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Targeting tumor associated macrophages (TAMs) *via* nanocarriers

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ABSTRACT

Recruitment of inflammatory cells to tumor has been well documented, with the most frequent inhabitants being macrophages termed as tumor associated macrophages, (TAMs). Their presence was thought to be an evidence of immune system initiating a fight response towards the tumor, *i.e.* immune surveillance. This is the case too initially, when TAMs majorly exhibit an M1 phenotype, but their continued presence in tumor microenvironment brings about their polarization to M2 phenotype, which not only participate in continued sustenance of existing tumor but also open up deleterious avenues for further progression and metastasis of cancer. Current perspective is built around this very premise and focuses specifically on TAMs and how they are being targeted by researchers working in annals of nanomedicine. To do so, we dwell into tumor microenvironment and focus on nanotechnology based drug delivery aspects which have either been already or can be potentially employed in the future to target tumor associated macrophages for improved immunoadjuvant therapy of cancer.

1. Introduction

Balance of homoeostatic machinery is an important regulator of human well-being. Even under exposition of harshest insults, homeostasis attempts to preserve body's integrity, *i.e.* it tries to restore basal structural and functional levels. An immune response can be purported as the perfect example, wherein, homeostatic machinery actively tries to cure the human body when faced with stress. Immune response, regardless of stimulant, scope, or context, is incessantly ubiquitous. However, there are circumstances when an intended immune response jeopardizes entire pretext for which that response was generated in the first place; case in point being etiologic role of immune system in progression of cancer. Immunoreactivity towards carcinoma cells is expressed by lymphoid infiltration into tumor microenvironment and regional lymphatic alterations, specifically phenotypic differentiation of macrophages present in tumor stroma [conveniently christened as tumor associated macrophages (TAMs)]. TAMs present two subtypes *i.e.* classically activated macrophages (M1) and alternatively activated macrophages (M2). The process of phenotypic conversion of one sub type into another is termed as polarization of TAMs. M1 cells have strong antigen presenting capability and facilitate immunological response against tumor. They raise proinflammatory cytokines such as TNFα, IL-12, and IL-23 and enhance release of reactive oxygen species (ROS) and nitric oxide (NO) which provides an antitumor response. Moreover, they also express high levels of major histocompatibility complex: class I and class II molecules, raising probability of recognizing tumor specific antigens. Contrarily M2 subset has strong anti-inflammatory activity. It enhances release of IL-4, IL-10, and IL-13 and has poor antigen presenting characteristics which suppresses T cells and generation of ROS and/or NO. IL-4 and IL-10 have negative

Abbreviations: TAMs, Tumor associated macrophages; Akt, Protein kinase B; CAT, Catalase; CCL, Chemokine (C-C motif) ligand; CD, Cluster of differentiation; COX, Cycloxygenase; CpG, Unmethylated sequences of DNA that have immunostimulatory properties; CSF, Colony-stimulating factor; CXCL, Chemokine (C-X-C motif) ligand; CXCL10, C-X-C motif chemokine 10 also known as IP10; CXCL9, Chemokine (C-X-C motif) ligand 9; CXCR1, Interleukin 8 receptor α; CXCR2, Interleukin 8 receptor β; EGF, Epidermal growth factor; EPR, Enhanced permeability and retention; ERK, Extracellular signal–regulated kinases; GPx1, Glutathione peroxidase 1; HIF, Hypoxia-inducible factors; IKK-β, Inhibitor of nuclear factor kappa-B kinase subunit beta; IL, Interleukin; IP10, Interferon gamma-induced protein 10; JNK, c-Jun N-terminal kinases; M1, M1 subtype of tumor associated macrophage; M2, M2 subtype of tumor associated macrophage; MAPK, Mitogen-activated protein kinase; M-CSF, Macrophage colony-stimulating factor also known as CSF1; MDC, Macrophage-derived chemokine also known as CCL22; MIG, Monokine induced by gamma interferon; MIP-1 β, A protein which in humans is encoded by the CCL4 gene; MMP, Matrix metalloproteinase; MRI, Magnetic resonance imaging; MYO_3A, A gene which encodes for the protein Myosin IIIA; NFκB, Nuclear factor kappa-light-chain-enhancer of activated B cells; NO, nitric oxide; p38, Class of mitogen-activated protein kinases; PD-L1, Programmed cell death 1; PEG, Polyethyleneglycol; PET, Positron emission tomography; PI3K, Phosphoinositide 3-kinase; ROS, Reactive oxygen species; SESN 3, A protein; SOD, Superoxide dismutase; STAT, Signal transducer and activator of transcription; TGF, Transforming growth factor; TRAIL, TNF-related apoptosis-inducing ligand; TNFα, Tumor necrosis factor alpha; VEGF, Vascular endothelial growth factor; VEGFR, Vascular endothelial growth factor receptor

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Table 1
Effect of M1 and M2 polarization of TAMs on expression of various receptors and production of cytokines and chemokines.

Cellular component		M1 phenotypic TAMs	M2 phenotypic TAMs	Effector Cytokines	References
Receptors	Membrane scavenger receptor A and receptor B	↓↓	↑↑	IL-10	[5,6]
	Membrane CD14	↓	↑↑	IL-10	[7]
	Membrane scavenger receptor CD163 and Fcε receptors (CD23)	↓↓	↑↑	IL-4 and IL-13	[8,9]
	Membrane mannose receptor CD206	↓↓	↑↑	IL-4, IL-10 and IL-13	[10,11]
	Membrane Toll-like receptor 2 (CD282) and 4 (CD284)	↑↑	↓	IFNγ	[12]
	Membrane Fcγ receptors (CD16, CD32, CD64)	↑↑	↓	IFNγ	[13]
	Membrane CD80	↑↑	↓	IFNγ	[14]
	Cytokine decoy Interleukin-1 R type II (CD121b)	↓↓	↑↑	IL-4, and IL-13	[15,16]
	C-C chemokine receptor type 7 (CCR7)	↑↑	↓↓	IFNγ	[17]
	C-C chemokine receptor type 2 (CCR2)	↓	↑↑	IL-10	[18]
	Interleukin 8 receptor α and β (CXCR1 and CXCR2)	↓	↑↑	IL-4 and IL-13	[19]
Cytokines	TNF-α, IL-1, IL-12 and Type I IFN	↑↑	↓↓	IFNγ	[20,21]
	IL-1ra	↓	↑↑	IL-4, IL-10 and IL13	[22]
	IL-10	↓	↑↑	IL-4, and IL-13	[23]
Chemokines	Chemokine (C-X-C motif) ligand 8 9, 10 and 11 (CXCL8, CXCL9, CXCL10 and CXCL11)	↑↑	↓↓	IFNγ	[24–27]
	Chemokine (C-C motif) ligand 2, 3, 4, and 5 (CCL2, CCL3, CCL4 and CCL5)				
	Chemokine (C-C motif) ligand 17, 22 and 24 (CCL17, CCL22 and CCL24)	↓↓	↑↑	IL-4 and IL-13	[23,28]
	Chemokine (C-C motif) ligand 18 (CCL18)	↓↓	↑↑	IL-4, IL-10 and IL-13	[29]

downstream effect on secretion of regulatory cytokines like IP-10 (CXCL10), and MIG (CXCL9) which stall Th1 immune response; whereas IL-4 and IL-10 released by them upregulates secretion of Th2 immune response mediators like eotaxin-2 (CCL24), CCL18 and MDC (CCL22) [1,2]. Known list of effectors, chemokines and receptors modulated by TAMs is very large, however, some selected ones which sub serve role in cancer dynamics are listed in Table 1.

Accumulation of TAMs in tumor stroma has significant physiological implications [3,4]. They initially inhibit, but later due to 'self and/or cross' talk promote tumor progression in various ways including retardation of antitumor immunologic response, stimulating angiogenesis, *etc.* It is therefore no wonder that scientists have tried to target this complex interplay, "of originally intended to be good immune cells, gone awry" to provide immunotherapy of cancer. Current perspective is built around this very premise and focuses specifically on TAMs and how they are being targeted by researchers working in annals of nanomedicine. To do so, we dwell deep into tumor microenvironment and try to locate chinks in tumor armory, which might be used to modulate activity of TAMs. We then focus on nanotechnology based drug delivery aspects which have either been already or can be potentially employed in the future to target tumor associated macrophages for improved immunoadjuvant therapy of cancer.

2. Recruitment of TAMs

Peyton Rous first coined the term "subthreshold neoplastic states" for chemical and viral carcinogenesis induced somatic changes because they involve irreversible DNA alterations [30]. But it was Rudolf Virchow who visualized recruitment of leukocytes in tumorous tissues and proposed a link between tumor and inflammation [31]. Later Karin substantiated Virchow's notion by elucidating the role of inflammation in tumorigenesis, particularly involvement of NFκB. This study eventually linked immunity and inflammation to cancer development and progression; as all known targets of NFκB inhibitor, IKK-β, such as TNF-α, IL-1, IL-6 were associated with carcinogenesis [32]. These observations strengthened the link between cancer related inflammation, the seventh hallmark of cancer, to genetic instability [33]. Thus recruitment of inflammatory cells to tumor became well documented with the most frequent inhabitants being tumor associated macrophages. Their presence was thought to be an evidence of body initiating a fight response towards tumor and referred to as immune surveillance [34]. In terms of lineage, TAMs are derived either directly from circulatory monocytes or pre-differentiated macrophages invading the periphery. Tymoszuk et al. have demonstrated that both monocyte recruitment and local macrophage proliferation determines TAMs pool size in HER2/Neu-positive mammary carcinomas [35]. Recruitment in tumor stroma is instigated by various factors like tumor induced local hypoxia, prevalent acidosis, cytokines, *etc.* (Fig. 1). For instance, the soluble chemokine CCL2, whose expression itself is regulated by activin A, is a known facilitator of monocyte or macrophage accumulation in inflammatory sites in vicinity of prostate [36] and breast cancer [37,38]. Apart from its chemotactic action, CCL2 also partakes in signaling which leads to polarization of monocytes to M2 subtype instigating Th2 immune response [39]. Mizutani K et al. have demonstrated CCL2 overexpressive luciferase-tagged PC3 cells attract monocytes in both *in vitro* and *in vivo* conditions using a xenograft model. They were even able to significantly reduce tumor burden by administering CCL2 neutralizing antibodies, which prevented recruitment of macrophages in tumor microenvironment [40]. Other chemokines such as CXCL1, CXCL8, CXCL12, CXCL15, CCL5, CCL17 and CCL22 are also observed in tumor environment, playing variety of overt, permissive or occult roles in initial inflammation, tumor progression, recruitment and polarization of TAMs [41–43].

Almost all oxygen-breathing species express a highly conserved transcriptional complex, hypoxia-inducible factor (HIF-1) that responds to decrease in available intracellular oxygen. In general, HIFs are vital to embryonic development and tissue repair. However, several reports suggest HIF-1 is deregulated in cancers due to distorted tumor milieu which makes chronic inflammation self-perpetuating and provides axis for further sustenance and metastasis of cancer [44–46]. HIF activity is required in angiogenesis which is mandatory to nurture rapidly dividing cancer cells, and consequently HIF inhibitors like acriflavine and phenethyl isothiocyanate are under the scanner of clinical investigation as probable anticancer drug candidates. HIF enhances expression of cytokines CXCR4 and CXCL12 which not only attract circulatory monocytes but also induce their polarization to M2 subtype by ERK signaling [47–50]. Tripathi et al. have elucidated the mechanism for recruitment of TAMs to hypoxic regions. They identified and described role of hypoxic cancer cell derived cytokines Oncostatin M and Eotaxin-1 in promoting recruitment of macrophages and their phenotypic switching to M2 subtype in solid tumors [51]. In a different study Wen Z et al. have shown that even transient elevation of 5-lipoxygenase (a metabolite produced by hypoxic cancer cells) enhances infiltration of TAMs. This hastening in migratory tendency of TAMs in response to local 5-lipoxygenase levels is mediated by matrix metalloproteinase (MMP)-7 *via* p38 pathway. The mechanism is so prudent that in *in vivo*