The role of macrophage phenotype in regulating the response to radiation therapy

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Increasing experimental and clinical evidence has revealed a critical role for myeloid cells in the development and progression of cancer. The ability of monocytes and macrophages to regulate inflammation allows them to manipulate the tumor microenvironment to support the growth and development of malignant cells. Recent studies have shown that macrophages can exist in several functional states depending on the microenvironment they encounter in the tissue. These functional phenotypes influence not only the genesis and propagation of tumors, but also the efficacy of cancer therapies, particularly radiation. Early classification of the macrophage phenotypes, or "polarization states," identified 2 major states, M1 and M2, that have cytotoxic and wound repair capacity, respectively. In the context of tumors, classically activated or M1 macrophages driven by interferon-gamma support antitumor immunity while alternatively activated or M2 macrophages generated in part from interleukin-4 exposure hinder antitumor immunity by suppressing cytotoxic responses against a tumor. In this review, we discuss the role that the functional phenotype of a macrophage population plays in tumor development. We will then focus more specifically on how macrophages and myeloid cells regulate the tumor response to radiation therapy.

Abbreviations: RT = radiation therapy; IFN-γ = interferon-γ; TLR = toll-like receptor; STAT = signal transducer and activator of transcription; NF-κB = nuclear factor-κB; CXCLs = C-X-C motif ligands; CCLs = C-C motif ligands; iNOS = inducible nitric oxide synthase; IL = interleukin; TNF = tumor necrosis factor; Arg-1 = arginase-1; VEGF = vascular endothelial growth factor; CSF1 = colony stimulating factor 1; TAMs = tumor-associated macrophages; M-CSF = macrophage colony-stimulating factor; PD-1 = programmed death-1; HIF-1α = hypoxia-inducible factor-1α; MMP = matrix metalloproteinase; DAMP = damage-associated molecular pattern; HMGB1 = high mobility group protein box 1; CRT = calreticulin; STING = stimulator of interferon genes; DCs = dendritic cells; Gy = Gray; miRNA = microRNA; MDSCs = myeloid-derived suppressor cells; IDO = indoleamine-2,2-dioxygenase; GATA3 = GATA-binding protein 3

INTRODUCTION

Macrophages arise from the myeloid cell lineage in the bone marrow. They begin life by entering the blood stream as monocytes and, in response to a variety of inflammatory stimuli, migrate into the tissue and become mature macrophages. Once in the tissue, they can differentiate into several different types of mature macrophages. These macrophages play pivotal roles in the initiation, propagation, and resolution of inflammation.[1] In the tissue, macrophages are highly responsive to environmental cues including cytokines and other inflammatory stimuli. Macrophages undergo phenotypic changes upon encountering these triggers to acquire functions that can support or inhibit an inflammatory response.[2] Tumors actively recruit myeloid cells and express various cytokines and cell surface molecules that push recruited myeloid cells to differentiate into macrophages that can support tumor growth and inhibit the tumor response to therapies such as chemo-

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therapy and radiation.[3] Tumor-associated macrophages inhibit the response to therapies through multiple mechanisms including inhibition of the antitumor immune response stimulated by therapy-induced cell death and production of vascular and epithelial growth factors. In this review, we will discuss the current understanding of macrophage phenotypes, the role of macrophages in cancer, and finally how macrophages and their functional phenotypes regulate the response to radiation therapy (RT).

MACROPHAGE POLARIZATION: FUNCTIONAL DIVERSITY OF MACROPHAGES

Recent studies have revealed tremendous functional diversity among macrophages ranging from their cytotoxic killing abilities to their central role in tissue repair. This diversity likely arises from the multitude of situations requiring phagocytic cells throughout the body. Found in virtually every tissue of the body, tissue- and bone marrow-derived macrophages encounter a tremendous number of agents they have to deal with to maintain tissue homeostasis. Thus, their diversity arises from the need to prepare macrophages for the things they will encounter anywhere from infected cells to damaged tissue. Early classification schemas attempted to categorize macrophage functional states into either the classically activated macrophages (M1) phenotype or the alternatively activated macrophages (M2) phenotype, mirroring the Th1/Th2 polarization states found in T-cells.[4] With respect to tumors, M1 macrophages owing to their cytotoxic capacity are often considered the "antitumor" phenotype, whereas the M2 macrophages owing to their immunosuppressive and angiogenic capacity are thought to be the "pro-tumor" phenotype. More recent studies examining macrophage populations in vivo have shown that the M1/M2 classification grossly oversimplifies the wide spectrum of functional macrophage phenotypes found in the body.[5,6] Nevertheless, for the purposes of this discussion, it remains helpful to broadly classify macrophages into their earlier M1/M2 nomenclature, recognizing that this subdivision likely does not capture a complete understanding of the macrophage phenotypes involved.

M1 MACROPHAGES: THE "ANTITUMOR" PHENOTYPE

Macrophages have long been recognized as the first line of defense against foreign pathogens in innate immunity.[7] Th1-related cytokines like interferon-γ (IFN-γ) and microbicidal stimuli such as lipopolysaccharide prime macrophages to produce a cytotoxic activation state that characterizes the M1 phenotype.[8] In these M1 macrophages, downstream signaling of IFNs and toll-like receptors (TLRs), through activation of signal transducer and activator of transcription 1 (STAT1) and nuclear factor-κB (NF-κB), drives the expression of a transcriptional program including the chemokines C-C motif ligand 15 (CCL15), C-X-C motif ligand 10 (CXCL10), chemokine receptors such as C-C chemokine receptor type 7 (CCR7), and reactive oxygen species (particularly inducible nitric oxide synthase [iNOS]).[9] M1 macrophages have been identified by both surface markers and expression of several key genes such as interleukin (IL)-12, IL-1, and tumor necrosis factor (TNF).[10-12] Studies examining the interaction between macrophages and cancer cells suggest that M1 macrophages both directly kill cancer cells and support the cytotoxic activity of other immune cells including T-cell and NK cells; thus, the M1 phenotype is often considered the "antitumor" phenotype.[13]

M2 MACROPHAGES: "PRO-TUMOR" PHENOTYPE

Originally identified in response to metazoan parasite infections and allergens, M2 macrophages form when macrophages encounter Th2-associated cytokines including IL-4 and IL-13, which activate STAT6, leading to expression of targets that were found to be key in mediating not only anti-helminthic immunity but also tissue repair.[14] M2-polarized macrophages possess higher levels of arginase (Arg-1) activity, allowing them to convert arginine to ornithine, a precursor of polyamines and collagen, contributing to the production of extracellular matrix.[15] M2 macrophages are also known to secrete other factors associated with wound healing such as vascular endothelial growth factor (VEGF), colony-stimulating factor 1 (CSF1), and IL-8, which promote angiogenesis, lymphangiogenesis, and fibrosis.[2,16] They are characterized by expression of immunosuppressive cytokines, chemokines, and surface markers such as IL-10, CCL17, and CD206.[9] Tumor-associated macrophages (TAMs) share many of the same expression patterns as M2 macrophages. TAMs play a crucial role in the initiation, promotion, and metastasis of cancer cells by encouraging angiogenesis and remodeling of the stromal matrix to help establish the premalignant niche. Thus, M2-polarized macrophages are often considered a "pro-tumor" phenotype.[17,18]

MYELOID-MACROPHAGE CELLS IN CANCER

Myeloid cells and macrophages have been associated with both the development and the progression of cancer.[16] Several larger retrospective clinical studies found that increasing numbers of TAMs correlate with higher grade tumors in multiple tumor types including breast, lung, and prostate.[19] Tumors actively recruit mac-