMs *in vitro* (data not shown) revealed that translation in TAMs during tumor growth partially depend on an M1- to M2-shift as the genes identified to be differently translated in the two phenotypes *in vitro*, also were found in the *in vivo* dataset. These results strengthened the hypothesis that genes found upregulated truly are important for the M2-phenotype.

To study the mechanism behind the identified translational regulation, we continued with *ex vivo* studies. Knowing that translation initiation factor eIF4E is an important step of regulating the speed of mRNA translation we performed western blot analyses for p-eIF4E and total eIF4E in M1- and M2-polarized BMDMs and found phosphorylation of eIF4E to be upregulated in M2- compared to M1-BMDMs without any significant differences in the

total protein. Interestingly, regulation of eIF4E has been found to be an important step of regulation for several factors involved in the pro-inflammatory immunity response. For instance, MEFs lacking functional eIF4e phosphorylation show enhanced activity of the transcription factor NF-κB (77), a factor known to skew macrophages towards an M1-phenotype in response to LPS stimulation.

To test whether blocking eIF4E phosphorylation would induce an M1-phenotype in M2-macrophages we used an inhibitor, cercosporamide, that inhibits the activity of MNK2, one of the kinases that phosphorylates eIF4E. The macrophage phenotype was evaluated by several markers, including transcription, cell surface markers and functional assays. Cercoporamide efficiently inhibited the phosphorylation of eIF4E in M2-polarized BMDMs and induced the expression of M1-associated cytokines and markers at the same time as the expression of M2-markers was reduced at transcriptional and cell surface level. Additionally, M2-macrophages treated with cercosporamide demonstrated reduced proliferation and enhanced capacity to activate T-cells, as displayed by increased production of IFN-γ in macrophage-T cell co-culture experiments. In conclusion, even though eIF4E is a general initiation factor, its activity seems to stimulate the translation of specific sets of mRNAs rather than global translation. The exact mechanisms are not fully elucidated.

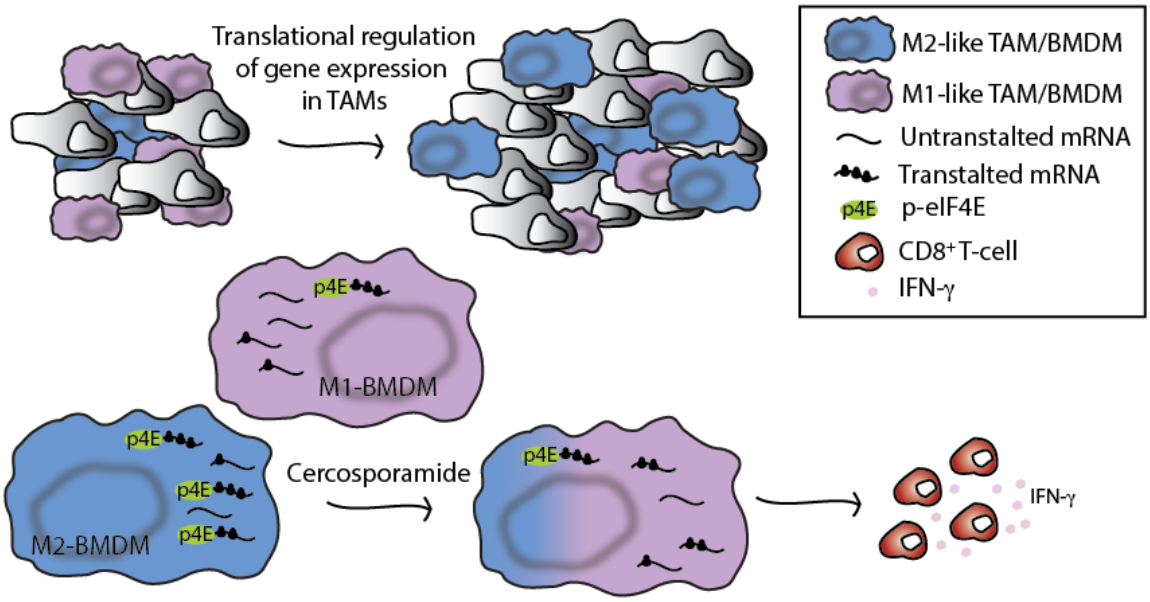

Figure 6. Summary of Study II. Translational regulation is an important mechanism regulating gene expression in TAMs during tumor growth. The phosphorylation of eIF4E is differentially regulated in M1- and M2-BMDMs and inhibition of eIF4E phosphorylation in M2-BMDMs induce a pro-inflammatory BMDM phenotype that activates $CD8^+$ T-cells.

In summary, we identified translational control as a mechanism regulating gene expression in TAMs during tumor growth. eIF4E phosphorylation is differentially regulated in M1- and M2-BMDMs and inhibition of eIF4E phosphorylation in M2-BMDMs induced a pro-inflammatory phenotype (**Figure 6**). Elucidating mechanisms that regulate the composition and function of TAMs during tumor growth is of great importance. Even though the speed and efficiency of mRNA translation have been identified as a significant step of regulation of gene-expression, only a very limited amount of studies investigate its importance in macrophages. By demonstrating selective translational control in M1- and M2-macrophages, we identified an additional mechanism possible to target therapeutically. Additionally, by generating a list of genes that are significantly regulated by translation, we have created opportunities to identify genes that have never been considered as important for the macrophage phenotype before.

6.4 STUDY III

Pro-Angiogenic microglia-induced expression of platelet-derived growth factor receptor beta in glioma cells promotes their migratory capacity

Gliomas are the most common tumor of the CNS and can arise from different cell types. Gliomas originating from oligodendrocytes, oligodendrogliomas, are typically of grade II and III. Gliomas originating from astrocytes, astrocytomas, can be divided into grade II-IV, where grade IV represent GBM that is the most common and aggressive form of glioma (111). In this study, we used the PDGFB driven N/tv-a;$Arf^{-/-}$ mouse glioma model. This model represents a subset of human gliomas where a perturbed PDGF signaling pathway is observed (PDGFB can bind both PDGFRA and PDGFRB). For instance, in human glioma, amplification of the *PDGFRA* gene is a commonly occurring event (112). The expression of PDGFRB in tumors is mostly associated to stromal cells, however, one study also reports that PDGFRB can be expressed in cultured patient derived GBM cells (113).

In the PDGFB-driven N/tv-a;$Arf^{-/-}$ model that we used, mice lacking the tumor suppressor $p19^{Arf}$ ($Arf^{-/-}$) and expressing the tv-a virus receptor in glial progenitor cells, were infected with retroviruses containing the RCAS vector expressing PDGFB. The virus can only infect glial progenitor cells that express the tv-a receptor and mice developed tumors in 1-3month after virus injection. Histopathological evaluation characterized the tumors into human like glioma of grade II-IV. Grade IV tumors displayed areas of necrosis and clusters of non-

perfused vessels, characteristics typical for GBM (114). In addition, grade IV tumors had accumulation of TAMMs.

TAMMs represent an interesting population to study because of its composition of macrophages with different origin. Under homeostatic conditions, the macrophages of the brain, the microglia, all originate from embryonic yolk sac progenitors. However, during pathological conditions such as glioma, the blood brain barrier is compromised (111) and BMDMs can infiltrate the tumor and contribute to the myeloid population (115). In fact, the majority of immune cells within brain tumors are macrophages, often comprising up to ~30% of the tumor mass (116). The accumulation of M2-polarized TAMMs positively correlates with the histological grade of human gliomas (117) and has been shown to be involved in the malignant progression from low- to high-grade tumors in mice (86).

When studying the localization of TAMMs, we found that they often were located to α-SMA$^+$NG2$^+$PDGFRB$^+$ pericytes. To our surprise, TAMMs were also found to co-localize to a population of glioma cells that also expressed pericyte markers α-SMA and PDGFRB. The PDGFRB$^+$ tumor cells could be found in all categories of tumors, and were physically tightly interlinked with TAMMs. When looking in human grade II and III astrocytomas, only a few PDGFRB$^+$ tumor cells were found, correlating to a low accumulation of TAMMs. However, human GBMs were found to have a high infiltration of TAMMs that correlated to an increased number of PDGFRB$^+$ tumor cells.

Because of the correlation between both the number and the localization of TAMMs and PDGFRB$^+$ tumor cells, we hypothesized that TAMMs could drive tumor cells to express PDGFRB. As described earlier, TAMMs are a very heterogeneous population and *in vitro*, we will never be able to create an equivalent heterogeneity, as the situation is *in vivo*. To resemble macrophages with different origins and grade of polarization, we used both microglia and BMDMs, polarized to an M1- and M2-phenotype with IFN-γ+LPS and IL4+IL10+TGF-β. By performing co-cultures and transwell assays, we could show that cell-to-cell contact with microglia, but not BMDMs, could induce PDGFRB expression in glioma cells. M2-polarized microglia were shown to induce the expression of PDGFRB to a bigger extent then M1-polarized microglia. Intriguingly, the upregulation of PDGFRB after co-culture with M2-microglia was shown to induce the migratory capacity of glioma cells towards serum. Importantly, the population of PDGFRB$^+$ tumor cells was observed also in

other experimental glioma models including gliomas driven by EGF and RAs/Akt (data not included in the manuscript) indicating that this phenotype is not only driven by a PDGFB signature.

It has become evident that microglia display a distinct molecular signature that differs from myeloid and other immune cells (118) and microglia and monocyte-derived myeloid cells can play distinct roles during neuropathological conditions (95). In the context of brain tumors, Bowman *et al* show that the transcriptional profile of tumor-associated microglia, tumor-associated monocyte derived macrophages, and microglia and monocytes from non-tumor bearing mice, separate into four distinct clusters. Further, the findings of the study demonstrate that both the chromatin landscapes established before tumor initiation and tumor-mediated education influence the transcription profile of TAMMs (115). Despite the described transcriptional differences, the functional difference between tumor-associated microglia and tumor-associated macrophages is poorly understood. Our finding that microglia, but not BMDMs, can induce PDGFRB expression in tumor cells *in vitro* demonstrated a functional difference between those cells. We did not explore the ontogeny of the macrophages that were in close proximity to PDGFRB expressing tumor cells *in vivo*, however, several specific markers that can be used to distinguish between microglia and BMDMs in gliomas are emerging in the literature (95, 115, 119).

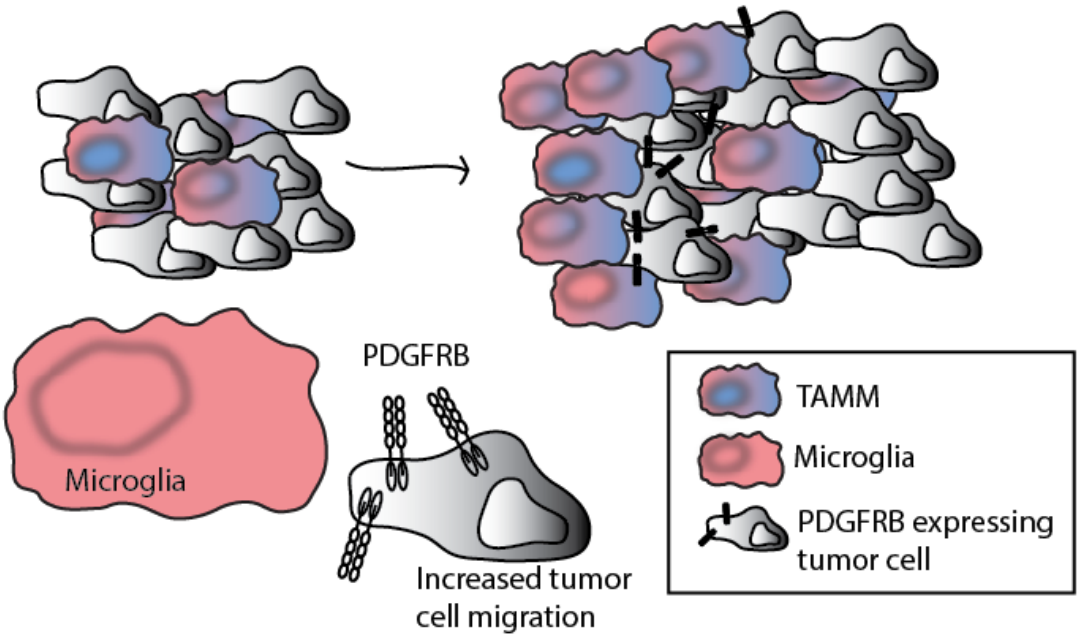

Figure 7. Summary of Study III. TAMM accumulation is correlating to accumulation of PDGFRB expressing tumor cells human and PDGFB driven N/tv-a;Arf-/- glioma growth. Microglia induce the expression of PDGFRB in glioma tumor cells increasing their migratory capacity *in vitro*.

Collectively, our data demonstrated a functional difference of TAMMs of different ontogeny. Microglia, but not BMDMs, could induce the expression of PDGFRB in tumor cells that fuels their migratory capacity towards serum (**Figure 7**). The findings of the study highlight the importance of dissecting the TAMM population and identify specific functional differences among the heterogeneous population. By doing this, therapeutic targeting of specific subtypes could be used for a more successful targeting of the TAMM population in glioma.

6.5 STUDY IV

Activated monocytes augment TRAIL-mediated cytotoxicity by human NK cells through release of IFN-γ

NK-cells are cytotoxic lymphocytes able to kill tumor cells without prior antigen stimulation. In hematological malignancies, NK-cell therapies have demonstrated clinical benefits, but for solid cancers the clinical responses are limited. Improving persistence and homing, as well as overcoming the suppressive tumor microenvironment are fields of research to improve NK-cell therapies to solid tumors.

In this study, we investigated the effect of ZA on the expression of TRAIL in human NK cells. ZA is a bisphosphonate used to prevent loss of bone and lower the risk of skeletal complications in patients with bone metastasis. Besides its main bone antiresorptive activity, ZA displays potential immune therapeutic properties by triggering the activation of γδ T-cells (120). In addition, ZA has also been shown to induce the expression of NKG2D on NK-cells *in vitro* (121).

Here, we could show that co-culture of human NK-cells with purified monocytes in the presence of ZA + IL-2 results in an upregulation of TRAIL on the NK-cells. Following co-culture, NK-cell cytotoxicity against TRAIL sensitive tumor cells was elevated compared to unprimed NK-cells. Transwell assays revealed that cell-to-cell contact was not necessary but instead the induced expression of TRAIL was shown to be dependent on IFN-γ produced by monocytes in the presence of ZA and IL-2 (**Figure 8**). Treatment with ZA increased the expression of IL-2Rα and IL-2Rβ transcripts in monocytes suggesting that ZA-treated monocytes may respond stronger to IL-2 stimulation and subsequently increase their IFN-γ production.

Interestingly, ZA has previously been shown to have an effect on macrophages including suppressing the expression and inhibiting the activity of MMP-9 in macrophages in cervical cancer. Inhibition of MMP-9 resulted in reduced VEGF-A mobilization and subsequent reduced angiogenesis and tumor growth (122). In concordance, cancer patients treated with a single dose of ZA display a long-lasting reduction of VEGF levels in serum (123).

In conclusion, our study highlights the importance of cells from the myeloid linage in regulating the ultimate killers, the cytotoxic cells.

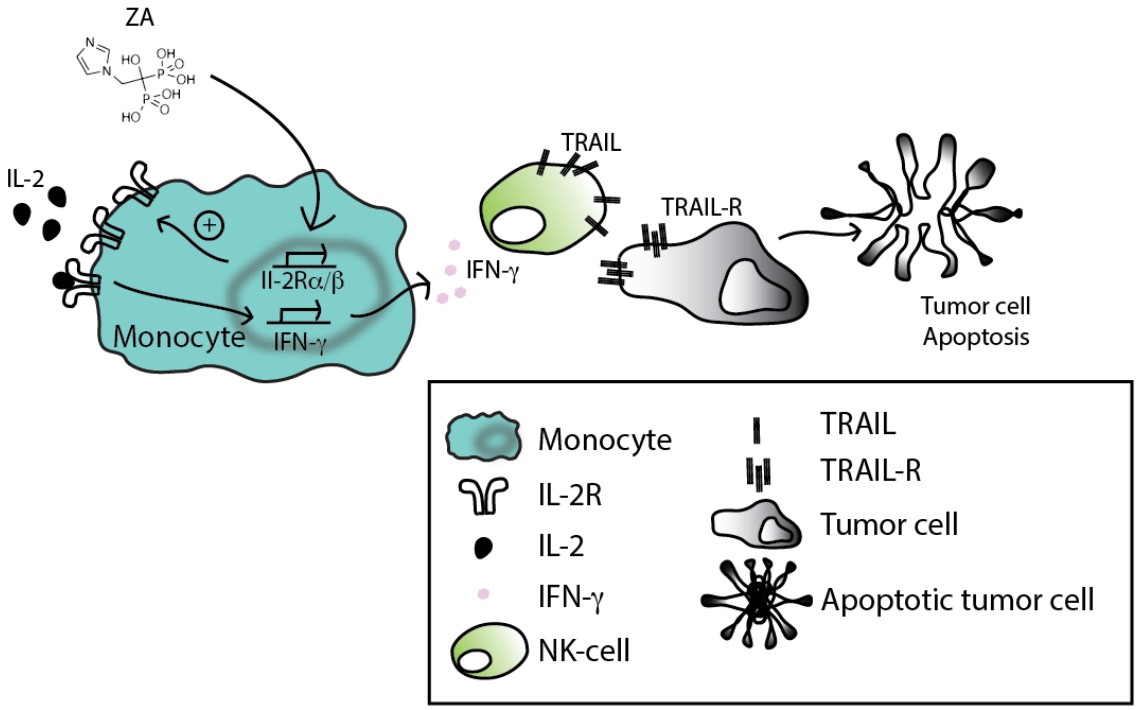

Figure 8. Summary of Study IV. ZA in combination with IL2 induce the expression of IFN-γ from monocytes leading to an up-regulation of TRAIL on NK-cells, inducing their cytotoxicity against tumor cells.

6.6 CONCLUSIONS, SIGNIFICANCES AND FURTHER PERSPECTIVES

Current immunotherapies towards the macrophage/monocyte linages show limited success. Strengthen by **Study I** and **IV** that highlight the importance of myeloid cells in regulating the innate and adaptive immunity to inhibit tumor growth, we believe that modulating the function of the existing pool of TAMs would be a more successful strategy than depleting the whole population. However, in order to do this, a more detailed insight into how TAMs are regulated is needed to identify new opportunities for therapeutic intervention. In this thesis, we identify control of proliferation (**study I and II**) and translation (**study II**) as

possible targets. Additionally, in **study III**, we identify a functional difference between macrophages of different origin showing another window for selective regulation.

As more advanced technologies are developed we will get a better insight into how the complex TAM population is regulated. The possibility to deeply study features of single cells will help us to understand more about the heterogeneity of the TAM population. Additionally, more reliable animal models for linage tracing experiments are emerging, creating new opportunities for studying the ontogeny of macrophages.

7 ACKNOWLEDGEMENTS

I am truly grateful that I have had the possibility to perform my PhD studies in the Rolny lab at the first floor of CCK. My main supervisor **Charlotte**, I could not have found a better place to spend these years, so thank you for letting me do my PhD in your lab. I admire your supportive supervision, it has been great to feel comfortable in that you are never more than a phone call or e-mail away. During the years in your lab you have helped me develop at so many levels, and you make me believe in myself.

My co-supervisors **Andreas** and **Arne**, thank you for taking time guiding me when it has been needed.

Present and former lab members. **Tatjana**, I cannot imagine a better colleague. Our collaborations are always so easy, and the way you have been there when I have needed it is amazing. So thankyou for being a wonderful colleague and friend. **Margarita**, I am happy you came to complete the PhD student crew. Thank you for interesting discussions about science, and everything else. You are a fantastic colleague and friend. **Jeanette** and **Emma**, thank you for valuable contributions to the work and also to great times in the lab. **Pradeepa**, thanks for the happiness you are spreading, it is impossible to not smile when you are around. **Stina, Laura,** and **Hui**, thank you for your positive attitudes and contributions to the group.

I would also like to thank all collaborators and co-authors from outside the Rolny lab that have contributed to the projects in this thesis. Without smooth collaborations, I would never be here today.

I have had the fortune get to know several colleagues by sharing cell-lab over the years. First, **Ola** and his lab-members. **Laia**, I am happy I got to know you when you were at CCK. I hope I did not make you concentrate more on speaking Swedish to me then to take care of your cells. **Vincent** and **Shuo**, thank you for interesting conversations during hours in front of the cell-hoods. **Nikos** and **Dimitris**, thank you for the joy you spread in the cell-lab and the foxes are still a favorite at home. Thank you **Ioannis** for a rewarding collaboration during the last year, it is difficult to not be contaminated by your enthusiasm.

Veronika, thanks for the welcome you gave me when first arriving to CCK, it was great sharing office with you. **Smaralda, Susanne** and **Sue-Lie**, thank you for many fruitful discussions when sharing office. **Kristina,** thank you always spreading positive energy. **Nina,** thank you for enjoyable conversations and fikas.

Dhifaf, Mao, Maarten, Erik, Tanja, Yago, Stina, Ulrika, Jeroen, Linnéa, Tom, Mohammad, Kia, Barbro, Ann and ALL present and former friends and colleagues at the first floor of CCK. Thank you all for creating the friendly atmosphere!

Elle, **Elisabeth**, **Sören**, **Eva-Lena** and **Fix-IT,** thank you for making it easy to concentrate on doing research on CCK, your expertise is invaluable.

Mina vänner utanför forskningen, tack för allt ni gjort under åren, det är så värdefullt att kunna fokusera på annat är forskning ibland. **Pia** och **Madde**, även om vi inte ses lika ofta som vi brukade så är det underbart att veta att ni finns där. **Lena, Simona**, **Marika**, **Sara** och resten av cykelgänget, med er har man aldrig tråkigt! **Sandra**, jag uppskattar verkligen våra lekdejter med tjejerna på helgerna, underbart att få komma ut och umgås så spontant.

Jag är tacksam för den fina stöttningen och välkomnandet jag fått i familjen Wallerius. **Jan** och **Bodil**, mer omtänksamma människor får man leta efter. Tack för att ni alltid finns där och ställer upp, vi är bortskämda med att få hjälp med Lovisa när det behövs trots att vi bor 30 mil ifrån varandra. **Therese**, **Joe**, **Lillie** och **Julia**, alltid lika kul att få skypa och följa tjejernas utveckling till två fantastiska individer.

Mamma och **Pappa**, tack för att ni alltid stöttat och pushat mig på rätt sätt. Ni har sedan tidigt gett mig bästa tänkbara förutsättningar för glädje till lärande och experimenterande.
Mina systrar med familjer, tack för att ni alltid finns där. **Karin**, **Petter** och **Emilia**, det är underbart att ha så mycket gemensamt med er och att vi faktiskt bor på ett avstånd som gör att vi kan umgås mer frekvent. Jag ser fram emot att våra familjer återigen växer i fas med varandra. **Maria** och **Fredrik**, det är alltid lika kul att träffas och få följa era äventyr.

Lovisa, min solstråle. Ingen kan få mig att vara så i nuet som du. Det finns inget bättre än att dras med av din glädje och nyfikenhet på livet. **Bebisarna i magen**, tack för att ni hållit mig sällskap under varje minut av skrivande på denna kappa. Vi ser så fram emot att lära känna er när ni är redo för livet utanför magen. **Tobias**, jag är så otroligt tacksam att jag

träffade just DIG. Tack för din förståelse och stöttning. Tack för alla de gånger du skjutsat mig tidiga mornar och helger, och hämtat mig sena kvällar. De gånger du kommit och hållt mig sällskap och gett mig mat när jag behövt sitta kvar längre än planerat vid FACSen. Tack för att du alltid lyckas göra mig glad och finns där och ställer upp oavsett vad det gäller, du är bäst!

8 REFERENCES

1. Yang L, Zhang Y. Tumor-associated macrophages: from basic research to clinical application. J Hematol Oncol. 2017;10(1):58.
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74.
3. World Health Organization. Cancer 2018 [Available from: http://www.who.int/mediacentre/factsheets/fs297/en/
4. Folkman J, Long DM, Jr., Becker FF. Growth and metastasis of tumor in organ culture. Cancer. 1963;16:453-67.
5. Coussens LM, Raymond WW, Bergers G, Laig-Webster M, Behrendtsen O, Werb Z, et al. Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. Genes Dev. 1999;13(11):1382-97.
6. Lin EY, Li JF, Gnatovskiy L, Deng Y, Zhu L, Grzesik DA, et al. Macrophages regulate the angiogenic switch in a mouse model of breast cancer. Cancer Res. 2006;66(23):11238-46.
7. De Palma M, Biziato D, Petrova TV. Microenvironmental regulation of tumour angiogenesis. Nat Rev Cancer. 2017;17(8):457-74.
8. Rak J, Yu JL, Klement G, Kerbel RS. Oncogenes and angiogenesis: signaling three-dimensional tumor growth. J Investig Dermatol Symp Proc. 2000;5(1):24-33.
9. Stylianopoulos T, Munn LL, Jain RK. Reengineering the Tumor Vasculature: Improving Drug Delivery and Efficacy. Trends Cancer. 2018;4(4):258-9.
10. Ruffell B, DeNardo DG, Affara NI, Coussens LM. Lymphocytes in cancer development: polarization towards pro-tumor immunity. Cytokine Growth Factor Rev. 2010;21(1):3-10.
11. Liu Y, Beyer A, Aebersold R. On the Dependency of Cellular Protein Levels on mRNA Abundance. Cell. 2016;165(3):535-50.
12. Piccirillo CA, Bjur E, Topisirovic I, Sonenberg N, Larsson O. Translational control of immune responses: from transcripts to translatomes. Nat Immunol. 2014;15(6):503-11.
13. Bhat M, Robichaud N, Hulea L, Sonenberg N, Pelletier J, Topisirovic I. Targeting the translation machinery in cancer. Nat Rev Drug Discov. 2015;14(4):261-78.
14. Parker MW, Rossi D, Peterson M, Smith K, Sikstrom K, White ES, et al. Fibrotic extracellular matrix activates a profibrotic positive feedback loop. J Clin Invest. 2014;124(4):1622-35.
15. Hershey JW, Sonenberg N, Mathews MB. Principles of translational control: an overview. Cold Spring Harb Perspect Biol. 2012;4(12).
16. Sonenberg N, Hinnebusch AG. Regulation of translation initiation in eukaryotes: mechanisms and biological targets. Cell. 2009;136(4):731-45.
17. Roux PP, Topisirovic I. Regulation of mRNA translation by signaling pathways. Cold Spring Harb Perspect Biol. 2012;4(11).
18. Ueda T, Watanabe-Fukunaga R, Fukuyama H, Nagata S, Fukunaga R. Mnk2 and Mnk1 are essential for constitutive and inducible phosphorylation of eukaryotic initiation factor 4E but not for cell growth or development. Mol Cell Biol. 2004;24(15):6539-49.
19. Waskiewicz AJ, Flynn A, Proud CG, Cooper JA. Mitogen-activated protein kinases activate the serine/threonine kinases Mnk1 and Mnk2. EMBO J. 1997;16(8):1909-20.
20. Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. Nature. 2013;496(7446):445-55.
21. Pollard JW. Macrophages define the invasive microenvironment in breast cancer. J Leukoc Biol. 2008;84(3):623-30.
22. Coffelt SB, Hughes R, Lewis CE. Tumor-associated macrophages: effectors of angiogenesis and tumor progression. Biochim Biophys Acta. 2009;1796(1):11-8.
23. Bremnes RM, Al-Shibli K, Donnem T, Sirera R, Al-Saad S, Andersen S, et al. The role of tumor-infiltrating immune cells and chronic inflammation at the tumor site on cancer development, progression, and prognosis: emphasis on non-small cell lung cancer. J Thorac Oncol. 2011;6(4):824-33.
24. Epelman S, Lavine KJ, Randolph GJ. Origin and functions of tissue macrophages. Immunity. 2014;41(1):21-35.
25. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science. 2010;330(6005):841-5.
26. Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. Nat Neurosci. 2007;10(12):1538-43.
27. Bain CC, Bravo-Blas A, Scott CL, Perdiguero EG, Geissmann F, Henri S, et al. Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. Nat Immunol. 2014;15(10):929-37.
28. Tamoutounour S, Guilliams M, Montanana Sanchis F, Liu H, Terhorst D, Malosse C, et al. Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. Immunity. 2013;39(5):925-38.

29. Ganesan AP, Johansson M, Ruffell B, Yagui-Beltran A, Lau J, Jablons DM, et al. Tumor-infiltrating regulatory T cells inhibit endogenous cytotoxic T cell responses to lung adenocarcinoma. Journal of immunology (Baltimore, Md : 1950). 2013;191(4):2009-17.
30. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity. 2014;41(1):14-20.
31. DeNardo DG, Barreto JB, Andreu P, Vasquez L, Tawfik D, Kolhatkar N, et al. CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. Cancer Cell. 2009;16(2):91-102.
32. Chiaramonte MG, Mentink-Kane M, Jacobson BA, Cheever AW, Whitters MJ, Goad ME, et al. Regulation and function of the interleukin 13 receptor alpha 2 during a T helper cell type 2-dominant immune response. J Exp Med. 2003;197(6):687-701.
33. Andreu P, Johansson M, Affara NI, Pucci F, Tan T, Junankar S, et al. FcRgamma activation regulates inflammation-associated squamous carcinogenesis. Cancer Cell. 2010;17(2):121-34.
34. Movahedi K, Laoui D, Gysemans C, Baeten M, Stange G, Van den Bossche J, et al. Different tumor microenvironments contain functionally distinct subsets of macrophages derived from Ly6C(high) monocytes. Cancer research. 2010;70(14):5728-39.
35. Baer C, Squadrito ML, Laoui D, Thompson D, Hansen SK, Kiialainen A, et al. Suppression of microRNA activity amplifies IFN-gamma-induced macrophage activation and promotes anti-tumour immunity. Nat Cell Biol. 2016;18(7):790-802.
36. Wallerius M, Wallmann T, Bartish M, Ostling J, Mezheyeuski A, Tobin NP, et al. Guidance Molecule SEMA3A Restricts Tumor Growth by Differentially Regulating the Proliferation of Tumor-Associated Macrophages. Cancer Res. 2016;76(11):3166-78.
37. Biswas SK, Sica A, Lewis CE. Plasticity of macrophage function during tumor progression: regulation by distinct molecular mechanisms. J Immunol. 2008;180(4):2011-7.
38. Karin M, Greten FR. NF-kappaB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol. 2005;5(10):749-59.
39. Zhu Y, Herndon JM, Sojka DK, Kim KW, Knolhoff BL, Zuo C, et al. Tissue-Resident Macrophages in Pancreatic Ductal Adenocarcinoma Originate from Embryonic Hematopoiesis and Promote Tumor Progression. Immunity. 2017;47(3):597.
40. Ohno S, Ohno Y, Suzuki N, Kamei T, Koike K, Inagawa H, et al. Correlation of histological localization of tumor-associated macrophages with clinicopathological features in endometrial cancer. Anticancer Res. 2004;24(5C):3335-42.
41. Burke B, Giannoudis A, Corke KP, Gill D, Wells M, Ziegler-Heitbrock L, et al. Hypoxia-induced gene expression in human macrophages: implications for ischemic tissues and hypoxia-regulated gene therapy. The American journal of pathology. 2003;163(4):1233-43.
42. Talks KL, Turley H, Gatter KC, Maxwell PH, Pugh CW, Ratcliffe PJ, et al. The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. The American journal of pathology. 2000;157(2):411-21.
43. Fang HY, Hughes R, Murdoch C, Coffelt SB, Biswas SK, Harris AL, et al. Hypoxia-inducible factors 1 and 2 are important transcriptional effectors in primary macrophages experiencing hypoxia. Blood. 2009;114(4):844-59.
44. Laoui D, Van Overmeire E, Di Conza G, Aldeni C, Keirsse J, Morias Y, et al. Tumor hypoxia does not drive differentiation of tumor-associated macrophages but rather fine-tunes the M2-like macrophage population. Cancer Res. 2014;74(1):24-30.
45. Guo X, Xue H, Shao Q, Wang J, Guo X, Chen X, et al. Hypoxia promotes glioma-associated macrophage infiltration via periostin and subsequent M2 polarization by upregulating TGF-beta and M-CSFR. Oncotarget. 2016;7(49):80521-42.
46. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, et al. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. Nature. 2014;513(7519):559-63.
47. Gocheva V, Wang HW, Gadea BB, Shree T, Hunter KE, Garfall AL, et al. IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion. Genes Dev. 2010;24(3):241-55.
48. Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. Immunity. 2003;19(1):71-82.
49. Carlin LM, Stamatiades EG, Auffray C, Hanna RN, Glover L, Vizcay-Barrena G, et al. Nr4a1-dependent Ly6C(low) monocytes monitor endothelial cells and orchestrate their disposal. Cell. 2013;153(2):362-75.
50. Hilgendorf I, Gerhardt LM, Tan TC, Winter C, Holderried TA, Chousterman BG, et al. Ly-6Chigh monocytes depend on Nr4a1 to balance both inflammatory and reparative phases in the infarcted myocardium. Circ Res. 2014;114(10):1611-22.
51. Movahedi K, Laoui D, Gysemans C, Baeten M, Stange G, Van den Bossche J, et al. Different Tumor Microenvironments Contain Functionally Distinct Subsets of Macrophages Derived from Ly6C(high) Monocytes. Cancer Res. 2010.

52. Amano SU, Cohen JL, Vangala P, Tencerova M, Nicoloro SM, Yawe JC, et al. Local proliferation of macrophages contributes to obesity-associated adipose tissue inflammation. Cell metabolism. 2014;19(1):162-71.
53. Robbins CS, Hilgendorf I, Weber GF, Theurl I, Iwamoto Y, Figueiredo JL, et al. Local proliferation dominates lesional macrophage accumulation in atherosclerosis. Nature medicine. 2013;19(9):1166-72.
54. Jenkins SJ, Ruckerl D, Cook PC, Jones LH, Finkelman FD, van Rooijen N, et al. Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation. Science. 2011;332(6035):1284-8.
55. Franklin RA, Liao W, Sarkar A, Kim MV, Bivona MR, Liu K, et al. The cellular and molecular origin of tumor-associated macrophages. Science. 2014;344(6186):921-5.
56. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer. 2004;4(1):71-8.
57. Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. Cancer research. 2006;66(2):605-12.
58. De Palma M, Venneri MA, Galli R, Sergi Sergi L, Politi LS, Sampaolesi M, et al. Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. Cancer Cell. 2005;8(3):211-26.
59. Rolny C, Mazzone M, Tugues S, Laoui D, Johansson I, Coulon C, et al. HRG inhibits tumor growth and metastasis by inducing macrophage polarization and vessel normalization through downregulation of PlGF. Cancer cell. 2011;19(1):31-44.
60. Lin EY, Nguyen AV, Russell RG, Pollard JW. Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. J Exp Med. 2001;193(6):727-40.
61. Harney AS, Arwert EN, Entenberg D, Wang Y, Guo P, Qian BZ, et al. Real-Time Imaging Reveals Local, Transient Vascular Permeability, and Tumor Cell Intravasation Stimulated by TIE2hi Macrophage-Derived VEGFA. Cancer Discov. 2015;5(9):932-43.
62. Goswami S, Sahai E, Wyckoff JB, Cammer M, Cox D, Pixley FJ, et al. Macrophages promote the invasion of breast carcinoma cells via a colony-stimulating factor-1/epidermal growth factor paracrine loop. Cancer research. 2005;65(12):5278-83.
63. Wyckoff JB, Wang Y, Lin EY, Li JF, Goswami S, Stanley ER, et al. Direct visualization of macrophage-assisted tumor cell intravasation in mammary tumors. Cancer Res. 2007;67(6):2649-56.
64. Bettinger I, Thanos S, Paulus W. Microglia promote glioma migration. Acta Neuropathol. 2002;103(4):351-5.
65. Wesolowska A, Kwiatkowska A, Slomnicki L, Dembinski M, Master A, Sliwa M, et al. Microglia-derived TGF-beta as an important regulator of glioblastoma invasion--an inhibition of TGF-beta-dependent effects by shRNA against human TGF-beta type II receptor. Oncogene. 2008;27(7):918-30.
66. Zhang J, Sarkar S, Cua R, Zhou Y, Hader W, Yong VW. A dialog between glioma and microglia that promotes tumor invasiveness through the CCL2/CCR2/interleukin-6 axis. Carcinogenesis. 2012;33(2):312-9.
67. Coniglio SJ, Eugenin E, Dobrenis K, Stanley ER, West BL, Symons MH, et al. Microglial stimulation of glioblastoma invasion involves epidermal growth factor receptor (EGFR) and colony stimulating factor 1 receptor (CSF-1R) signaling. Mol Med. 2012;18:519-27.
68. Oberg A, Samii S, Stenling R, Lindmark G. Different occurrence of CD8+, CD45R0+, and CD68+ immune cells in regional lymph node metastases from colorectal cancer as potential prognostic predictors. International journal of colorectal disease. 2002;17(1):25-9.
69. Oosterling SJ, van der Bij GJ, Meijer GA, Tuk CW, van Garderen E, van Rooijen N, et al. Macrophages direct tumour histology and clinical outcome in a colon cancer model. The Journal of pathology. 2005;207(2):147-55.
70. Gottlieb CE, Mills AM, Cross JV, Ring KL. Tumor-associated macrophage expression of PD-L1 in implants of high grade serous ovarian carcinoma: A comparison of matched primary and metastatic tumors. Gynecol Oncol. 2017;144(3):607-12.
71. Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, et al. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. Nature. 2017;545(7655):495-9.
72. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med. 2004;10(9):942-9.
73. Colina R, Costa-Mattioli M, Dowling RJ, Jaramillo M, Tai LH, Breitbach CJ, et al. Translational control of the innate immune response through IRF-7. Nature. 2008;452(7185):323-8.
74. Cook KD, Miller J. TCR-dependent translational control of GATA-3 enhances Th2 differentiation. J Immunol. 2010;185(6):3209-16.
75. Gigoux M, Lovato A, Leconte J, Leung J, Sonenberg N, Suh WK. Inducible costimulator facilitates T-dependent B cell activation by augmenting IL-4 translation. Mol Immunol. 2014;59(1):46-54.

76. Xu H, Zhu J, Smith S, Foldi J, Zhao B, Chung AY, et al. Notch-RBP-J signaling regulates the transcription factor IRF8 to promote inflammatory macrophage polarization. Nat Immunol. 2012;13(7):642-50.
77. Herdy B, Jaramillo M, Svitkin YV, Rosenfeld AB, Kobayashi M, Walsh D, et al. Translational control of the activation of transcription factor NF-kappaB and production of type I interferon by phosphorylation of the translation factor eIF4E. Nat Immunol. 2012;13(6):543-50.
78. Nikolcheva T, Pyronnet S, Chou SY, Sonenberg N, Song A, Clayberger C, et al. A translational rheostat for RFLAT-1 regulates RANTES expression in T lymphocytes. J Clin Invest. 2002;110(1):119-26.
79. Schott J, Reitter S, Philipp J, Haneke K, Schafer H, Stoecklin G. Translational regulation of specific mRNAs controls feedback inhibition and survival during macrophage activation. PLoS Genet. 2014;10(6):e1004368.
80. Liepelt A, Mossanen JC, Denecke B, Heymann F, De Santis R, Tacke F, et al. Translation control of TAK1 mRNA by hnRNP K modulates LPS-induced macrophage activation. RNA. 2014;20(6):899-911.
81. Piecyk M, Wax S, Beck AR, Kedersha N, Gupta M, Maritim B, et al. TIA-1 is a translational silencer that selectively regulates the expression of TNF-alpha. EMBO J. 2000;19(15):4154-63.
82. Su X, Yu Y, Zhong Y, Giannopoulou EG, Hu X, Liu H, et al. Interferon-gamma regulates cellular metabolism and mRNA translation to potentiate macrophage activation. Nat Immunol. 2015;16(8):838-49.
83. Robichaud N, Hsu BE, Istomine R, Alvarez F, Blagih J, Ma EH, et al. Translational control in the tumor microenvironment promotes lung metastasis: Phosphorylation of eIF4E in neutrophils. Proc Natl Acad Sci U S A. 2018;115(10):E2202-E9.
84. Aharinejad S, Paulus P, Sioud M, Hofmann M, Zins K, Schafer R, et al. Colony-stimulating factor-1 blockade by antisense oligonucleotides and small interfering RNAs suppresses growth of human mammary tumor xenografts in mice. Cancer Res. 2004;64(15):5378-84.
85. Ries CH, Cannarile MA, Hoves S, Benz J, Wartha K, Runza V, et al. Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. Cancer cell. 2014;25(6):846-59.
86. Pyonteck SM, Akkari L, Schuhmacher AJ, Bowman RL, Sevenich L, Quail DF, et al. CSF-1R inhibition alters macrophage polarization and blocks glioma progression. Nat Med. 2013;19(10):1264-72.
87. Van Overmeire E, Stijlemans B, Heymann F, Keirsse J, Morias Y, Elkrim Y, et al. M-CSF and GM-CSF Receptor Signaling Differentially Regulate Monocyte Maturation and Macrophage Polarization in the Tumor Microenvironment. Cancer Res. 2016;76(1):35-42.
88. DeNardo DG, Brennan DJ, Rexhepaj E, Ruffell B, Shiao SL, Madden SF, et al. Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. Cancer discovery. 2011;1(1):54-67.
89. Xu J, Escamilla J, Mok S, David J, Priceman S, West B, et al. CSF1R signaling blockade stanches tumor-infiltrating myeloid cells and improves the efficacy of radiotherapy in prostate cancer. Cancer research. 2013;73(9):2782-94.
90. Patwardhan PP, Surriga O, Beckman MJ, de Stanchina E, Dematteo RP, Tap WD, et al. Sustained inhibition of receptor tyrosine kinases and macrophage depletion by PLX3397 and rapamycin as a potential new approach for the treatment of MPNSTs. Clinical cancer research : an official journal of the American Association for Cancer Research. 2014;20(12):3146-58.
91. Priceman SJ, Sung JL, Shaposhnik Z, Burton JB, Torres-Collado AX, Moughon DL, et al. Targeting distinct tumor-infiltrating myeloid cells by inhibiting CSF-1 receptor: combating tumor evasion of antiangiogenic therapy. Blood. 2010;115(7):1461-71.
92. Mok S, Koya RC, Tsui C, Xu J, Robert L, Wu L, et al. Inhibition of CSF-1 receptor improves the antitumor efficacy of adoptive cell transfer immunotherapy. Cancer research. 2014;74(1):153-61.
93. Zhu Y, Knolhoff BL, Meyer MA, Nywening TM, West BL, Luo J, et al. CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. Cancer research. 2014;74(18):5057-69.
94. Georgoudaki AM, Prokopec KE, Boura VF, Hellqvist E, Sohn S, Ostling J, et al. Reprogramming Tumor-Associated Macrophages by Antibody Targeting Inhibits Cancer Progression and Metastasis. Cell Rep. 2016;15(9):2000-11.
95. Lahmar Q, Keirsse J, Laoui D, Movahedi K, Van Overmeire E, Van Ginderachter JA. Tissue-resident versus monocyte-derived macrophages in the tumor microenvironment. Biochim Biophys Acta. 2016;1865(1):23-34.
96. Zacchigna S, Pattarini L, Zentilin L, Moimas S, Carrer A, Sinigaglia M, et al. Bone marrow cells recruited through the neuropilin-1 receptor promote arterial formation at the sites of adult neoangiogenesis in mice. J Clin Invest. 2008;118(6):2062-75.
97. Carrer A, Moimas S, Zacchigna S, Pattarini L, Zentilin L, Ruozi G, et al. Neuropilin-1 identifies a subset of bone marrow Gr1- monocytes that can induce tumor vessel normalization and inhibit tumor growth. Cancer Res. 2012;72(24):6371-81.

98. Casazza A, Fu X, Johansson I, Capparuccia L, Andersson F, Giustacchini A, et al. Systemic and Targeted Delivery of Semaphorin 3A Inhibits Tumor Angiogenesis and Progression in Mouse Tumor Models. Arterioscler Thromb Vasc Biol. 2011;31(4):741-9.
99. Casazza A, Laoui D, Wenes M, Rizzolio S, Bassani N, Mambretti M, et al. Impeding macrophage entry into hypoxic tumor areas by Sema3A/Nrp1 signaling blockade inhibits angiogenesis and restores antitumor immunity. Cancer Cell. 2013;24(6):695-709.
100. Neufeld G, Mumblat Y, Smolkin T, Toledano S, Nir-Zvi I, Ziv K, et al. The role of the semaphorins in cancer. Cell Adh Migr. 2016;10(6):652-74.
101. Jiang H, Qi L, Wang F, Sun Z, Huang Z, Xi Q. Decreased semaphorin 3A expression is associated with a poor prognosis in patients with epithelial ovarian carcinoma. Int J Mol Med. 2015;35(5):1374-80.
102. Li K, Chen MK, Li LY, Lu MH, Shao Ch K, Su ZL, et al. The predictive value of semaphorins 3 expression in biopsies for biochemical recurrence of patients with low- and intermediate-risk prostate cancer. Neoplasma. 2013;60(6):683-9.
103. Bagci T, Wu JK, Pfannl R, Ilag LL, Jay DG. Autocrine semaphorin 3A signaling promotes glioblastoma dispersal. Oncogene. 2009;28(40):3537-50.
104. Tang C, Gao X, Liu H, Jiang T, Zhai X. Decreased expression of SEMA3A is associated with poor prognosis in gastric carcinoma. Int J Clin Exp Pathol. 2014;7(8):4782-94.
105. Staton CA, Shaw LA, Valluru M, Hoh L, Koay I, Cross SS, et al. Expression of class 3 semaphorins and their receptors in human breast neoplasia. Histopathology. 2011;59(2):274-82.
106. DuPre SA, Redelman D, Hunter KW, Jr. The mouse mammary carcinoma 4T1: characterization of the cellular landscape of primary tumours and metastatic tumour foci. Int J Exp Pathol. 2007;88(5):351-60.
107. Lin EY, Jones JG, Li P, Zhu L, Whitney KD, Muller WJ, et al. Progression to malignancy in the polyoma middle T oncoprotein mouse breast cancer model provides a reliable model for human diseases. Am J Pathol. 2003;163(5):2113-26.
108. Geeraerts X, Bolli E, Fendt SM, Van Ginderachter JA. Macrophage Metabolism As Therapeutic Target for Cancer, Atherosclerosis, and Obesity. Front Immunol. 2017;8:289.
109. Forrest GL, Gonzalez B. Carbonyl reductase. Chem Biol Interact. 2000;129(1-2):21-40.
110. Wang T, Liu G, Wang R. The Intercellular Metabolic Interplay between Tumor and Immune Cells. Front Immunol. 2014;5:358.
111. Dubois LG, Campanati L, Righy C, D'Andrea-Meira I, Spohr TC, Porto-Carreiro I, et al. Gliomas and the vascular fragility of the blood brain barrier. Front Cell Neurosci. 2014;8:418.
112. Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR, et al. The somatic genomic landscape of glioblastoma. Cell. 2013;155(2):462-77.
113. Kim Y, Kim E, Wu Q, Guryanova O, Hitomi M, Lathia JD, et al. Platelet-derived growth factor receptors differentially inform intertumoral and intratumoral heterogeneity. Genes Dev. 2012;26(11):1247-62.
114. Urbanska K, Sokolowska J, Szmidt M, Sysa P. Glioblastoma multiforme - an overview. Contemp Oncol (Pozn). 2014;18(5):307-12.
115. Bowman RL, Klemm F, Akkari L, Pyonteck SM, Sevenich L, Quail DF, et al. Macrophage Ontogeny Underlies Differences in Tumor-Specific Education in Brain Malignancies. Cell Rep. 2016;17(9):2445-59.
116. Quail DF, Joyce JA. The Microenvironmental Landscape of Brain Tumors. Cancer Cell. 2017;31(3):326-41.
117. Komohara Y, Ohnishi K, Kuratsu J, Takeya M. Possible involvement of the M2 anti-inflammatory macrophage phenotype in growth of human gliomas. J Pathol. 2008;216(1):15-24.
118. Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, Gabriely G, et al. Identification of a unique TGF-beta-dependent molecular and functional signature in microglia. Nat Neurosci. 2014;17(1):131-43.
119. Bennett ML, Bennett FC, Liddelow SA, Ajami B, Zamanian JL, Fernhoff NB, et al. New tools for studying microglia in the mouse and human CNS. Proc Natl Acad Sci U S A. 2016;113(12):E1738-46.
120. Hebbeler AM, Cairo C, Cummings JS, Pauza CD. Individual Vgamma2-Jgamma1.2+ T cells respond to both isopentenyl pyrophosphate and Daudi cell stimulation: generating tumor effectors with low molecular weight phosphoantigens. Cancer Immunol Immunother. 2007;56(6):819-29.
121. Maniar A, Zhang X, Lin W, Gastman BR, Pauza CD, Strome SE, et al. Human gammadelta T lymphocytes induce robust NK cell-mediated antitumor cytotoxicity through CD137 engagement. Blood. 2010;116(10):1726-33.
122. Giraudo E, Inoue M, Hanahan D. An amino-bisphosphonate targets MMP-9-expressing macrophages and angiogenesis to impair cervical carcinogenesis. J Clin Invest. 2004;114(5):623-33.
123. Santini D, Vincenzi B, Dicuonzo G, Avvisati G, Massacesi C, Battistoni F, et al. Zoledronic acid induces significant and long-lasting modifications of circulating angiogenic factors in cancer patients. Clin Cancer Res. 2003;9(8):2893-7.