Toby Lawrence
Thorsten Hagemann *Editors*

Tumour-Associated Macrophages

Springer

Tumour-Associated Macrophages

Toby Lawrence • Thorsten Hagemann
Editors

Tumour-Associated Macrophages

Springer

Editors
Toby Lawrence
Centre d'Immunologie Marseille Luminy
Inserm-CNRS-Universitie de Mediteranee
Inflammation Biology Group
Parc Scientifique de Luminy, Case 906
Marseille Cedex 9, France
lawrence@ciml.univ-mrs.fr

Thorsten Hagemann
Centre for Cancer and Inflammation
Barts Cancer Institute, Barts and the
London School of Medicine and Dentistry
Queen Mary University of London
London, EC1M 6BQ, UK
t.hagemann@qmul.ac.uk

ISBN 978-1-4614-0661-7 e-ISBN 978-1-4614-0662-4
DOI 10.1007/978-1-4614-0662-4
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2011937444

Preface

Macrophages are tissue resident phagocytes derived from blood monocytes; they have diverse functions in development and immunity and display enormous phenotypic heterogeneity. Macrophages in different tissues have specialized and specific functions that support organ development and physiology, for example, kupffer cells in the liver filter debris from the blood and aid liver regeneration after injury, Langerhans cells in the skin are important immune sentinel cells and mediate immune surveillance, osteoclasts mediate bone morphogenesis, and microglia in the brain support the development and maintenance of neuronal networks. In response to inflammation or injury, monocytes are recruited into tissue and differentiate locally into macrophages and depending on the nature of the insult or injury these macrophages may acquire distinct phenotypes. Tumours are frequently infiltrated by large number of macrophages and in most cases this is linked with tumour progression and poor prognosis. Macrophage polarization is a poorly defined phenomenon; the mediators and mechanisms that maintain the phenotype of distinct macrophage subsets in both physiology and disease remain to be described. Based primarily on in vitro studies, two particular macrophage phenotypes have been described: "classically" activated or M1 macrophages are characterized by the production of pro-inflammatory cytokines and increased microbicidal or even tumouricidal activity. The second, "alternatively" activated or M2 macrophages, in contrast produce anti-inflammatory cytokines and are linked with angiogenesis, tissue repair, and remodeling. These polarized phenotypes have been described based on in vitro stimulation of macrophages with either interferon (IFN) γ, in the case of M1 macrophages, or interleukin (IL)-4 for M2 macrophages. It still is not clear what correlates these populations have in vivo and their physiological relevance remains ambiguous. While these classifications have been useful in that they allow the functional grouping of different macrophage phenotypes, M1 macrophages being pro-inflammatory cells and M2 macrophages linked with trophic functions and wound healing, there are undoubtedly several intermediates between these polarized phenotypes. However, this classification is too restrictive and it is clear that the functional diversity macrophages in vivo may not be associated with these distinct phenotypic subsets. In fact, the question remains in the context of inflammation and

tumours if “the macrophage” merely displays functional plasticity within tissue responding to environmental cues, or distinct stable subsets of macrophages exist with specialized functions. This issue is particularly pertinent in the case of TAM; these cells often display an M2-like phenotype associated with trophic functions promoting tumour angiogenesis, invasion, and metastasis. However, TAM also often produce pro-inflammatory cytokines and have been associated with the promotion of inflammation-associated cancer. This volume provides an overview of current research on the form and function of TAM, highlighting both the mechanistic roles they play in carcinogenesis and tumour progression as well as the molecular mechanisms that control their phenotype and function.

Marseille, France — Toby Lawrence
London, UK — Thorsten Hagemann

Contents

Contributors

Frann Antignano The Terry Fox Laboratory, B.C. Cancer Agency, Vancouver, BC V5Z 1L3, Canada

Claudia Binder Department of Hematology/Oncology, University of Göttingen, Göttingen 37099, Germany

Vincenzo Bronte Verona University Hospital and Department of Pathology, Immunology Section, Piazzale L.A. Scuro 10, 37134 Verona, Italy

Mee Young Chang Lankenau Institute for Medical Research, Wynnewood, PA, USA

Mariacristina Chioda Istituto Oncologico Veneto (IOV), IRCCS, Via Gattamelata 64, 35128 Padova, Italy

Oscar R. Colegio Department of Immunobiology, Howard Hughes Medical Institute, Yale University, New Haven, CT, USA

Thorsten Cramer Medizinische Klinik mit Schwerpunkt Hepatologie und Gastroenterologie and Molekulares Krebsforschungszentrum, Charité – Universitätsmedizin Berlin, Campus Virchow-Klinikum, Augustenburger Platz 1, 13353 Berlin, Germany

Giacomo Desantis Department of Oncology and Surgical Sciences, University of Padova, Via Gattamelata 64, 35128 Padova, Italy

Hsin-Yu Fang Academic Unit of Inflammation & Tumour Targeting, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX, UK

Siamon Gordon Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK

Thorsten Hagemann Centre for Cancer and Inflammation, Barts Cancer Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London EC1M 6BQ, UK

Simon Hallam Centre for Cancer and Inflammation, Barts Cancer Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London EC1M 6BQ, UK

Melisa J. Hamilton The Terry Fox Laboratory, B.C. Cancer Agency, Vancouver, BC V5Z 1L3, Canada

Victor W. Ho The Terry Fox Laboratory, B.C. Cancer Agency, Vancouver, BC V5Z 1L3, Canada

Russell Hughes Academic Unit of Inflammation & Tumour Targeting, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX, UK

Randall S. Johnson Molecular Biology Section, Division of Biology, University of California, San Diego, CA 92093, USA

Gerald Krystal The Terry Fox Laboratory, B.C. Cancer Agency, Vancouver, BC V5Z 1L3, Canada

Etsushi Kuroda The Terry Fox Laboratory, B.C. Cancer Agency, Vancouver, BC V5Z 1L3, Canada

Vivian Lam The Terry Fox Laboratory, B.C. Cancer Agency, Vancouver, BC V5Z 1L3, Canada

Claire E. Lewis Academic Unit of Inflammation & Tumour Targeting, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX, UK

Hsi-Hsien Lin Department of Microbiology and Immunology, College of Medicine, Chang Gung University, 259 Wen-Hwa 1st Road, Kwei-Shan, Tao-Yuan, Taiwan

Alberto Mantovani Istituto Clinico Humanitas IRCCS, via Manzoni 56, 20089 Rozzano, Italy

Department of Translational Medicine, University of Milan, Milan, Italy

Ruslan Medzhitov Department of Immunobiology, Howard Hughes Medical Institute, Yale University, New Haven, CT, USA

Richard Metz New Link Genetics Corporation, Ames, IA, USA

Alexander J. Muller Lankenau Institute for Medical Research, Wynnewood, PA, USA

Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA, USA

Munitta Muthana Academic Unit of Inflammation & Tumour Targeting, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX, UK

Suzanne Ostrand-Rosenberg Department of Biological Sciences, University of Maryland at Baltimore, Baltimore, MD, USA

Melissa Phillips Centre for Molecular Oncology and Imaging, Institute of Cancer, Barts and The London School of Medicine, Queen Mary College, John Vane Science Centre, Charterhouse Square, London EC1M 6BQ, UK

George C. Prendergast Lankenau Institute for Medical Research, Wynnewood, PA, USA

Department of Pathology, Anatomy & Cell Biology, Jefferson Medical School, Philadelphia, PA, USA

Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA, USA

Nadine Rohwer Medizinische Klinik mit Schwerpunkt Hepatologie und Gastroenterologie and Molekulares Krebsforschungszentrum, Charité – Universitätsmedizin Berlin, Campus Virchow-Klinikum, Augustenburger Platz 1, 13353 Berlin, Germany

Jens Ruschmann The Terry Fox Laboratory, B.C. Cancer Agency, Vancouver, BC V5Z 1L3, Canada

Antonio Sica Istituto Clinico Humanitas IRCCS, via Manzoni 56, 20089 Rozzano, Italy

DiSCAFF, University of Piemonte Orientale A. Avogadro, 28100 Novara, Italy

Courtney Smith Lankenau Institute for Medical Research, Wynnewood, PA, USA

Christian Stockmann Molecular Biology Section, Division of Biology, University of California, San Diego, CA 92093, USA

Institut für Physiologie, University of Duisburg-Essen, Duisburg-Essen, Germany

Peter W. Szlosarek Centre for Molecular Oncology and Imaging, Institute of Cancer, Barts and The London School of Medicine, Queen Mary College, John Vane Science Centre, Charterhouse Square, London EC1M 6BQ, UK

Serena Zilio Department of Oncology and Surgical Sciences, University of Padova, Via Gattamelata 64, 35128 Padova, Italy

Istituto Oncologico Veneto (IOV), IRCCS, Via Gattamelata 64, 35128 Padova, Italy

Part I
Form and Function

Chapter 1
Macrophage Phenotype in Tumours

Hsi-Hsien Lin and Siamon Gordon

Introduction

Monocytes and macrophages are a prominent component of the host response to, and manipulation by, tumour cells (Gordon and Martinez 2010; Mantovani et al. 2008). Together with other myeloid and lymphoid cells, they influence tumour development, both positively and negatively. Although the factors that determine outcome of the host–tumour relationship are not well understood, many tumours recruit immature myelomonocytic cells, block their differentiation, subvert their cytotoxicity, suppress lymphoid effector cells, and induce peripheral tolerance. In addition, they mimic and utilise macrophage functions to enhance growth, produce a stroma and promote angiogenesis, local invasion of their micro-environment and metastasis (Qian and Pollard 2010). In particular, the uptake of apoptotic tumour cells can suppress anti-tumour inflammatory responses by TGF-beta and prostaglandins. The macrophage growth factor CSF-1 stimulates macrophage recruitment and modulates its phenotype, limiting the activation of cytotoxic effector functions; Interleukin-4 and -13, acting through common and specific receptors, induce a trophic, alternative M2 activation phenotype, distinct from cytotoxic M1, classically activated (Interferon-gamma-dependent) macrophages (Reviewed by Gordon and Martinez 2010). Interleukin-10 is a potent deactivator of macrophage inflammatory properties, whereas TGF-beta, another deactivator, promotes fibrosis and vascular remodelling.

H.-H. Lin
Department of Microbiology and Immunology, College of Medicine,
Chang Gung University, 259 Wen-Hwa 1st Road, Kwei-Shan, Tao-Yuan, Taiwan

S. Gordon (✉)
Sir William Dunn School of Pathology, University of Oxford,
South Parks Road, Oxford OX1 3RE, UK
e-mail: siamon.gordon@path.ox.ac.uk

T. Lawrence and T. Hagemann (eds.), *Tumour-Associated Macrophages*,
DOI 10.1007/978-1-4614-0662-4_1, © Springer Science+Business Media, LLC 2012

A wide range of chemokines such as MCP-1, often produced by tumour cells, attract mononuclear and myeloid cells. TNF-alpha has also been implicated in tumourigenesis (Mantovani et al. 2008).

Monocyte-macrophages express a wide range of plasma membrane receptors which govern their response to chemokines, cytokines, growth factors and other tumour- and host-derived ligands (Taylor et al. 2005). Other membrane molecules regulate cellular responses to diverse agonists, inhibiting or enhancing macrophage effector mechanisms. These molecules provide useful markers for the presence, characterisation and possible functions of tumour-associated monocyte/macrophages, and targets for therapeutic intervention. In this review, we present a range of possible molecular markers for in situ characterisation, with special reference to the EGF-TM7 family of myeloid G protein-coupled receptors (GPCRs) with large extracellular domains. Their potential is reviewed in the context of macrophage heterogeneity and plasticity (Auffray et al. 2009; Gordon and Taylor 2005) and the experimental analysis of macrophage phenotype in tumours.

Macrophage Heterogeneity in Tumours

Some of the earliest studies on the presence and possible role of macrophages in tumours were undertaken by Evans and Alexander, Mantovani, Pollard and their collaborators (Mantovani et al. 2008; Qian and Pollard 2010). The topic received renewed impetus in recent years with the work of Bronte (Peranzoni et al. 2010) and Gabrilovitch (Gabrilovich and Nagaraj 2009) and their groups. Important contributions came from Balkwill (Mantovani et al. 2008), Lewis (Coffelt et al. 2009), Karin (Grivennikov et al. 2010) and Coussens (Coussens and Werb 2002), Rosenberg (Domachowske et al. 2000) and Joyce (Joyce and Pollard 2009). A great deal of confusion has resulted from myeloid cell heterogeneity and terms such as TAMs (tumour-associated macrophages) and MDSC (myeloid-derived suppessor cells) are currently in wide use. The former embraces cells with macrophage-restricted markers such as F4/80 and alternative activation markers such as Arginase-1 (Gordon and Martinez 2010); the latter term includes cells with immature monocytic phenotype (Gr-1 low) and granulocyte characteristics (Gr-1 high). Mononuclear phagocyte heterogeneity associated with stages of differentiation and activation status gives rise to considerable plasticity within and among cell populations. Studies by Geissmann (Geissmann et al. 2010) and Jung (Varol et al. 2009) have utilised the fractalkine receptor, in combination with other chemokine receptors, to define precursors of tissue macrophages during development, adult life, physiologically and in various inflammatory and pathologic states. Studies by Nussenzweig (Dudziak et al. 2007), Merad (Merad 2010; Merad and Manz 2009) and their colleagues have helped to clarify the origins and population dynamics of myeloid dendritic cells, vis-à-vis monocyte/macrophages. Their fluorescence and transgenic methods will be useful to trace precursors of myelomonocytic cells in mouse tumours.

Tumours are obviously heterogeneous themselves, not only in their ability to invade (benign or malignant), but also in their micro-environment (lung, liver, bone and lymph nodes), origin (epithelial, mesenchymal and haemopoietic), vascularisation, within individual tumours as well as among different primary or secondary tumour populations. Other differences pertain as tumours induce matrix synthesis and catabolism, undergo hypoxia, apoptosis and necrosis. The concomitant presence of $CD4^+$, $CD8^+$ lymphocytes, FoxP3 positive suppressor cells, as well as innate lymphoid cells (NKT and NK cells) modulates myeloid cells, reciprocally. Tumour cells themselves often express characteristic properties of leukocytes that can contribute to their migration and invasion. Macrophages can also be tolerogenic and contribute to lymphocyte suppression by cell contact or secretory products. Dendritic cell maturation and antigen presentation can also be subverted by tumour- or other myeloid-derived products.

Apart from the above considerations, many difficulties hinder experimental analysis of macrophage phenotype in tumours. Ideally, one should study naturally occurring tumours in situ, rather than transplantable models. Isolation of myeloid cells, especially macrophages, is difficult and prone to artefact, particularly if FACS analysis is not combined with immunocytochemistry in situ. The use of oncogenic transgenes, e.g. by Hanahan and colleagues (Hanahan 1989) made it possible to synchronise defined stages of experimental tumours. Mouse models do not necessarily replicate human tumours, often studied at late stages, or after chemotherapy and irradiation. Finally, macrophage markers used in the mouse and human may differ markedly between species.

The interactions between macrophages and tumour cells result in novel gene expression profiles in both cell types, only partially reproduced during co-cultivation in vitro. Microarray and proteomic analyses, while powerful indicators of signatures, e.g. of type 1 interferon activation pathways, need refinement. The traditional methods of morphologic, diagnostic pathology are undergoing rapid advances, but have not yet progressed to interpret function at the single-cell level sufficiently.

Membrane Markers for Macrophages in Tumours

Given the above caveats, we present a list of validated and candidate antigen markers to define macrophage heterogeneity in tumours (Table 1.1). We feel that the present focus reported in the literature is too narrow, that FACS analysis of isolated macrophages is insufficient and that whilst antigens are reasonably well-defined in the mouse, markers for human antigens are limited and not sufficiently characterised. Monoclonal and polyclonal antibodies for FACS and western blotting are not necessarily suitable for immunocytochemistry. Tissue preservation, antigen stability and antibody staining need to be optimised for each epitope. Table 1.1 includes members of a range of molecular families, varying in cell specificity. Markers include opsonic and non-opsonic phagocytic receptors, lectins and scavenger receptors, as well as cytokine receptors and other differentiation antigens, with some functional correlates.

Table 1.1 Selected membrane markers for macrophages in tumours

Molecule	Property	Comment
F4/80	EGF-TM7/adhesion-GPCR	Peripheral tolerance, Mϕ subpopulation
CD97	EGF-TM7/adhesion-GPCR	Myeloid, other cells
EMR2	EGF-TM7/adhesion-GPCR	Human, not mouse, aberrant in breast cancers
CD68	LAMP family	Pan-Mϕ and DC, some tumours
Gr-1	Ly-6 family	PMN, immature monocytes
7/4	Ly-6 family (Rosas et al. 2010)	Polymorphic, PMN, immature monocytes
Siglec-1	IgSF	Sialyl-ligand, e.g. Muc-1
CD163	SRCR family	Glucocorticoid, IL-10 induced
CD200/CD200R	IgSF	Receptor/ligand pair, negative regulator
FcR	IgSF	Activatory/inhibitory
CR3	Beta-2 integrin	Opsonic and non-opsonic phagocytosis Adhesion
SR-A	SRCR family	Clearance apoptotic cells, CSF-1 upregn
MARCO	SRCR family	Adhesion, induced via TLRs
CD36	Bispanner SR-B	Ox-LDL, Apoptotic, Thrombospondin R
MR	C-type lectin	Alternative activation marker
Dectin-1	C-type lectin-like	Beta-glucan R, ITAM-like domain
Dectin-2	C-type lectin-like	Subset macrophages, Mannose-ligand
TLRs	Leucine-rich repeat	Sensor exogenous, host ligands
IL-4/13 R	Cytokine R	Common, specific R, alternative activation
CSF-1R	Receptor tyrosine kinase	fms
GM-CSF R	Haemopoietic R	Fc-GMCSF chimeric ligand (Rosas et al. 2007)
CX3CR1	GPCR	Membrane bound fractalkine R
CCR2	GPCR	MCP-1 ligand

Curiously, in some cases, e.g. CD68, non-haemopoietic tumour cells are able to express leukocyte markers ectopically. Giant cells and hybrids arising from fusion of tumour cells and macrophages provide another mechanism for aberrant marker expression.

Some of these markers have been utilised in inflammatory and infectious models in the mouse but not in tumours. The need for co-localisation and double/multiple labelling, so useful in FACS, is more difficult to achieve in immunohistochemistry, which also lacks quantitation. Laser capture microscopy and tissue arrays may overcome some of these difficulties.

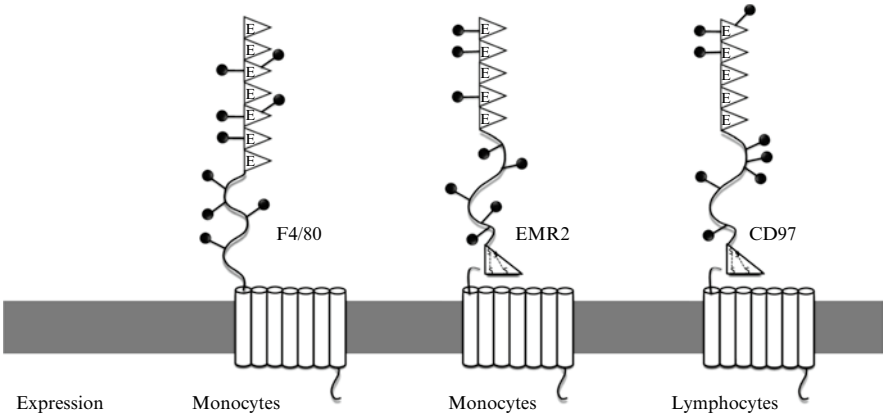
EGF-TM7 Receptors and Tumour-Associated Macrophages

The mouse differentiation antigen F4/80 is a well-characterised marker for mouse macrophages and has been implicated in peripheral tolerance in a non-tumour model (see below for references). The mouse and human antigen CD97 is not only associated

with myeloid cell differentiation and activation, but has also been studied in a variety of tumour settings in vivo. The closely related antigen EMR2 provides a sensitive marker for macrophage identification in human tissues. We review the common and selective characteristics of these molecules in detail, in relation to tissue specificity and as potential markers of macrophage heterogeneity and function in tumour–host interactions.

Common Characteristics of the EGF-TM7 Receptors

F4/80, EMR2 and CD97 all belong to the group of EGF-TM7 molecules that make up the second largest GPCR sub-family in man, the adhesion-GPCRs (Fig. 1.1) (McKnight and Gordon 1996; McKnight and Gordon 1998; Stacey et al. 2000; Yona et al. 2008a; Bjarnadottir et al. 2004; Bjarnadottir et al. 2007). The EGF-TM7 receptors share many common characteristics in protein structure, cellular function



	F4/80	EMR2	CD97
Expression	Monocytes Resident tissue macrophages (Kupffer cells, microglia, etc.) Immature dendritic cells Eosinophils TAMs	Monocytes Macrophages Dendritic cells Neutrophils	Lymphocytes Monocytes Macrophages Dendritic cells Neutrophils Smooth muscle cells Tumour cells
EGF-like domains	7	5	5
GPS proteolysis	No	Yes	Yes
Ligand(s)	Unknown	Chondroitin sulfate	CD55, Chondroitin sulfate Integrins (α5β1/αvβ3)
Function(s)	Induction of Peripheral tolerance	Priming of neutrophils	Migration and homeostasis of PMNs, HSC mobilization, CD4+ T cell co-stimulation, Angiogenesis, Tumour invasior

Fig. 1.1 Characteristics of F4/80, EMR2 and CD97. The three receptors are represented schematically. The EGF-like (E) motifs are shown as *triangles*, the GPS motif as a *triangle with two disulfide bonds* and the 7TM domain is represented by *seven cylinders*