Johanna A. Joyce

CURRICULUM VITAE

Institution: Department of Oncology
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Date of Birth: 28th March 1972

Nationality: Irish

Education

1990–1994	B.A. (Hons) in Genetics, Trinity College Dublin, Ireland
1995–1999	Ph.D. in Biology, University of Cambridge, UK

Employment History – Research Experience

Present	Full Professor, Department of Oncology, University of Lausanne, Switzerland
Present	Full Member, Ludwig Institute for Cancer Research, Lausanne Branch, Switzerland
2014–2015	Full Member, Memorial Sloan Kettering Cancer Center, New York, USA
2014–2015	Full Professor, Weill Cornell Graduate School of Medical Sciences, NY, USA
2010–2014	Associate Member, Memorial Sloan Kettering Cancer Center, New York, USA
2010–2014	Associate Professor, Weill Cornell Graduate School of Medical Sciences, NY, USA
2005–2010	Assistant Professor, Weill Cornell Graduate School of Medical Sciences, NY, USA
2004–2010	Assistant Member, Memorial Sloan Kettering Cancer Center, New York, USA

1999–2004	Postdoctoral Fellow, Laboratory of Prof. Douglas Hanahan, Dept. of Biochemistry, University of California at San Francisco, California, USA
1995–1999	Ph.D. Student, Laboratory of Dr. Paul Schofield, University of Cambridge, UK
1994–1995	Postgraduate Researcher, Lab of Prof. Eamonn Maher, University of Cambridge, UK

Honours and Awards

2018	Cloëtta Prize
2017	EMBO Member
2017	Pandolfi Award for Women in Cancer Research (Harvard Medical School)
2017	Swiss Bridge Award
2017	Elected Fellow of European Academy of Cancer Sciences
2012	American Cancer Society Scholar
2011	Boyer Young Investigator Award
2007	Geoffrey Beene Junior Faculty Chair
2005	Rita Allen Foundation Scholar
2005	Sidney Kimmel Foundation Scholar
2005	V Foundation Scholar
2001	Leukemia and Lymphoma Foundation Fellow
1998	Cambridge Philosophical Society Fellow
1995	British Biological Sciences Research Council Fellow

Editorial and International Advisory Boards

2017–present	Editorial Board of *The Journal of Experimental Medicine*
2017–present	Editorial Board of *Genes and Development*
2015–present	Editorial Board of *Trends in Cancer*
2011–present	Editorial Board of *Cell Reports*
2018–present	Scientific Advisory Board, CRUK Cambridge Institute and Cancer Center, UK

2016–present	Scientific Advisory Board, IRB Institute, Barcelona, Spain
2016–2017	Elected Chairperson of AACR Tumor Microenvironment Working Group
2014–2018	Steering Committee, AACR Tumor Microenvironment Working Group
2007–2011	Elected to Council of the International Proteolysis Society

Selected Funding

Swiss Bridge Award (1/1/2018–31/12/2019)
Investigating and Therapeutically Targeting Neutrophils in Brain Metastasis

Swiss Cancer League (1/1/2017–31/12/2019)
Targeting Tumor-Associated Macrophages to Enhance Therapeutic Efficacy in Gliomas

Cancer Research UK Grand Challenge Team Award (1/5/2017–30/4/2023)
IMAXT: Imaging and Molecular Annotation of Xenografts and Tumors

Breast Cancer Research Foundation (1/10/2009–present)
Microenvironmental Regulation of Breast Cancer Metastasis and Therapeutic Response

National Cancer Institute, R01 grant (1/7/2014–30/6/2019)
Investigating and Targeting TAMs in the Glioma Microenvironment

Roche Strategic Alliance (1/11/2016–31/10/2018)
Combinatorial Strategies for Therapeutically Targeting the Glioma Microenvironment

Alan and Sandra Gerry Foundation (1/8/2014–7/31/2016)
Investigation of the Microenvironmental Transcriptome in Breast to Brain Metastasis

ONO Pharmaceuticals (1/7/2015–30/6/2016)
Small Molecule Inhibitor Screen for M2 Macrophage Reprogramming

American Cancer Society (1/1/2012–31/12/2015)
Interleukins, Cathepsin Proteases and Macrophages in the Tumor Microenvironment

Rosenkranz Foundation (1/1/2014–31/12/2015)
Investigating Tumor-Stromal Interactions in Pancreatic Neuroendocrine Tumors

Health Research Science Board of New York (1/9/2013–31/8/2015)
Targeting Interactions Between Cancer and the Microenvironment in Breast to Brain Metastasis

National Cancer Institute, Center for Cancer Systems Biology team grant (1/2/2010–31/1/2015)
Systems Biology of Diversity in Cancer

National Cancer Institute, R01 grant (1/7/2007–31/5/2012)
Dissecting the Function of Cysteine Cathepsins in the Tumor Microenvironment

National Cancer Institute, U54 team grant (1/10/2006–30/9/2011)
Tumor-Host Interactions in the Tissue Microenvironment of Brain Tumors and Metastases

Geoffrey Beene Foundation, Endowed Junior Faculty Chair (1/3/2007–31/12/2011)

Emerald Foundation (1/1/2007–31/12/2010)
Understanding and Targeting the Roles of Cathepsin Proteases in Cancer Growth and Metastasis

Rita Allen Foundation, Scholar Award (1/9/2005–31/8/2008)
Molecular Dissection of the Tumor Microenvironment

The V Foundation for Cancer Research, Scholar Award (1/9/2005–31/8/2007)
Dissecting the Role of Cysteine Cathepsins in Cancer

Sidney Kimmel Foundation for Cancer Research, Scholar Award (1/7/2005–30/6/2007)
Genetic Analysis of Cathepsins in Cancer Development

SELECTED PUBLICATIONS

1. Quail DF*, Olson OC*, Bhardwaj P, Walsh LA, Akkari L, Quick M, Chen IC, Wendel N, Ben-Chetrit N, Walker J, Holt PR, Dannenberg AJ, Joyce JA (2017). Obesity alters the lung myeloid cell landscape to enhance breast cancer metastasis via IL5 and GM-CSF. *Nature Cell Biology* 19: 974–987.

2. Olson OC, Kim H, Quail DF, Foley EA, Joyce JA (2017). Tumor-associated macrophages suppress the cytotoxic activity of antimitotic agents. *Cell Reports* 19: 101–113.

3. Quail D and Joyce JA (2017). The microenvironmental landscape of brain tumors. *Cancer Cell* 31: 326–341.

4. Quail DF, Bowman RL, Akkari L, Quick ML, Schuhmacher AJ, Huse JT, Holland EC, Sutton JC, Joyce JA (2016). The tumor microenvironment underlies acquired resistance to CSF-1R inhibition in gliomas. *Science* 352: aad3018.

5. Bowman RL, Klemm F, Akkari L, Pyonteck SM, Sevenich L, Quail DF, Dhara S, Simpson K, Gardner EE, Iacobuzio-Donahue C, Brennan CW, Tabar V, Gutin PH, Joyce JA (2016). Macrophage ontogeny underlies differences in tumor-specific education in brain malignancies. *Cell Reports* 17: 2445–2459.

6. Sevenich L, Bowman R*, Mason SD*, Quail DF, Rapaport F, Elie BT, Brogi E Brastianos P, Hahn WC, Holsinger L, Massague J, Leslie CS, Joyce JA (2014). Analysis of tumour and stroma-supplied proteolytic networks reveals a brain metastasis-promoting role for cathepsin S. *Nature Cell Biology* 16: 876–888.

7. Pyonteck SM*, Akkari L*, Schuhmacher AJ*, Bowman RL, Sevenich L, Quail D, Olson OC, Quick M, Huse J, Teijeiro V, Setty M, Leslie C, Oei Y, Pedraza A, Zhang J, Brennan CW, Sutton JC, Holland EC, Daniel D, Joyce JA (2013). CSF-1R inhibition alters macrophage polarization and blocks glioma progression. *Nature Medicine* 19: 1264–1272.

8. Quail DF and Joyce JA (2013). Microenvironmental regulation of tumor progression and metastasis. *Nature Medicine* 19: 1423–1437.

9. Shree T*, Olson OC*, Elie BT, Kester JC, Garfall AL, Simpson K, Bell-McGuinn KM, Zabor EC, Brogi E, Joyce JA (2011). Macrophages and cathepsin proteases blunt chemotherapeutic response in breast cancer. *Genes and Development* 25: 2465–2479.

10. Gocheva V*, Wang HW*, Gadea BB, Shree T, Hunter KE, Garfall A, Berman T, Joyce JA (2010). IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion. *Genes and Development* 24: 241–255.

(Full publication list and PDFs of all published articles: http://joycelab.org/publications)

EXPLORING AND THERAPEUTICALLY EXPLOITING THE TUMOR MICROENVIRONMENT

Johanna Joyce

Summary

Cancers do not arise within a vacuum; rather they develop and grow within complex organs and tissue environments that critically regulate the fate of tumor cells at each sequential step of malignant progression. The tumor microenvironment (TME) can be viewed as an intricate ecosystem populated by diverse innate and adaptive immune cell types, stromal cells, extracellular matrix, blood and lymphatic vessel networks that are embedded along with the cancer cells. While bidirectional communication between cells and their microenvironment is critical for normal tissue homeostasis, this active dialog can become subverted in cancer leading to tumor initiation and progression. Through their exposure to tumor-derived molecules, normal cells can become "educated" to actually promote cancer development. As a consequence of this tumor-mediated education, TME cells produce a plethora of growth factors, chemokines, and matrix-degrading enzymes that together enhance the proliferation and invasion of the tumor. Moreover, these conscripted normal cells also provide a support system for cancer cells to fall back on following traditional therapies such as chemotherapy and radiation, and additionally contribute to a general immune-suppressive state, thus limiting the efficacy of immunotherapies. Consequently, multi-targeted approaches in which co-opted cells in the microenvironment are "re-educated" to actively fight the cancer represent a promising strategy for the effective long-term treatment of this devastating disease.

Introduction

Tumors contain diverse cell types and inflammatory mediators within their TME, including endothelial cells, fibroblasts, tissue-resident and peripherally-derived immune cells, among others (Joyce, 2005; Quail and Joyce, 2013; Quail and Joyce, 2017c) (Fig. 1). Depending on the organ, there are also unique compositions of tissue-specific resident cell types and extracellular matrix molecules, which can affect tumor development in different ways (Joyce and Pollard, 2009; Quail and Joyce, 2017a). Indeed, tumor progression is not only dictated by genetic alterations within the cancer cells, but also by whether the surrounding niche is permissive to growth at each stage of disease. Thus, a full mechanistic understanding of both tumor cell-intrinsic and -extrinsic mediators of malignant progression is critical to optimize therapeutic strategies against cancer.

Interactions between tumor cells and the associated stroma and cells of the immune system profoundly influences cancer initiation, progression and patient prognosis. The link between chronic inflammation and tumorigenesis was first proposed by Rudolf Virchow in 1863 following his seminal observation that infiltrating leukocytes are a hallmark of tumors (Virchow, 1863). Another clinical investigator of that era, Stephen Paget, specifically recognized the importance of the microenvironment in determining the organ-tropism of metastasis, leading to his seminal "seed and soil" hypothesis published in 1889 (Paget, 1889). Paget stated that "when a plant goes to seed, its seeds are carried in all directions; but they can only live and grow if they fall in congenial soil". However, despite these and other key findings dating back to the late 19th century, for many subsequent decades the TME was overlooked, as researchers predominantly focused on identifying the genetic drivers of cancer.

In recent years the TME field has exploded, with a plethora of studies contributing to a molecular and cellular understanding of the importance and complexity of the TME, further complicating the already challenging task of understanding and treating cancer. Thus, while cancer was long viewed as a heterogeneous disease driven by DNA mutations and genomic alterations in tumor cells, it is now evident that tumors are similarly diverse by nature of their microenvironmental composition. Moreover, in response to evolving environmental conditions and oncogenic

signals from growing tumors, the TME continually changes over the course of cancer progression and in the context of therapeutic intervention. This underscores the need to investigate the influences of the TME as a dynamic process and to understand how cancer cells drive the construction and evolution of their own niche.

In contrast to cancer cells, immune and stromal cell types within the TME are genetically stable and thus represent an attractive therapeutic target with reduced risk of resistance and tumor recurrence (Joyce, 2005; Quail and Joyce, 2013). However, specifically disrupting the pro-tumorigenic TME is a challenging task, as the TME has diverse capacities to induce either beneficial or adverse consequences for tumorigenesis, in a context- and stage-dependent manner. Indeed, the microenvironment is capable of normalizing cancer cell behavior, leading to the notion that re-education of immune and stromal cells, rather than their targeted ablation *per se,* could be a more effective strategy for effectively treating cancer (Quail and Joyce, 2013; Bowman and Joyce, 2014).

In this review, I will discuss the importance of the TME as a potent regulator of cancer development, metastasis, and therapeutic response. In the general overviews of each of these processes I have referred to several reviews I have written on these topics with different members of my lab and other colleagues over the years. I have included examples of my lab's contributions to understanding these different processes during the past decade and more, following the tradition of the Max Cloëtta Series.

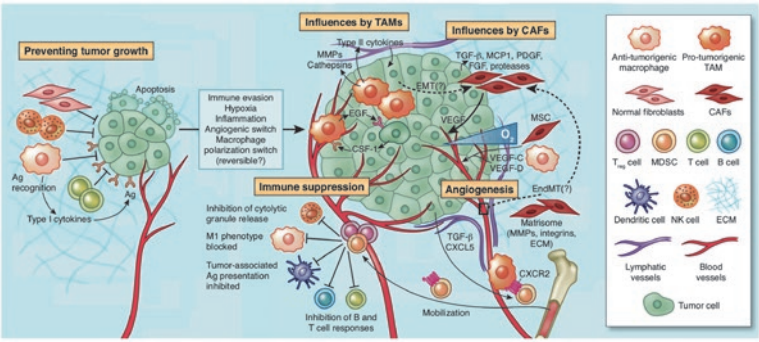

Figure 1: *Multiple stromal cell types converge to support a tumorigenic primary niche. After circumventing cell-intrinsic mechanisms of apoptosis, tumor cells are subject to elimination pressures by the immune system. Tumor cell-specific antigens have a role during this process, which are recognized by cytotoxic immune cells, leading to their destruction. Fibroblasts and macrophages within the tumor microenvironment (TME) also contribute to a growth-suppressive state; however, these cells may later become educated by the tumor to acquire pro-tumorigenic functions. For instance, tumor-associated macrophages (TAMs) support diverse phenotypes within the primary tumor, including growth, angiogenesis and invasion, by secreting a plethora of pro-tumorigenic proteases, cytokines and growth factors. As tumors grow, immune-suppressor cells, including myeloid-derived suppressor cells (MDSCs) and regulatory T cells are mobilized into the circulation in response to activated cytokine axes that are induced by tumorigenesis, and infiltrate the growing tumor to disrupt immune surveillance through multiple mechanisms, including, but not limited to, disruption of antigen presentation by dendritic cells, inhibition of T and B cell proliferation and activation, or inhibition of natural killer (NK) cell cytotoxicity. Cancer-associated fibroblasts (CAFs), which become activated by tumor-derived factors, secrete extracellular matrix (ECM) proteins and basement membrane components, regulate differentiation, modulate immune responses and contribute to deregulated homeostasis. In addition to cellular contributions, several extracellular properties contribute to tumor progression, including low oxygen tension, high interstitial fluid pressure and changes in specific components of the ECM. From Quail and Joyce, Nature Medicine (2013).*

Tumor-associated macrophages are key regulators of cancer initiation and progression

One of the critical regulatory cell types in the TME are tumor-associated macrophages (TAMs) (Noy and Pollard, 2014), which can constitute up to 30-50 % of the tumor mass in some cancers. Analysis of clinical samples has shown that in the vast majority of malignancies, high TAM numbers are associated with more aggressive disease and poor patient prognosis, indicating tumor-promoting functions for these cells (Bingle et al., 2002; Zhang et al., 2012; Fridman et al., 2017). At the time of initiating our research program on TAMs over a decade ago, there was a limited understanding of precisely how TAMs regulate tumorigenesis. This led us to ask several critical questions: How are normal macrophages converted or "educated" to TAMs? What are the molecular and cellular changes that characterize TAMs? What are the mechanisms by which TAMs then regulate tumor progression, and can TAMs be therapeutically targeted? Do TAMs modulate the response to traditional or molecularly targeted anti-cancer agents, and if so, will combinatorial targeting approaches enhance therapeutic efficacy?

To answer these questions, we have investigated distinct TMEs and devised complementary experimental strategies by integrating the analysis of patient samples, diverse mouse models, *in vivo* cell lineage tracing, *ex vivo* tissue and cell culture systems, and a comprehensive panel of computational analyses. We have focused on primary tumors in the brain, breast, and pancreas, in addition to investigating metastases that disseminate to the brain, lung, or bone. Through these diverse and illuminating methodologies, we have been fortunate to make a number of key conceptual advances in the TME field as summarised below (see Figure 2 for schematic).

We have identified key molecular mechanisms driving the education of tumor-promoting macrophages in the pancreas and breast (Gocheva et al., 2010b; Yan et al., 2016), and uncovered the epigenetic and transcriptomic regulatory machinery underlying differential ontogeny and tumor-mediated education between distinct macrophage populations in the brain (Bowman et al., 2016). We found that a critical molecular difference between normal macrophages and TAMs is the increased activity

of matrix-degrading enzymes, specifically cathepsin proteases and heparanase (Gocheva et al., 2010b; Hunter et al., 2014), and identified the upstream cytokines responsible for their induction (Yan et al., 2016) (Figure 2, and discussed further below).

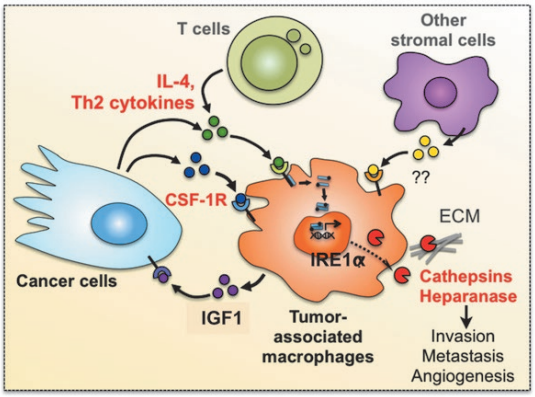

Figure 2: *Model of reciprocal interactions between cancer cells, tumor-associated macrophages and additional cells in the tumor microenvironment that enhance malignant progression. IL-4 and other Th2 cytokines are produced by cancer cells and T cells in the TME, leading to an increase in protease activity in tumor-associated macrophages (TAMs) that promotes several hallmarks of cancer, including angiogenesis, invasion and metastasis. Schematic depicts data compiled from: Gocheva, Wang et al, Genes Dev (2010); Pyonteck, Akkari, Schuhmacher et al, Nat Med (2013); Akkari et al, Genes Dev (2014); Hunter et al, Oncogene (2014); Sevenich et al, Nat Cell Biol (2014); Yan, Wang, Bowman and Joyce, Cell Reports (2016); Quail et al, Science (2016).*

Through our exploration of the molecular differences between TAMs and their normal counterparts, we also became intrigued as to whether tissue-resident macrophages, such as microglia in the brain, differ from peripherally-recruited macrophages in terms of gene expression, epigenetic regulation and tumorigenic functions. To address this question, Robert Bowman, a graduate student in my lab, used complementary cell lineage-tracing genetic models to selectively distinguish resident microglia (MG) from bone marrow-derived macrophages (BMDMs) recruited from the periphery (Bowman et al., 2016). Using this strategy, he investigated the epigenetic and transcriptomic regulatory machinery underlying dif-

ferential ontogeny and tumor-mediated education between MG and BMDMs in multiple brain malignancies, including gliomas and breast-to-brain metastasis. Interestingly, we found there are distinct transcriptional networks in MG and BMDMs associated with tumor-mediated education, which are also influenced by differential chromatin landscapes that are established before tumor initiation (Figure 3). We showed that microglia specifically repress the integrin subunit Itga4 (CD49D), enabling its utility as a discriminatory marker between BMDMs and MG in primary and metastatic disease in mouse models and patient samples. We concluded that while macrophages have been shown to acquire tissue-resident traits upon entry into an organ (Lavin et al., 2014; Lavin et al., 2015), an inflammatory microenvironment, such as in the context of cancer or neuroinflammation, can further amplify differences between cell populations leading to diverse functional outcomes for tissue-resident and peripherally-derived macrophage populations. These results (Bowman et al., 2016) collectively have important implications for targeting TAMs in brain malignancies, and other cancers.

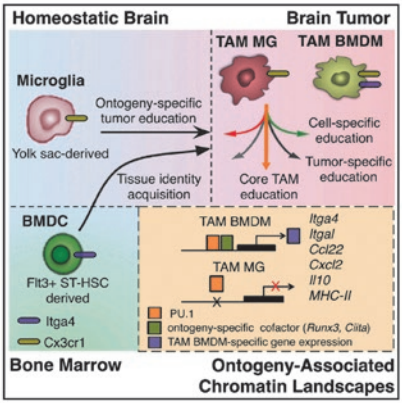

Figure 3: *Macrophage ontogeny underlies differences in tumor-specific education in brain malignancies. Genetic lineage tracing models were used to interrogate the ontogeny of tumor-associated macrophages in brain malignancy. We found that bone-marrow-derived macrophages (BMDMs) and tissue-resident microglia (MG) are present in glioma and brain metastases, and show distinct transcriptional and chromatin states. We identified a number of differentially-expressed genes, as indicated in this schematic, which clearly distinguish these cell populations. From Bowman et al, Cell Reports (2016).*

Therapeutic targeting of TAMs in cancer

To evaluate the therapeutic potential of targeting TAMs, we have taken a number of different approaches, including pharmacological inhibition or genetic ablation of colony stimulating factor-1 (CSF-1) signaling (Quail and Joyce, 2017b), which is a key mediator of macrophage survival and differentiation (Noy and Pollard, 2014). We began by investigating the consequences of CSF-1 deletion using a pancreatic neuroendocrine cancer mouse model (RIP1-Tag2), as we had simultaneously revealed key functions for TAM-supplied cathepsins in RIP1-Tag2 mice (see below). Stephanie Pyonteck, a graduate student in my lab, found that CSF-1 deletion led to TAM depletion in the pancreas and a substantial reduction in cumulative tumor burden (Pyonteck et al., 2012). Interestingly, she determined that this resulted from a significant decrease in the initial angiogenic switching of progenitor lesions and subsequent development of tumors, rather than an evident effect on tumor growth. This study thus revealed important functions for TAMs at the earliest stages of tumor initiation, thereby expanding the repertoire of TAM functions beyond the promotion of advanced malignancy and metastasis. In collaboration with our colleague Laura Tang, at MSKCC in New York, we also analyzed a cohort of tissue samples from human pancreatic neuroendocrine tumors (PanNETs). We found that elevated TAM number in the pancreas increased with tumor grade, as in the mouse model, and importantly showed that this can be prognostic for PanNET patients that develop liver metastases (Pyonteck et al., 2012) (Figure 4).

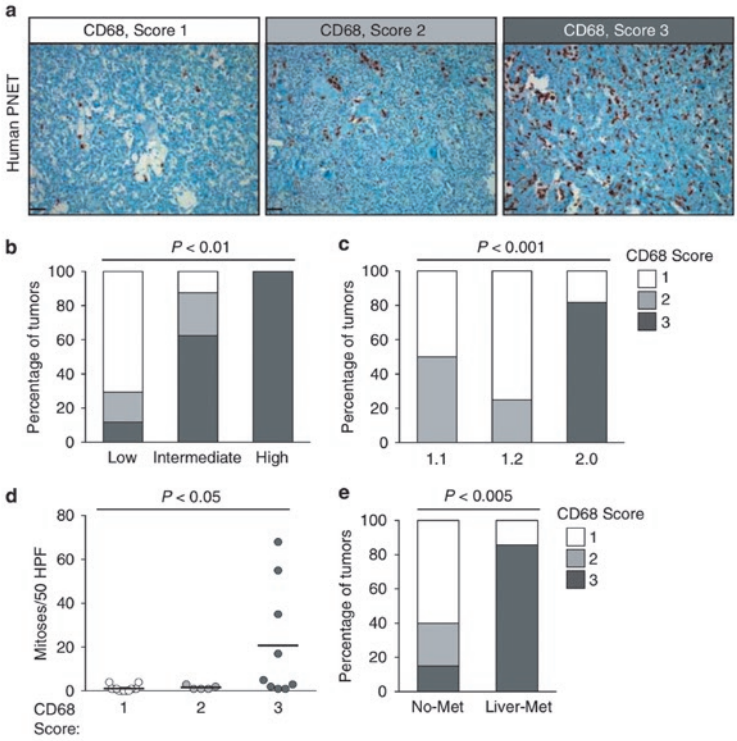

Figure 4: *Macrophage infiltration positively correlates with aggressiveness of human pancreatic neuroendocrine tumors (PanNETs). (a) PanNET patient tissue sections were immunohistochemically stained for macrophages with a CD68 antibody (brown color). Stained tissue sections were then blindly scored by two independent investigators for the CD68+ macrophage density and classified into low (CD68 score 1), medium (score 2) or high (score 3). Representative images are depicted in (a). Scale bar, 50 um. (b–e) CD68 scores for each tumor were then de-coded and matched with their corresponding clinicopathological data: (b) histological tumor grade; (c) WHO tumor stage; (d) number of mitoses per 50 high-powered fields (HPF); (e) the absence and presence of distant metastasis to the liver. Fisher's exact test was used for statistical analyses. From Pyonteck et al., Oncogene (2012).*

Another tumor type in which TAMs are highly abundant and associated with aggressive disease are gliomas that arise in the brain. We and others have found that tumor-associated macrophages and microglia together can comprise up to 30% of the total tumor mass in glioblastomas, thus representing the most abundant non-cancerous cell type in this high-grade malignancy (Hussain et al., 2006; Komohara et al., 2008; Bowman et al., 2016; Quail and Joyce, 2017a). Most therapeutic approaches directly targeting tumor cells in glioblastoma have failed. We therefore proposed an alternative strategy: to target TAMs in the brain TME. Stephanie Pyonteck, Leila Akkari, Alberto Schuhmacher and several other key lab members teamed up to work on this exciting project. We used an inhibitor of the CSF-1 receptor, CSF-1R, to target TAMs in mouse glioblastoma models developed by our collaborator Eric Holland, who was also then at MSKCC. Treatment with this selective inhibitor (BLZ945 from Novartis) regressed established high-grade tumors, even after just one week of treatment (Pyonteck et al., 2013). We found that CSF-1R inhibition markedly increased tumor cell apoptosis and phagocytosis *in vivo,* while decreasing proliferation and glioma malignancy, and significantly extending survival (Figure 5).

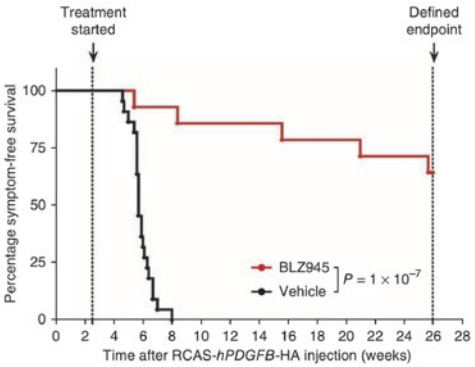

Figure 5: *CSF-1R inhibition specifically targets macrophages, improves survival and decreases glioma malignancy in the transgenic PDGF-driven glioma (PDG) mouse model. Symptom-free survival curves are shown for PDG mice treated in an early intervention trial with a CSF-1R inhibitor BLZ945 (red) or vehicle (black), demonstrating a dramatic increase in survival following CSF-1R inhibition. From Pyonteck, Akkari, Schuhmacher et al, Nature Medicine (2013).*

Surprisingly, we found that while microglia in the normal brain were depleted, as expected, TAM numbers were not reduced in gliomas of the treated mice. Instead, we identified glioma-secreted factors, including GM-CSF and IFN-γ, which facilitated TAM survival in the face of CSF-1R inhibition (Pyonteck et al., 2013). Gene expression analysis of these surviving TAMs revealed a decrease in alternatively activated/ M2-like macrophage markers, consistent with their impaired tumor-promoting functions and enhanced capacity to phagocytose glioma cells. CSF-1R blockade additionally slowed intracranial tumor growth of multiple patient-derived glioma xenografts. Subsequent preclinical trials by Dongyao Yan, a postdoc in my lab, using a chemically-distinct CSF-1R inhibitor (PLX3397 from Plexxikon) showed a similar therapeutic efficacy and macrophage reprogramming (Yan et al., 2017).

Together, our results revealed a new therapeutic strategy for targeting the TME. Rather than depleting TME cells, as had been the goal with many microenvironment-targeted therapies up to that point, we proposed that "re-educating" these cells has the potential to not only abolish their tumor-promoting functions but also actively enlist them as suppressors of tumorigenesis (Quail and Joyce, 2013; Bowman and Joyce, 2014). This body of research has had an important impact in the TME field, and on the therapeutic evaluation of CSF-1R inhibitors in glioma patients.

This representative study (Pyonteck et al., 2013) and many others from colleagues in the TME field indicate that therapies targeted against the TME offer a promising approach for targeting cancer (Quail and Joyce, 2013; Binnewies et al., 2018). However, it remained unclear whether resistance may develop to TME therapies over time. Given that TME-targeted agents are increasingly being evaluated in the clinic, it was critical to mechanistically define how resistance may evolve in response to these therapies in order to provide long-term disease management for patients. We therefore addressed this important question by further investigating the case of CSF-1R inhibition of TAMs in gliomas, and extended the original preclinical trial design to treat mice over many months following the development of high-grade bulky glioblastoma. In this case, Daniela Quail, the postdoc in my lab who led this study, found that while overall survival was dramatically prolonged following CSF-1R inhibition, tumors eventually recurred in ~50 % of mice (Quail et al., 2016), allowing us

to explore the underlying mechanisms. Interestingly, upon isolation and transplantation of tumor cells from recurrent gliomas into naïve animals, Daniela found that sensitivity to CSF-1R inhibition was re-established, indicating that the resistance was in fact driven by the microenvironment.

Through RNA-sequencing of glioma cells and TAMs purified from treated tumors, and *ex vivo* cell culture assays, we discovered an elevation in PI3K pathway activity in recurrent glioblastoma following CSF-1R inhibition, which was driven by macrophage-derived IGF-1 and tumor cell IGF-1R (Quail et al., 2016) (Figure 6). Consequently, combining IGF-1R or PI3K blockade with continuous CSF-1R inhibition in recurrent tumors dramatically extended overall survival. By contrast, monotherapy with IGF-1R or PI3K inhibitors in rebound or treatment-naïve tumors was minimally effective, indicating the necessity of combination therapy to expose PI3K signaling dependency in recurrent disease. Mechanistically, Daniela found that T cell-derived IL4 led to macrophage activation in recurrent tumors, and elevated STAT6 and NFAT signaling upstream of IGF-1 induction. Similarly, inhibition of any of these pathways *in vivo* was also sufficient to significantly extend survival when combined with CSF-1R inhibition (Quail et al., 2016). Given that PI3K signaling is aberrantly activated in a substantial proportion of glioma patients, including through mutations in PTEN and other PI3K pathway components, it is possible that this pathway could also contribute to intrinsic resistance to CSF-1R inhibition. Our findings thus revealed the importance of continuous bidirectional feedback between cancer cells and the TME, and support the notion that although immune and stromal cells are less susceptible to genetic mutation than are cancer cells, a tumor can nonetheless acquire a resistant phenotype by exploiting its extracellular environment (Quail et al., 2016; Quail and Joyce, 2017b).

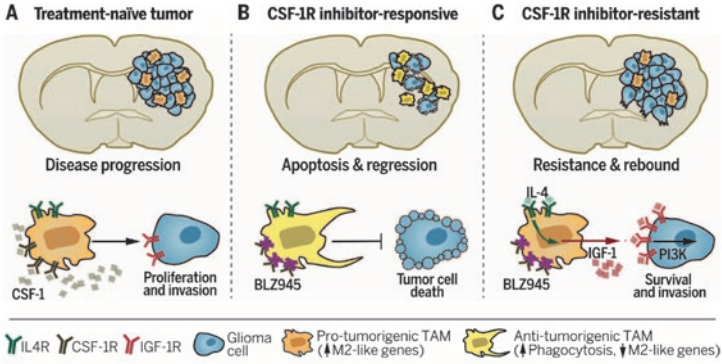

Figure 6: *Resistance to CSF-1R inhibition in gliomas. (A) Macrophages contribute to glioblastoma progression by creating a protumorigenic niche associated with M2-like gene expression. CSF-1R is a critical receptor for macrophage biology and is under clinical evaluation as a therapeutic target in glioma. (B) Targeting CSF-1R early in gliomagenesis significantly prolongs survival in mouse models (using CSF-1R inhibitors e.g. BLZ945). CSF-1R inhibition reprograms macrophages to become antitumorigenic by down-regulating M2-like genes and enhancing phagocytosis. Tumor-derived survival factors sustain macrophage viability despite CSF-1R blockade (Pyonteck et al., Nat Med 2013). (C) After prolonged treatment, a subset of glioblastomas acquire resistance to CSF-1R inhibition, and tumors recur. This is driven by elevated macrophage-derived IGF-1 and high IGF-1R on tumor cells, resulting in PI3K pathway activation and enhanced glioma cell survival and invasion. Blocking this pathway in combination with CSF-1R in preclinical trials resulted in a pronounced survival benefit. Adapted from Quail et al., Science (2016).*

Matrix-degrading enzymes in the TME: cathepsin proteases and heparanase

All tissues require extracellular matrix (ECM) to provide structural support and to facilitate the continuous intercellular communication that maintains tissue homeostasis (Mouw et al., 2014; Vogel, 2018). The ECM comprises secreted macromolecules including collagens, fibronectin, laminin, etc., and the precise composition can vary considerably in a cell type- and organ-dependent manner. In cancer, ECM production, composition and turnover are often aberrantly regulated by comparison to the normal tissue, contributing to enhanced invasion and proliferation of cancer cells (Pickup et al., 2014). Interestingly, through our investigation of the molecular differences between normal macrophages and TAMs, we found that upregulation of key matrix-degrading enzymes, specifically

cysteine cathepsin proteases and heparanase, was a prominent feature of TAMs in multiple TMEs (Gocheva et al., 2010b; Hunter et al., 2014).

In earlier experiments, dating back to my postdoc in Doug Hanahan's lab at UCSF, we had found that expression of a subset of 6 of 11 cathepsin family members were progressively upregulated during pancreatic cancer progression (Joyce et al., 2004) in the RIP1-Tag2 mouse model introduced above. Cathepsins are typically lysosomal enzymes, which are critical for terminal protein degradation (Olson and Joyce, 2015). To explore whether they might have extra-lysosomal functions in cancer, we used activity-based probes developed by Matthew Bogyo, a chemical biologist and long-standing collaborator now at Stanford University. We successfully imaged global cathepsin activity *in vivo* in several mouse models of cancer and found a specific increase in TAMs within the TME of PanNETs, breast cancer, and lung metastases (Joyce et al., 2004; Gocheva et al., 2010b) (Figure 7).

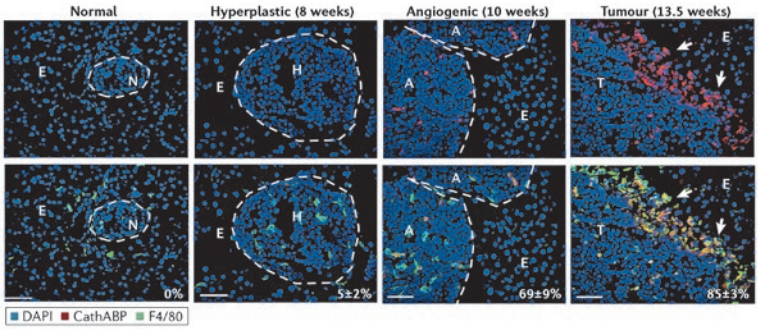

Figure 7: *Increase in cathepsin activity in TAMs during pancreatic islet tumor development in the RIP1-Tag2 mouse model. Cathepsins are highly activated in infiltrating macrophages during tumor progression at the angiogenic islet and tumor stages of RT2 tumorigenesis. Mice were injected with the cathepsin activity-based probe (Cath-ABP), and the resulting tissues stained with a F4/80 antibody to visualize macrophages. Normal (N) Tag+ islets were analyzed at 4–7 wks of age, hyperplastic (H) islets at 8 wks, angiogenic (A) islets at 10 wks, and tumors (T) at 13.5 wks of age. The percentage of F4/80+ cells that were Cath-ABP+ was determined by image analysis and is indicated in the representative image for each stage. Macrophages present in the normal adjacent exocrine (E) pancreas did not show high levels of cathepsin activity. Arrows represent the invasive tumor front. Adapted from Gocheva, Wang et al., Genes and Development (2010).*

This led Leny Gocheva and Hao-Wei Wang, two graduate students in my lab, along with Bedrick Gadea and several other key lab members (Gocheva et al., 2010b), to explore how cathepsin activity is elevated in TAMs, and investigate the mechanisms by which cathepsin proteases supplied by TAMs contribute to tumorigenesis. To discover the factors that upregulate cathepsin activity in macrophages, Hao-Wei developed a novel cell-based assay to initially focus on cancer cell-secreted proteins, and identified interleukin (IL)-4 as a critical inducer of cathepsin activity. Consistently, he found that deletion of IL-4 *in vivo* resulted in a significant reduction in cathepsin-positive TAMs in tumors. In parallel, Leny asked whether the increase in active cathepsins in TAMs contributed to tumor progression by performing a series of reciprocal bone marrow transplantation (BMT) experiments using different cathepsin knockout mice (as either donors or recipients). She found that removal of BM-derived cathepsin B or S, but not C or L, significantly reduced pancreatic tumor growth and invasion. We employed co-culture assays to show that macrophage-supplied cathepsins B and S significantly promote the invasive behavior of tumor cells. Together, these results established IL-4 as an important regulator, and specific cathepsin proteases as critical mediators, of the cancer-promoting functions of TAMs (Gocheva et al., 2010b; Wang and Joyce, 2010).

We subsequently sought to identify the precise molecular mechanisms by which cathepsins are secreted from TAMs, and address whether this new extracellular location was critical for their tumor-promoting functions. Dongyao Yan, Hao-Wei Wang and Bobby Bowman in the lab teamed up and began by asking whether other Th2 cytokines in addition to IL-4 could increase cathepsin secretion. Whole-genome expression analyses of macrophages revealed that IL-4 synergizes with the Th2 cytokines IL-6 or IL-10 to activate the unfolded protein response (UPR) via STAT6 and STAT3, which resulted in a potent upregulation of cathepsin secretion (Yan et al., 2016). We found that pharmacological inhibition of IRE1-alpha, a UPR sensor, blocked cathepsin secretion and consequently blunted macrophage-mediated cancer cell invasion. Critically, genetic deletion of STAT3 and STAT6 signaling components also impaired tumor development and invasion *in vivo*. Together, these findings revealed that cytokine-activated STAT3 and STAT6 cooperate to promote a secretory phenotype in macrophages that leads to enhanced tumor progression and invasion in a cathepsin-dependent manner (Yan et al., 2016) (Figure 8).

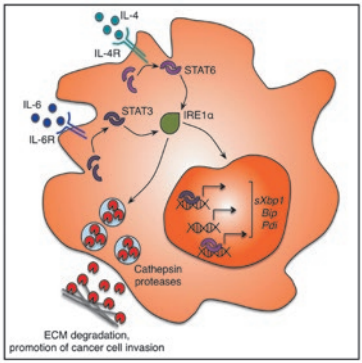

Figure 8: *STAT3 and STAT6 signaling pathways synergize to promote cathepsin secretion from macrophages via IRE1-alpha activation. We found that the Th2 cytokine IL-4 synergizes with IL-6 and IL-10 in macrophages to promote pancreatic neuroendocrine tumor growth and invasion. This synergy depends on STAT3 and STAT6 interaction to activate IRE1-alpha, leading to a pronounced secretion of cathepsin proteases and induction of unfolded protein response-related genes. From Yan, Wang, Bowman and Joyce, Cell Reports (2016).*

Cathepsin proteases are potent regulators of multiple hallmarks of cancer

To gain insights into the mechanisms by which cathepsins regulate different hallmark capabilities of cancer, we devised a comprehensive genetic strategy to delete individual cathepsins (alone and in combination) and determine the consequences for tumor proliferation, angiogenesis, and invasion. Using the RIP1-Tag2 model we first sought to identify the key tumor-promoting family members from the six that we found upregulated (B, C, H, L, S, and Z) from whole genome expression analyses (Joyce et al., 2004). There were several compelling reasons for undertaking this genetic analysis. First, to fully understand how cathepsins promote tumorigenesis it was critical to determine how each family member individually regulates tumor growth, invasion and angiogenesis. Second, from a translational perspective, when using pan-family inhibitors, there is the possibility of undesirable effects if some family members are actually tumor suppressors (Lopez-Otin and Matrisian, 2007). Thus, identifying the tumor-promoting proteases, and developing selective inhibitors that only target these enzymes is critical; as we have also addressed pharmacologically (Sadaghiani et al., 2007; Elie et al., 2010; Sevenich et al., 2014).

Our comprehensive analysis of multiple tumorigenic processes in the individual mutants, led initially by Leny Gocheva and subsequently by Leila Akkari, revealed specialized functions, in addition to phenotypes that were regulated by several cathepsins, as summarized in Table 1 (Gocheva et al., 2006; Gocheva and Joyce, 2007; Gocheva et al., 2010a; Akkari et al., 2014; Prudova et al., 2016). Importantly, cathepsin C, which was similarly upregulated during tumorigenesis (Joyce et al., 2004) had no impact when deleted (Gocheva et al., 2006), underlying the importance of rigorous genetic analyses for functional validation of whole genome expression data. We also made compound mutants of cathepsins B, S and Z, uncovering both additive and overlapping roles in tumorigenesis (Akkari et al., 2016). This body of work was critically enabled by the generosity of Thomas Reinheckel, Christoph Peters and other collaborators in sharing cathepsin mutants, and represented the first comprehensive genetic analysis of a family of proteases in cancer (Olson and Joyce, 2015). As a result, we successfully identified the key tumor-promoting family members, elucidated their different tumorigenic roles, and revealed that many of their tumorigenic functions are mediated via TAMs, rather than cancer cells (Gocheva et al., 2010b; Akkari et al., 2014).

	$B^{-/-}$ RT2	$H^{-/-}$ RT2	$L^{-/-}$ RT2	$S^{-/-}$ RT2	$Z^{-/-}$ RT2	$B^{-/-}S^{-/-}$ RT2	$B^{-/-}S^{-/-}Z^{-/-}$ RT2	$C^{-/-}$ RT2
Angiogenic switching	24% ↓	32% ↓	No change	24% ↓	53% ↓	60% ↓	*Not evaluated*	No change
Tumor volume	72% ↓	40% ↓	88% ↓	47% ↓	63% ↓	51% ↓	58% ↓	No change
Proliferation	44% ↓	No change	58% ↓	No change	86% ↓	85% ↓	53% ↓	No change
Apoptosis	2.3 fold ↑	2.0 fold ↑	3.4 fold ↑	1.6 fold ↑	1.8 fold ↑	No change	3.8 fold ↑	No change
Tumor vascularization	56% ↓	32% ↓	No change	48% ↓	No change	No change	*Not evaluated*	No change
Invasion	Significant reduction	No change	Significant reduction	Significant reduction	Significant reduction	No change	Significant reduction	No change

Table 1: *Summary of the effects of cathepsin deletion on multiple tumorigenic processes. Each of the six cathepsin knockout RIP1-Tag2 lines (single and compound mutants) was compared to wild-type RIP1-Tag2 littermates. Significant changes for each tumorigenic process are indicated in black, with no change indicated in grey. Data compiled from Gocheva et al, Genes Dev (2006); Gocheva et al, Biol Chem (2010); Akkari et al, Genes Dev (2014); Akkari, Gocheva et al, Genes Dev (2016).*

To unravel the molecular mechanisms by which cathepsins promote the different hallmarks of cancer summarized above we employed both candidate-based strategies and unbiased proteomic screens to reveal their substrates within the TME (Figure 9). For example, Leny Gocheva identified cleavage of the cell adhesion protein E-cadherin by the pro-invasive cathepsins B, L and S as a key mechanism that contributes to tumor invasion (Gocheva et al., 2006). Lisa Sevenich discovered a brain metastasis-promoting function for cathepsin S via shedding of the junctional adhesion molecule, JAM-B, which facilitates extravasation of tumor cells into the brain across the blood-brain barrier (BBB) (Sevenich et al., 2014). Leila Akkari, with Leny Gocheva and other lab members, found that cathepsin Z promotes cancer cell invasion and proliferation through a unique RGD-binding motif in its pro-domain, which promotes attachment via integrins to different ECM components, in a FAK/Src-dependent manner (Akkari et al., 2014). Moreover, we demonstrated that for this cathepsin family member, its tumor-promoting functions are actually independent of its enzymatic activity, and instead rely on ECM-mediated signaling. In collaboration with the Vlodavsky lab in Israel, cathepsin L was identified as the major protease responsible for activation of the key matrix-degrading enzyme, heparanase (Abboud-Jarrous et al., 2008). In an interesting convergence, we had previously shown that inhibition of heparanase (Joyce et al., 2005) disrupts several of the same tumorigenic pathways as pan-cathepsin inhibitors (Joyce et al., 2004; Bell-McGuinn et al., 2007; Elie et al., 2010). Karen Hunter, a graduate student in my lab, thus investigated how genetic modulation of heparanase levels regulates tumor progression using heparanase knockout and heparanase-overexpressing mice in the RIP1-Tag2 model, and thereby identified critical roles for heparanase in promoting lymphangiogenesis and tumor invasion (Hunter et al., 2014).

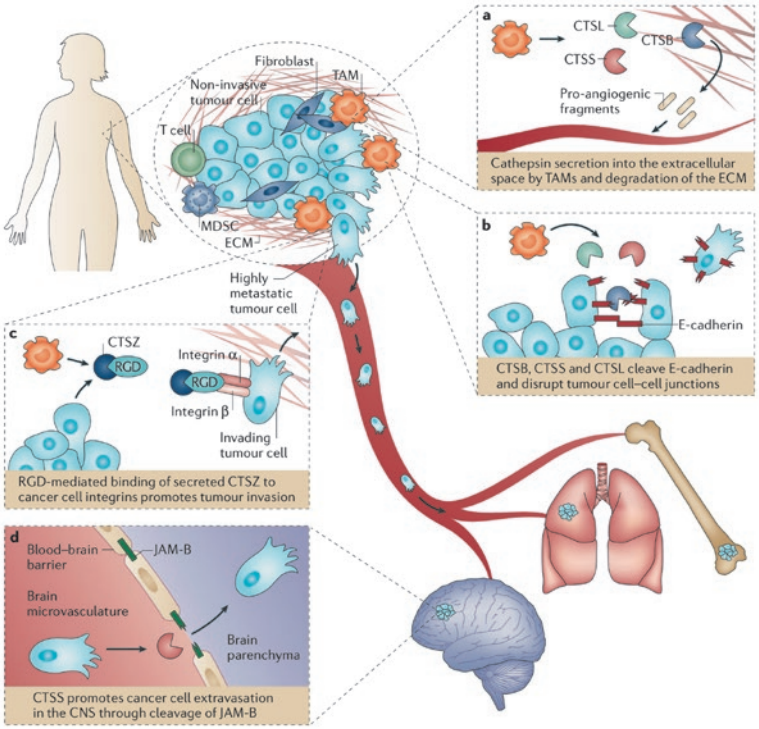

Figure 9: *Cathepsin proteases in tumor progression and the metastatic cascade. (a) Cathepsins can be supplied from multiple cellular sources within the tumor microenvironment, including cancer cells and infiltrating immune cells such as TAMs. Cathepsins have crucial roles both intracellularly and extracellularly in the promotion of tumor progression, for example, by ECM degradation. (b) Secreted cathepsin B (CTSB), CTSL and CTSS can cleave the cell adhesion molecule E-cadherin, promoting cancer cell invasion into the surrounding tissue. (c) The pro-form of CTSZ, secreted by either TAMs or cancer cells, binds to cancer cell integrins through the Arg-Gly-Asp (RGD) domain to promote invasion. (d) Secretion of CTSS by circulating breast cancer cells has been shown to be crucial for their ability to cross the blood-brain barrier (BBB) and metastasize to the central nervous system. Cancer cells use this proteolytic activity to cleave junctional adhesion molecules, specifically JAM-B, in order to disrupt the integrity of the BBB and allow for their extravasation. From Olson and Joyce, Nat Rev Cancer (2015), depicting data compiled from Gocheva et al, Genes Dev (2006); Akkari et al, Genes Dev (2014); Sevenich et al, Nat Cell Biol (2014).*

In addition to these targeted candidate approaches, which were each very fruitful in identifying *bona fide* cathepsin substrates, we also collaborated with the lab of Chris Overall in Vancouver to perform unbiased proteomics screens *in vivo* (Prudova et al., 2016). By applying 8-plex iTRAQ terminal amine isotopic labeling of substrates (TAILS), a systems-level N-terminome degradomics approach, we identified cleavage sites for *in vivo* substrates of cathepsins B, H, L, S, and Z within the TME by taking advantage of the different cathepsin knockouts we had generated in the RIP1-Tag2 background (Table 1). We validated several of the substrates using independent experimental approaches, including the glycolytic enzyme pyruvate kinase M2 associated with the Warburg effect, the ER chaperone GRP78, and the oncoprotein prothymosin-alpha.

Collectively, our studies over the past decade have revealed novel, unexpected roles for cathepsin proteases as critical processing and activation enzymes, functioning as "master regulators" at the apex of multiple protease networks, thereby greatly expanding their functions in cancer beyond simple matrix degradation (Mason and Joyce, 2011; Sevenich and Joyce, 2014; Olson and Joyce, 2015).

In parallel with the findings discussed here, we have also had many successful collaborations with colleagues exploring the roles of tumor-associated macrophages and myeloid cells in numerous diverse contexts. This includes investigating TAM metabolism within the TME with Carlos-Carmona Fontaine and Joao Xavier; exploring how the senescence-associated secretory program in the liver TME impacts macrophage polarization with Amaia Lujambio, Leila Akkari and Scott Lowe; targeting TAMs in thyroid cancer with Mabel Ryder and Jim Fagin; working with Matej Krajcovic and Mike Overholtzer on phagocytosis, lysosome fission and nutrient uptake; Rich Bakst and Rich Wong on the promotion of perineural cancer invasion by inflammatory monocytes; imaging of TAMs in breast cancer with Avigdor Leftin, Nir Ben-Chetrit and Jason Koutcher, and lipid flux in macrophages with Prakrit Jena and Dan Heller; and injury-related brain inflammation with Nduka Amankulor and Eric Holland (Amankulor et al., 2009; Carmona-Fontaine et al., 2013; Krajcovic et al., 2013; Lujambio et al., 2013; Ryder et al., 2013; Bakst et al., 2017; Carmona-Fontaine et al., 2017; Jena et al., 2017; Leftin et al., 2017), among other studies.

Microenvironmental regulation of therapeutic efficacy

While the TME is now recognized to critically modulate cancer progression, our understanding of its potential role in regulating treatment response is still in its infancy (Klemm and Joyce, 2015). Solid tumors respond to conventional anti-cancer therapies, including chemotherapy and radiation, with many acute changes. Unfortunately, tumors frequently recover from these assaults and re-establish growth. Several years ago, we postulated that there are specific needs for stromal cells and TME-supplied factors under these conditions to enhance tumor cell survival and drive ECM remodeling and revascularization, thus re-establishing a favorable environment for growth. Similar processes at work during different stages of tumor progression have been shown to require the trophic functions of TAMs (Noy and Pollard, 2014). We therefore reasoned that TAMs and their associated products are ideal candidate modulators of response to therapy.

Indeed, Tanaya Shree and Oakley Olson, two graduate students in my lab, found increased TAM accumulation and cathepsin protease levels in breast tumors from patients and mouse models following Taxol chemotherapy (Shree et al., 2011). Cathepsin-expressing macrophages protected against Taxol-induced tumor cell death in co-culture, an effect fully reversed by cathepsin inhibition and mediated partially by cathepsins B and S. They also found that macrophages protected against tumor cell death induced by additional chemotherapies from a broader panel that they investigated, specifically etoposide and doxorubicin. Critically, combining cathepsin inhibition with chemotherapy *in vivo* significantly enhanced efficacy against primary and metastatic tumors (Shree et al., 2011), supporting the therapeutic relevance of this effect.

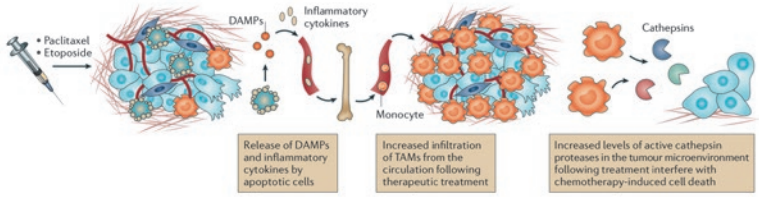

Figure 10: *Cathepsin proteases and therapeutic resistance. Adaptive upregulation of cathepsins can occur through the increased recruitment of cathepsin-high TAMs in response to chemotherapeutic agents such as paclitaxel or etoposide. These adaptive increases in intratumoural cathepsin activity levels blunt therapeutic efficacy, which can accordingly be improved by cathepsin inhibition. Schematic from Olson and Joyce, Nat Rev Cancer (2015), depicting data from Shree, Olson et al, Genes Dev (2011).*

We recently extended this initial finding (Figure 10) by incorporating live cell imaging to investigate precisely how TAMs impact Taxol-induced alterations in the mitotic arrest of cancer cells, through a collaboration with Emily Foley at MSKCC, that was led by Oakley Olson in my lab. Oakley found that macrophages suppress the duration of Taxol-induced mitotic arrest in breast cancer cells and promote earlier mitotic slippage (Olson et al., 2017a). This correlated with a decrease in the phosphorylated form of histone H2AX (γH2AX), decreased p53 activation, and reduced cancer cell death in interphase. He found that acute and specific depletion of major histocompatibility complex class II (MHCII)-low TAMs increased Taxol-induced DNA damage and apoptosis in cancer cells, leading to greater efficacy in preclinical intervention trials. Oakley's mechanistic investigations also revealed the importance of the MAPK/ERK kinase (MEK) pathway in this protective effect (Figure 11), and MEK inhibition blocked the protective capacity of TAMs and phenocopied the effects of TAM depletion on Taxol treatment *in vivo* (Olson et al., 2017a). Thus, we found that TAMs suppress the cytotoxic effects of Taxol, in part through cell non-autonomous modulation of mitotic arrest in cancer cells, and consequently targeting TAM-cancer cell interactions potentiates Taxol efficacy (Shree et al., 2011; Olson et al., 2017a).

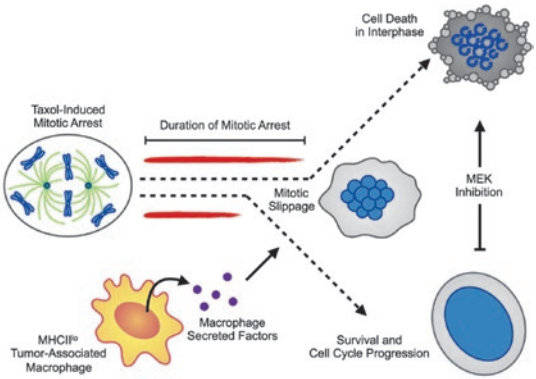

Figure 11: *Tumor-associated macrophages (TAMs) suppress the cytotoxic activity of anti-mitotic agents. We investigated how TAMs suppress the duration of Taxol-induced mitotic arrest in breast cancer cells using live cell imaging. We found that TAMs promote cancer cell viability following mitotic slippage through a mechanism that is sensitive to MEK inhibition. Acute depletion of MHCII-low TAMs in a preclinical breast cancer model increased the ability of Taxol to induce apoptosis and improved therapeutic response. From Olson et al, Cell Reports (2017).*

In addition to our investigation of TAMs in breast cancer, we are actively exploring how the TME changes dynamically in response to therapeutic intervention in brain cancers, and consequently determining which TME components to target for combination therapies. One recent example of this analysis relates to gliomas, where we have shown that TAMs interfere with the efficacy of molecularly-targeted tyrosine kinase inhibitors (TKIs) *in vivo* (Yan et al., 2017). Dongayo Yan in my lab found that while these inhibitors effectively killed glioma cells in culture, they showed minimal effects in mice; indicating that a TME-medicated resistance mechanism may be involved. Indeed, we showed that the CSF-1R inhibitor PLX3397 restored the sensitivity of glioma cells to TKIs *in vivo* in preclinical drug combination trials. Together, these representative studies highlight the importance of TAMs and the microenvironment in modulating therapeutic response, a concept that has been demonstrated in additional cancers by a number of other groups (reviewed in Klemm and Joyce, 2015; Ruffell and Coussens, 2015), and which may have important translational relevance for patients.

Microenvironmental regulation of metastasis

Cancer cells in an aggressive primary tumor are adept at exploiting their local tissue environment. By contrast, when metastatic cells leave these favorable surroundings, they must possess or acquire traits that will allow them to survive and colonize foreign, potentially hostile tissue environments (Figure 12). The obstacles that metastasizing tumor cells encounter vary from organ to organ, and are highly influenced by cells of the TME (Joyce and Pollard, 2009; Quail and Joyce, 2013). Indeed, dissemination can occur to multiple organs, yet metastatic tumors typically grow in only one or a few sites, indicating critical roles for the microenvironment in this process, as already appreciated by Paget in the late 19th century (Paget, 1889).

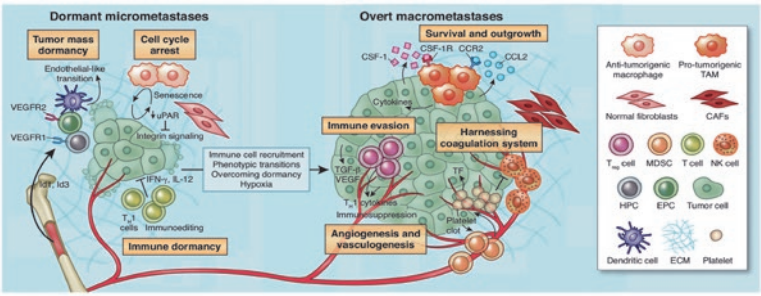

Figure 12: *Initiation of secondary outgrowth in metastatic niches. Dormant micrometastases are held in check by several mechanisms including tumor mass dormancy, or angiogenic dormancy, when proliferation is balanced by apoptosis because of a lack of vasculature and limited supply of nutrients and oxygen. Multiple TME cell types contribute to the re-establishment of vascularity at the secondary site, including myeloid and endothelial cell progenitors and TAMs. In addition, tumor cells can enter immune-induced dormancy whereby immunogenic cells are cleared, and cells that are able to survive enter a state of equilibrium. Immune suppressor cells are recruited to tumors in response to this process and contribute to the establishment of an immunosuppressive state within secondary tissues. Once micrometastases overcome dormancy, they become receptive to signals and cell types within the TME to further support their expansion. For example, TAMs are abundant in metastases of multiple cancer types and support different tumorigenic processes to allow for outgrowth, including vascularization, impaired immunogenicity and enhanced survival in overt metastases. Platelets, and components of the coagulation system are also important mediators of metastatic outgrowth, as they interfere with the ability of natural killer (NK) cells to destroy micrometastases and support clot formation, which in turn causes the recruitment of myeloid suppressor cells. From Quail and Joyce, Nature Medicine (2013).*

To gain insights into how different tissue environments influence metastasis we analyzed tumor–microenvironment interactions that modulate organ tropism of brain, bone and lung metastasis. We took advantage of organ-specific models of breast cancer metastasis to these sites which had been previously developed by our collaborator Joan Massagué at MSKCC, and investigated gene expression in a tissue- and stage-dependent manner (Sevenich et al., 2014). The "HuMu" screens we performed focused on analysis of proteases and their endogenous inhibitors, that we and others had shown to be important in the primary cancer TME (reviewed in Mason and Joyce, 2011; Sevenich and Joyce, 2014), but which were relatively understudied in metastasis.

We identified numerous differentially expressed proteases and inhibitors that were regulated in either a stage- or tissue-specific manner in different metastatic TMEs (Sevenich et al., 2014). By querying whether expression of these genes in primary breast cancer patients was associated with metastasis-free survival in brain, bone or lung, we were able to apply an additional filter that allowed restriction of the gene lists to only those that showed a significant correlation with survival. One such protease was cathepsin S in which high expression in breast cancer patients correlated with decreased brain metastasis-free survival. Lisa Sevenich, a postdoc in my lab who led this study along with Bobby Bowman and Steve Mason (Sevenich et al., 2014), found that both TAMs and tumor cells produce cathepsin S, and only their combined depletion significantly reduced brain metastasis *in vivo*. Lisa discovered that cathepsin S specifically mediates blood-brain barrier penetration through proteolytic processing of the junctional adhesion molecule, JAM-B, thereby enabling endothelial cell transmigration (Sevenich et al., 2014) (Figure 9d). Interestingly, cathepsin S is typically predominantly produced by immune cells during homeostasis (Olson and Joyce, 2015). In brain metastasis, we therefore proposed that the induction of cathepsin S expression in cancer cells of epithelial origin may indicate a type of "leukocytic mimicry" whereby metastatic tumor cells could implement immune-cell-like expression programs that enhance mobilization and cell motility (Sevenich et al., 2014); a hypothesis that may extend to other components of the brain TME (Quail and Joyce, 2017a).

Beyond the local TME, an inflammatory systemic environment can also affect disease outcome, by perturbing homeostasis within multiple tissues throughout the body. This becomes particularly important during metastasis, where systemic alterations can modify the tissue landscape of distant organs and support tumor cell colonization by establishing a pre-metastatic niche (McAllister and Weinberg, 2014). Indeed, chronic inflammation can significantly increase cancer risk and disease progression (Quail and Joyce, 2013). Investigation into how the systemic environment affects tumor biology is therefore critical for an integrated understanding of cancer. As such, we have begun to explore how the systemic microenvironment modulates tumorigenesis and metastasis. We first chose to assess the clinically relevant case of obesity-associated chronic inflammation, as it can disrupt homeostasis within tissue microenvironments (Olson et al., 2017b). Given the correlation between obesity and increased relative risk of death from breast cancer, we focused on determining whether obesity-associated inflammation promotes metastatic progression (Quail et al., 2017).

In this study, Daniela Quail and Oakley Olson together showed that obesity causes lung neutrophilia in otherwise-normal individuals (Quail et al., 2017). They found this occurred independently of diet content; rather it was directly related to increased adiposity and the production of IL5 by adipose tissue. They found that IL5 increases *Csf2* (GM-CSF) expression by IL5R+ monocytes, and enhances neutrophil trafficking to lung (Figure 13). Furthermore, elevated serum GM-CSF promotes myelopoiesis, leading to an expansion of peripheral neutrophils. In mouse models, obesity-associated lung neutrophilia enhanced breast cancer metastasis to this organ, and depletion of Gr1+ neutrophils in obese animals reversed this effect (Olson et al., 2017b; Quail et al., 2017). We also found that GM-CSF is predominantly expressed in the lungs of obese mice, and that GM-CSF blockade *in vivo* reverses the pro-metastatic effects of obesity. Interestingly, weight loss was equally effective at reversing all these phenomena in mice, including breast-to-lung metastasis.

In collaboration with Andrew Dannenberg, Peter Holt and colleagues in their labs in New York, we had the opportunity to analyze human serum from morbidly obese individuals who had undergone a 10 % weight loss following diet restriction. This weight loss was associated with reduced

serum IL5 and GM-CSF, concomitant with decreased circulating neutrophils. Collectively, our findings also have implications for the long-term management of obese breast cancer patients, as lung inflammation may have prognostic value. Clinical studies are thus needed to appropriately manage the obese cancer patient population, and to uncouple the comorbidity of obesity and cancer (Olson et al., 2017b).

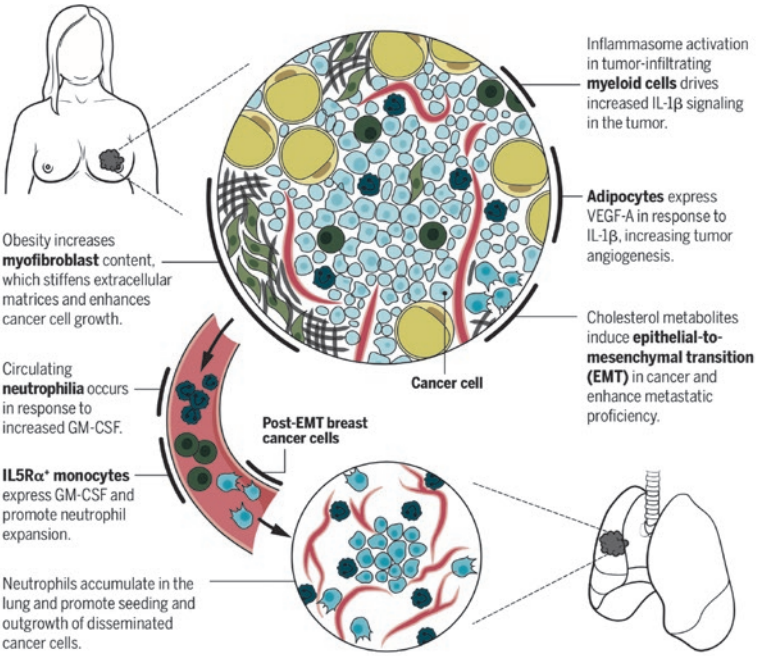

Figure 13: *Obesity drives alterations in the local tumor microenvironment, and systemic changes, which enhance cancer progression and metastasis. The effects of obesity on cancer progression are depicted using breast cancer as a representative example, based on studies from mice and humans, including our own research (Quail, Olson et al, Nat Cell Biol 2017). Obesity promotes both primary tumor growth and metastatic progression through systemic alterations that affect tissue homeostasis. From Olson, Quail and Joyce, Science (2017).*

Conclusions and perspectives

We have been fortunate to gain important insights into many of the questions posed when I initiated my lab's research program over a decade ago, as highlighted by the representative studies discussed here. We have identified several mechanisms of TAM education, elucidated processes by which TAMs, neutrophils and other immune cells promote tumorigenesis, discovered that TAMs have potent protective functions in blocking therapeutic efficacy, identified the TME as a major mediator of resistance to TAM therapies, and have helped to illuminate the interplay between the TME and cancer cells during different stages of the metastatic process.

Going forward, we are focusing much of our efforts in my lab on understanding and therapeutically targeting brain malignancies and metastatic disease, both from the perspective of the TME (Figure 14). Glioblastomas and brain metastases are among the most lethal of cancers, with an average lifespan of a year or less following diagnosis. Given this dismal patient prognosis, we became very interested in studying these particular brain malignancies several years ago, and in investigating both the similarities and differences between primary and metastatic brain cancers, which may have important implications for understanding differential immunotherapy efficacy, for example. While we have been able to make several important insights into the brain TME from our recent studies (Pyonteck et al., 2013; Bowman and Joyce, 2014; Sevenich et al., 2014; Bowman et al., 2016; Quail et al., 2016; Yan et al., 2017), as a field we have much to discover and understand about the unique and particularly challenging microenvironment of brain cancers (Quail and Joyce, 2017a).

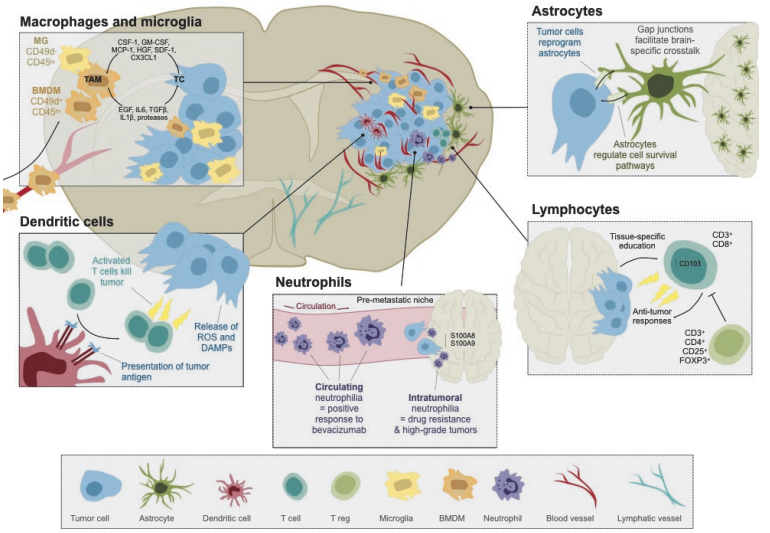

Figure 14: *The microenvironmental landscape of brain cancers. Brain tumors are composed of diverse cellular players, ranging from peripherally-derived immune cells to various specialized organ-resident cell types, such as astrocytes. Each of these cell types contributes to brain tumor biology in unique ways. For example, tumor-associated macrophages and microglia (TAMs) arise from two distinct sources, including the periphery (bone marrow-derived macrophages, BMDMs; CD49d+) or the yolk sac (microglia, MG; CD49d-). TAMs engage in significant bidirectional crosstalk with tumor cells (TC) in the brain, whereby brain tumor cells release cytokines and chemoattractants to recruit TAMs to the microenvironment, and TAMs in turn supply pro-tumorigenic, pro-survival factors. Adapted from Quail and Joyce, Cancer Cell (2017).*

It will be essential to advance our current knowledge of individual brain TME components into a more complex microenvironmental landscape in which we analyze these cellular and non-cellular components as part of an integrated whole. Moreover, investigating the evolution of the brain TME as a dynamic process, incorporating detailed timecourse analyses in patients and live imaging of TME cells and components in mice, will reveal critical information that single timepoints cannot capture. Similarly, major insights can be expected from a detailed comparison of how distinct molecular sub-types or genetic drivers in cancer cells may differentially sculpt their microenvironment during the course of cancer pro-

gression. Although as a field it is widely recognized that there are cancer cell-intrinsic differences in tumor evolution and response to therapy by virtue of different molecular subtypes, appropriate dissection of the different TME determinants of therapeutic response is still in its infancy, and largely untapped clinically. Going forward, it will therefore be critical to determine the many differences in microenvironmental composition between distinct tumor subtypes in order to achieve a comprehensive understanding of tumor biology, including consideration of matrix stiffness, tumor-stromal interactions, and immune cell landscapes.

Moreover, it will be important to globally address how all aspects of the TME are affected by both standard of care therapy and new investigational therapies across all brain tumors and their respective molecular subtypes. From a practical perspective, we need to engage actively with medicinal chemists to improve drug delivery into the brain; a perennial challenge for all brain-targeted therapies, including those directed against the TME. We need to understand how the generally immunosuppressive environment of the brain is further exacerbated in the context of brain cancers in order to devise therapies to overcome this. Finally, if we cannot take a "one size fits all" approach for targeting the TME in different brain malignancies, we will need to determine where the vulnerable points are to attack at a more personalized level. Given the current advances being made in the immunotherapy and TME fields, however, we can also expect an exciting and illuminating time ahead for basic research and clinical translation in brain cancers, and for microenvironment biology as a whole.

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References

Abboud-Jarrous, G., Atzmon, R., Peretz, T., Palermo, C., Gadea, B.B., Joyce, J.A., and Vlodavsky, I. (2008). Cathepsin L is responsible for processing and activation of proheparanase through multiple cleavages of a linker segment. **Journal of Biological Chemistry** 283, 18167–18176.

Akkari, L., Gocheva, V., Kester, J.C., Hunter, K.E., Quick, M.L., Sevenich, L., Wang, H.W., Peters, C., Tang, L.H., Klimstra, D.S., Reinheckel, T., and Joyce, J.A. (2014). Distinct functions of macrophage-derived and cancer cell-derived cathepsin Z combine to promote tumor malignancy via interactions with the extracellular matrix. **Genes and Development** 28, 2134–2150.

Akkari, L., Gocheva, V., Quick, M.L., Kester, J.C., Spencer, A.K., Garfall, A.L., Bowman, R.L., and Joyce, J.A. (2016). Combined deletion of cathepsin protease family members reveals compensatory mechanisms in cancer. **Genes and Development** 30, 220–232.

Amankulor, N.M., Hambardzumyan, D., Pyonteck, S.M., Becher, O.J., Joyce, J.A., and Holland, E.C. (2009). Sonic hedgehog pathway activation is induced by acute brain injury and regulated by injury-related inflammation. **Journal of Neuroscience** 29, 10299–10308.

Bakst, R.L., Xiong, H., Chen, C.H., Deborde, S., Lyubchik, A., Zhou, Y., He, S., McNamara, W., Lee, S.Y., Olson, O.C., Leiner, I.M., Marcadis, A., Keith, J., Al-Ahmadie, H., Katabi, N., Gil, Z., Vakiani, A., Joyce, J.A., Pamer, E., and Wong, R.J. (2017). Inflammatory monocytes promote perineural invasion via CCL2-mediated recruitment and cathepsin B expression. **Cancer Research** 77, 6400–6414.

Bell-McGuinn, K.M., Garfall, A.L., Bogyo, M., Hanahan, D., and Joyce, J.A. (2007). Inhibition of cysteine cathepsin protease activity enhances chemotherapy regimens by decreasing tumor growth and invasiveness in a mouse model of multistage cancer. **Cancer Research** 67, 7378–7385.

Bingle, L., Brown, N.J., and Lewis, C.E. (2002). The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. **Journal of Pathology** 196, 254–265.

Binnewies, M., Roberts, E.W., Kersten, K., Chan, V., Fearon, D.F., Merad, M., Coussens, L.M., Gabrilovich, D.I., Ostrand-Rosenberg, S., Hedrick, C.C., Vonderheide, R.H., Pittet, M.J., Jain, R.K., Zou, W., Howcroft, T.K., Woodhouse, E.C., Weinberg, R.A., and Krummel, M.F. (2018). Understanding the tumor immune microenvironment (TIME) for effective therapy. **Nature Medicine** 24, 541–550.

Bowman, R.L., and Joyce, J.A. (2014). Therapeutic targeting of tumor-associated macrophages and microglia in glioblastoma. **Immunotherapy** 6, 663–666.

Bowman, R.L., Klemm, F., Akkari, L., Pyonteck, S.M., Sevenich, L., Quail, D.F., Dhara, S., Simpson, K., Gardner, E.E., Iacobuzio-Donahue, C.A., Brennan, C.W., Tabar, V., Gutin, P.H., and Joyce, J.A. (2016). Macrophage ontogeny underlies differences in tumor-specific education in brain malignancies. **Cell Reports** 17, 2445–2459.

Carmona-Fontaine, C., Bucci, V., Akkari, L., Deforet, M., Joyce, J.A., and Xavier, J.B. (2013). Emergence of spatial structure in the tumor microenvironment due to the Warburg effect. **Proc Natl Acad Sci U S A** 110, 19402–19407.

Carmona-Fontaine, C., Deforet, M., Akkari, L., Thompson, C.B., Joyce, J.A., and Xavier, J.B. (2017). Metabolic origins of spatial organization in the tumor microenvironment. **Proc Natl Acad Sci U S A** 114, 2934–2939.

Elie, B.T., Gocheva, V., Shree, T., Dalrymple, S.A., Holsinger, L.J., and Joyce, J.A. (2010). Identification and pre-clinical testing of a reversible cathepsin protease inhibitor reveals anti-tumor efficacy in a pancreatic cancer model. **Biochimie** 92, 1618-1624.

Fridman, W.H., Zitvogel, L., Sautes-Fridman, C., and Kroemer, G. (2017). The immune contexture in cancer prognosis and treatment. **Nature Reviews Clinical Oncology** 14, 717–734.

Gocheva, V., Zeng, W., Ke, D., Klimstra, D., Reinheckel, T., Peters, C., Hanahan, D., and Joyce, J.A. (2006). Distinct roles for cysteine cathepsin genes in multistage tumorigenesis. **Genes and Development** 20, 543–556.

Gocheva, V., and Joyce, J.A. (2007). Cysteine cathepsins and the cutting edge of cancer invasion. **Cell Cycle** 6, 60–64.

Gocheva, V., Chen, X., Peters, C., Reinheckel, T., and Joyce, J.A. (2010a). Deletion of cathepsin H perturbs angiogenic switching, vascularization and growth of tumors in a mouse model of pancreatic islet cell cancer. **Biological Chemistry** 391, 937–945.

Gocheva, V., Wang, H.W., Gadea, B.B., Shree, T., Hunter, K.E., Garfall, A.L., Berman, T., and Joyce, J.A. (2010b). IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion. **Genes and Development** 24, 241–255.

Hunter, K.E., Palermo, C., Kester, J.C., Simpson, K., Li, J.P., Tang, L.H., Klimstra, D.S., Vlodavsky, I., and Joyce, J.A. (2014). Heparanase promotes lymphangiogenesis and tumor invasion in pancreatic neuroendocrine tumors. **Oncogene** 33, 1799–1808.

Hussain, S.F., Yang, D., Suki, D., Aldape, K., Grimm, E., and Heimberger, A.B. (2006). The role of human glioma-infiltrating microglia/macrophages in mediating antitumor immune responses. **Neuro Oncology** 8, 261–279.

Jena, P.V., Roxbury, D., Galassi, T.V., Akkari, L., Horoszko, C.P., Iaea, D.B., Budhathoki-Uprety, J., Pipalia, N., Haka, A.S., Harvey, J.D., Mittal, J., Maxfield, F.R., Joyce, J.A. and Heller, D.A. (2017). A carbon nanotube optical reporter maps endolysosomal lipid flux. **ACS Nano** 11, 10689–10703.

Joyce, J.A., Baruch, A., Chehade, K., Meyer-Morse, N., Giraudo, E., Tsai, F.Y., Greenbaum, D.C., Hager, J.H., Bogyo, M., and Hanahan, D. (2004). Cathepsin cysteine proteases are effectors of invasive growth and angiogenesis during multistage tumorigenesis. **Cancer Cell** 5, 443–453.

Joyce, J.A. (2005). Therapeutic targeting of the tumor microenvironment. **Cancer Cell** 7, 513–520.

Joyce, J.A., Freeman, C., Meyer-Morse, N., Parish, C.R., and Hanahan, D. (2005). A functional heparan sulfate mimetic implicates both heparanase and heparan sulfate in tumor angiogenesis and invasion in a mouse model of multistage cancer. **Oncogene** 24, 4037–4051.

Joyce, J.A., and Pollard, J.W. (2009). Microenvironmental regulation of metastasis. **Nature Reviews Cancer** 9, 239–252.

Klemm, F., and Joyce, J.A. (2015). Microenvironmental regulation of therapeutic response in cancer. **Trends in Cell Biology** 25, 198–213.

Komohara, Y., Ohnishi, K., Kuratsu, J., and Takeya, M. (2008). Possible involvement of the M2 anti-inflammatory macrophage phenotype in growth of human gliomas. **Journal of Pathology** 216, 15–24.

Krajcovic, M., Krishna, S., Akkari, L., Joyce, J.A., and Overholtzer, M. (2013). mTOR regulates phagosome and entotic vacuole fission. **Mol Biol Cell** 24, 3736–3745.

Lavin, Y., Mortha, A., Rahman, A., and Merad, M. (2015). Regulation of macrophage development and function in peripheral tissues. **Nature Reviews Immunology** 15, 731–744.

Lavin, Y., Winter, D., Blecher-Gonen, R., David, E., Keren-Shaul, H., Merad, M., Jung, S., and Amit, I. (2014). Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. **Cell** 159, 1312–1326.

Leftin, A., Ben-Chetrit, N., Klemm, F., Joyce, J.A., and Koutcher, J.A. (2017). Iron imaging reveals tumor and metastasis macrophage hemosiderin deposits in breast cancer. **PLoS One** 12, e0184765.

Lopez-Otin, C., and Matrisian, L.M. (2007). Emerging roles of proteases in tumour suppression. **Nature Reviews Cancer** 7, 800–808.

Lujambio, A., Akkari, L., Simon, J., Grace, D., Tschaharganeh, D.F., Bolden, J.E., Zhao, Z., Thapar, V., Joyce, J.A., Krizhanovsky, V., and Lowe, S.W. (2013). Non-cell-autonomous tumor suppression by p53. **Cell** 153, 449–460.

Mason, S.D., and Joyce, J.A. (2011). Proteolytic networks in cancer. **Trends in Cell Biology** 21, 228–237.

McAllister, S.S., and Weinberg, R.A. (2014). The tumour-induced systemic environment as a critical regulator of cancer progression and metastasis. **Nature Cell Biology** 16, 717–727.

Mouw, J.K., Ou, G., and Weaver, V.M. (2014). Extracellular matrix assembly: a multiscale deconstruction. **Nature Reviews Molecular and Cell Biology** 15, 771–785.

Noy, R., and Pollard, J.W. (2014). Tumor-associated macrophages: from mechanisms to therapy. **Immunity** 41, 49–61.

Olson, O.C., and Joyce, J.A. (2015). Cysteine cathepsin proteases: regulators of cancer progression and therapeutic response. **Nature Reviews Cancer** 15, 712–729.

Olson, O.C., Kim, H., Quail, D.F., Foley, E.A., and Joyce, J.A. (2017a). Tumor-associated macrophages suppress the cytotoxic activity of antimitotic agents. **Cell Reports** 19, 101–113.

Olson, O.C., Quail, D.F., and Joyce, J.A. (2017b). Obesity and the tumor microenvironment. **Science** 358, 1130–1131.

Paget, S. (1889). The distribution of secondary growths in cancer of the breast. **The Lancet** 133, 571–573.

Pickup, M.W., Mouw, J.K., and Weaver, V.M. (2014). The extracellular matrix modulates the hallmarks of cancer. **EMBO Reports** 15, 1243–1253.

Prudova, A., Gocheva, V., Auf dem Keller, U., Eckhard, U., Olson, O.C., Akkari, L., Butler, G.S., Fortelny, N., Lange, P.F., Marc, J.C., Joyce, J.A. and Overall, C.M. (2016). TAILS N-terminomics and proteomics show protein degradation dominates over proteolytic processing by cathepsins in pancreatic tumors. **Cell Reports** 16, 1762–1773.

Pyonteck, S.M., Akkari, L., Schuhmacher, A.J., Bowman, R.L., Sevenich, L., Quail, D.F., Olson, O.C., Quick, M.L., Huse, J.T., Teijeiro, V., Setty, M., Leslie, C., Oei, Y., Pedraza, A., Zhang, J., Brennan, C.W., Sutton, J.C., Holland, E.C., Daniel, D. and Joyce, J.A. (2013). CSF-1R inhibition alters macrophage polarization and blocks glioma progression. **Nature Medicine** 19, 1264–1272.

Pyonteck, S.M., Gadea, B.B., Wang, H.W., Gocheva, V., Hunter, K.E., Tang, L.H., and Joyce, J.A. (2012). Deficiency of the macrophage growth factor CSF-1 disrupts pancreatic neuroendocrine tumor development. **Oncogene** 31, 1459–1467.

Quail, D.F., Bowman, R.L., Akkari, L., Quick, M.L., Schuhmacher, A.J., Huse, J.T., Holland, E.C., Sutton, J.C., and Joyce, J.A. (2016). The tumor microenvironment underlies acquired resistance to CSF-1R inhibition in gliomas. **Science** 352, aad3018.

Quail, D.F., and Joyce, J.A. (2013). Microenvironmental regulation of tumor progression and metastasis. **Nature Medicine** 19, 1423–1437.

Quail, D.F., and Joyce, J.A. (2017a). The microenvironmental landscape of brain tumors. **Cancer Cell** 31, 326–341.

Quail, D.F., and Joyce, J.A. (2017b). Molecular Pathways: Deciphering mechanisms of resistance to macrophage-targeted therapies. **Clinical Cancer Research** 23, 876–884.

Quail, D.F., and Joyce, J.A. (2017c). Multifaceted effects of the microenvironment on tumour progression. **Nature Reviews Cancer** https://www.nature.com/nrc/posters/microenvironment/micro_poster.pdf

Quail, D.F., Olson, O.C., Bhardwaj, P., Walsh, L.A., Akkari, L., Quick, M.L., Chen, I.C., Wendel, N., Ben-Chetrit, N., Walker, J., Holt, P.R., Dannenberg, A.J., and Joyce, J.A. (2017). Obesity alters the lung myeloid cell landscape to enhance breast cancer metastasis through IL5 and GM-CSF. **Nature Cell Biology** 19, 974–987.

Ruffell, B., and Coussens, L.M. (2015). Macrophages and therapeutic resistance in cancer. **Cancer Cell** 27, 462–472.

Ryder, M., Gild, M., Hohl, T.M., Pamer, E., Knauf, J., Ghossein, R., Joyce, J.A., and Fagin, J.A. (2013). Genetic and pharmacological targeting of CSF-1/CSF-1R inhibits tumor-associated macrophages and impairs BRAF-induced thyroid cancer progression. **PLoS One** 8, e54302.

Sadaghiani, A.M., Verhelst, S.H., Gocheva, V., Hill, K., Majerova, E., Stinson, S., Joyce, J.A., and Bogyo, M. (2007). Design, synthesis, and evaluation of in vivo potency and selectivity of epoxysuccinyl-based inhibitors of papain-family cysteine proteases. **Chemistry and Biology** 14, 499–511.

Sevenich, L., Bowman, R.L., Mason, S.D., Quail, D.F., Rapaport, F., Elie, B.T., Brogi, E., Brastianos, P.K., Hahn, W.C., Holsinger, L.J., Massagué, J., Leslie, C.S., and Joyce, J.A (2014). Analysis of tumour- and stroma-supplied proteolytic networks reveals a brain-metastasis-promoting role for cathepsin S. **Nature Cell Biology** 16, 876–888.

Sevenich, L., and Joyce, J.A. (2014). Pericellular proteolysis in cancer. **Genes and Development** 28, 2331–2347.

Shree, T., Olson, O.C., Elie, B.T., Kester, J.C., Garfall, A.L., Simpson, K., Bell-McGuinn, K.M., Zabor, E.C., Brogi, E., and Joyce, J.A. (2011). Macrophages and cathepsin proteases blunt chemotherapeutic response in breast cancer. **Genes and Development** 25, 2465–2479.

Virchow, R. (1863). Die Krankhaften Geschwülste, Berlin, Germany.

Vogel, V. (2018). Unraveling the mechanobiology of extracellular matrix. **Annual Reviews in Physiology** 80, 353–387.

Wang, H.W., and Joyce, J.A. (2010). Alternative activation of tumor-associated macrophages by IL-4: priming for protumoral functions. **Cell Cycle** 9, 4824–4835.

Yan, D., Kowal, J., Akkari, L., Schuhmacher, A.J., Huse, J.T., West, B.L., and Joyce, J.A. (2017). Inhibition of colony stimulating factor-1 receptor abrogates microenvironment-mediated therapeutic resistance in gliomas. **Oncogene** 36, 6049–6058.

Yan, D., Wang, H.W., Bowman, R.L., and Joyce, J.A. (2016). STAT3 and STAT6 signaling pathways synergize to promote cathepsin secretion from macrophages via IRE1alpha activation. **Cell Reports** 16, 2914–2927.

Zhang, Q.W., Liu, L., Gong, C.Y., Shi, H.S., Zeng, Y.H., Wang, X.Z., Zhao, Y.W., and Wei, Y.Q. (2012). Prognostic significance of tumor-associated macrophages in solid tumor: a meta-analysis of the literature. **PLoS One** 7, e50946.