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Back to the Problem Of Tumor-Associated Macrophages: A Role For Diagnostic Imaging?

Abstract

Inflammation is intrinsically bound to cancer, and the host immune system, particularly represented by monocytes and macrophages, takes active part in tumor growth and spread. Tumor associated macrophages (TAMs) are the relatively new problem in current oncology research, since their presence in tumor site is strongly correlated with negative prognosis for the patient. TAM-targeted therapies as well as clinical imaging and quantification of TAM presence at the tumor site represent the questions of outmost importance. Current paper attempts to give a short overview of the problem of TAMs and briefly discusses potential diagnostic imaging strategies with a scope for further research actions.

Keywords: Cancer; Tumor-associated macrophages; TAM; Imaging; Nanoparticles; Biomarkers

Mini Review

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Abbreviations: TAM: Tumor Associated Macrophages; LPS: Lipopolysaccharides; IL: Interleukin; TNF: A - Tumor Necrosis Factor- A; CCL: C-C Motif Ligand; MCP: Monocyte Chemotactic Protein; CSF: Colony Stimulating Factor; EGF: Epidermal Growth Factor; G-CSF: Granulocyte Stimulating Factor; GM-CSF: Granulocyte-Monocyte Stimulating Factor; M-CSFR : Macrophage Colony Stimulating Factor Receptor; Mabs: Monoclonal Antibodies; NF-Kb: Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells; STAT: Signal Transducer and Activator of Transcription; HRG: Histidine-Rich Glycoprotein; MDXAA: 5,6-Dimetyhlxanthenone-4-Acetic Acid; PPZ: Pantoprazole Proton Pump Inhibitor; Cpg-ODN: Cpg Oligodeoxynucleotides; SPECT: Single Photon Emission Tomography; PET: Positron Emission Tomography; NIRF: Near Infrared Fluorescence; MMR: Macrophage Mannose Receptor; USPIO: Ultrasmall Superparamagnetic Iron Oxide; SPIO: Superparamagnetic Iron Oxide; MMTV-Pymt: Mouse Mammary Tumor Virus - Polyoma Middle T Antigen; PEG: Polyethylene Glycol; CT: Computed Tomography; MRI: Magnetic Resonance Imaging

Introduction

There has been increasing epidemiological evidence that inflammation, often as a result of incipient infection can act as a promoter of tumorigenesis [1]. Experimental studies in mice supported this hypothesis by showing that cells of the innate immune system (particularly macrophages) play central role in inflammatory responses also play crucial roles in tumor progression [1]. Macrophages are immune cells, playing a significant role in tissue homeostasis [2]. Resident in tissues and inflammatory macrophages take their origin from circulating bone-marrow derived monocytes. After extravasating from the blood into the tissues, they differentiate and polarize according to existing microenvironment [2]. Polarization creates a specific cytokine/enzyme/surface receptor profile of a macrophage, which generally falls in one of the two categories: M1 or M2 [2]. M1 phenotype is driven by Th1 cytokines, like interferon-γ, LPS and Toll-like receptor agonists [2]. M1 macrophages are a critical component of inflammatory response and antitumor immunity and are characterized by production of pro-inflammatory factors like IL-6, IL-12, IL-23, (TNF-a), as well as histocompatibility complex class I and II molecules (the latter are required for presentation of tumor-specific antigens). Unlike highly bactericidal and tumorocidal M1 macrophages, M2 macrophages, whose profiling is stimulated by Th2 cytokines, express anti-inflammatory and pro-tumorogenic properties, providing a favorable environment for tumor survival, growth and angiogenesis [2]. Tumor associated M2 macrophages are also known to contribute to radio-protective effects, support the dissemination of tumor cells from primary to secondary sites and control self-renewal and chemotherapeutic resistance of cancer stem cells [2]. Macrophages (specifically M2-like macrophages) represent a predominant population of inflammatory cells in solid tumors [3]. Important is the fact that macrophage phenotype shifts are dependent on the tumor progression stage and a shift to M2 or anti-inflammatory, “repair”-oriented and angiogenic state is observed in advanced tumor stages [4]. The recruitment of monocytes/macrophages into the tumor is regulated by cytokines, chemokines (like CCL-2\MCP-1) and growth factors present in the tumor microenvironment, as well as by hypoxic states in the tumor [2]. There is evidence that growth factors, like CSF-1 (colony stimulating factor), play major role in tumor macrophage infiltration and subsequent pro-angiogenic TAM changes. Mechanistically, tumor cells produce certain mediators (like CSF-1) in order to promote macrophage migration along with associated macrophage changes and subsequently TAM-derived mediators or active substances (like EGF) enhance tumor cell invasion [5]. For example, overexpression of CSF-1 along with significant TAM presence in tumor, are associated with poor

prognosis in breast, ovarian, endometrial and prostatic cancers [1]. There is some evidence that even exogenous application of CSFs (like G-CSF and GM-CSF), which is commonly used to correct therapy-induced neutropenia and mucositis in oncologic patients, despite ameliorating mucositis, neutropenia and promoting wound healing, might impair disease control and cause tumor progression [6].

The abovementioned indicates TAMs as a most important target for strategies aimed at controlling/preventing tumor growth and metastatic dissemination.

Since tumor-promoting effects of TAMs are directly correlated with their accumulation and activation in tumor tissues, TAM-targeted approaches are could be grouped as [3]:

I. Inhibiting macrophage recruitment [3]
   a. inhibitors of CCL2/CCR2
   b. inhibitors of M-CSF/M-CSFR
   c. inhibitors of other chemoattractants and their receptors
   d. inhibitors of the pathways for macrophage recruitment

II. Supressing TAM survival [3]
   a. macrophage-depleting chemical drugs (e.g. bisphosphonates)
   b. immunotoxin-conjugated mAbs, targeting TAM membrane molecules
   c. attenuated bacteria (e.g. Shigella flexneri), inducing macrophage apoptosis
   d. agents that induce macrophages to express molecules to be targeted by cytotoxic T lymphocytes

III. Enhancing tumoricidal activity of TAMs [3]
   a. agonists of NF-kB
   b. agonists of STAT1 (e.g. interferon)
   c. agonists of other M1 pathways
   d. other agents (e.g. IL-12 and thymosin α1)

IV. Blocking M2 tumor-promoting activity of TAMs [3]
   a. inhibitors of STAT3 (e.g. sunitinib)
   b. inhibitors of STAT6
   c. inhibitors of other M2 pathways
   d. other agents (e.g. HRG,cuNg, MDXAA, silibinin and PPZ)

Although majority of the strategies are currently unavailable, still there has been approved a number of drugs with TAM-targeting properties, like Zoledronic acid, Trabecitidin, Dasatinib, CpG-ODN, Interleukin-12, Sunitinib and others [3].

Discussion

Recognition of TAMs as a promising target for cancer therapy has led to increasing number of studies during the past years. However there is still a long journey ahead, regarding the tumor-macrophage interactions and signaling pathways as well as biochemical aspects of TAM phenotype profiling. Unraveling the whole spectrum of M1 macrophage profiling mechanisms could open a way for the most promising TAM therapy strategy, aimed at reprogramming or enhancing macrophage tumorocidal activity. Taking into consideration the role of TAMs in negative prognosis and outcome of cancer, non-invasive quantification of TAMs at the tumor site is the question of vital importance. Given the opportunity for clinically applicable non-invasive, preferably non-ionizing (to minimize the hazards of further follow-ups) diagnostic method it might be possible to expand the classical tumor staging approach with an additional disease prognosis scale and create an effective monitoring tool for TAM-targeted therapies. For example, patients with breast cancer and marked macrophage infiltration could be directed to novel macrophage-antagonist or immune reprogramming therapies, while patients with node-negative tumors and without significant macrophage infiltration could be treated alternatively [7].

To achieve selective visualization of TAMs in a tumor site it would be absolutely logical to target TAM-specific receptors, for example with marked antibodies (we would ignore the questions of cost-effectiveness in this paper). For example, it was discovered that strongly pro-angiogenic TAMs, residing in hypoxic areas of the tumor overexpress Macrophage Mannose Receptor (MMR) [8], which served as a target for a number of in-vivo imaging studies, utilizing marked antibodies to achieve selective receptor binding and selective imaging of TAMs using SPECT, PET and near infrared fluorescence imaging (NIRF) [8-10]. However, antibody-based imaging methods may come across the problem of immunogenicity during the process of clinical translation [11]. Besides, specificity (in terms of selective antibody-receptor binding), might also turn into a weakness, once the tumor cells "lose" a certain receptor.

An alternative visualization approach could rely on natural endocytic properties of macrophages (either phago- or pinocytosis), and could utilize particle formulations that are easily ingested by macrophages for delivery of contrast agents to TAMs. Engineered nanoparticles (NPs) have a lot to offer in this sense, starting from a wide range of NP categories, presented by carbon-based NPs (like carbon nanotubes and inorganic NPs), ones based on the metal oxides (iron oxide, cerium oxide) metals (gold and silver) and semiconductor NPs or so called quantum dots [12]. There are numerous publications on nanoparticle interaction with cells and specifically macrophages and it is well known that besides size of the particle, other characteristics, like shape, surface charge and chemical base of the particle play crucial role in its ingestion by a macrophage [13].

Moreover these characteristics account for the particle toxicity profile. For example, smaller nanoparticles have a higher probability to be internalized by passive uptake than larger ones [14] and at the same time smaller NPs are also reported to be more likely to cause toxic cellular responses [14]. A number of authors have already investigated NPs as imaging biomarkers. For example, a study by Daldrup-Link et al. [15] investigated in vivo MR imaging of TAMs with clinically applicable iron oxide nanoparticles. The goal of the study was to utilize novel ultra-small superparamagnetic iron oxide nanoparticle compounds

(USPIO) to develop an immediately clinically applicable molecular imaging approach for enhanced imaging of TAMs in breast cancer. Intravenously injected SPIO were consumed by macrophages in various target tissues depending on the particle size and composition [15]. It was stated that large SPIO particles with hydrodynamic diameters in range from 80 to 150 nm are rapidly ingested by macrophages of the reticulo-endothelial system (RES), while ultra-small particles with diameters of less than 50 nm are able to escape RES endocytosis to some extent, leading to a prolonged blood pool circulation, accumulation and macrophage ingestion in inflamed tissues and tumors [15]. The study assessed three USPIO compounds with hydrodynamic diameter <30 nm: Ferumoxytol (Feraheme, AMAG Pharmaceuticals Inc.), P904 (Guebert, France) and P1133 (Guebert, France). Animal model constituted of MMTV-PyMT mice that spontaneously developed multifocal, multiclonal mammary adenocarcinomas [15]. The results of the study showed that USPIO particles were predominantly ingested by macrophages and not by breast cancer tumor cells. Ferumoxytol was found to be responsible for persistent tumor enhancement on delayed MR images, which corresponded to specific nanoparticle retention in TAMs.

Another study by Shi et al. [16] analyzed different USPIO compound named GEH121333 as a potential imaging biomarker for TAMs. It was concluded that GEH121333 (USPIO agent with an iron oxide core and ahydroxyphosphonate-PEG shell) has better toleration, provides longer blood half-life and better TAM visualization compared with Ferumoxytol [16].

There are also publications on radioactive labeling of SPIO compounds, which makes possible PET/CT or PET/MRI investigation of tissues with presence of activated macrophages [17].

The questions that yet have to be answered regarding the use of NPs as biomarkers include the concepts of dose, accumulation, toxicity and both short- and long-term biological effects. It is now established that engineered NPs may cause cell death. Moreover NPs that are well tolerated by the cells could also possess "latent toxicity" [18]. The potential danger of NP could first of all be related to their size, since they have a potential to accumulate nearly anywhere in a biological system [12] and estimation of the real nanotoxic effects could be a very uneasy matter. The first and the very important concept of dose still remains a matter of discussion, while for any clinical application the nominal/delivered/cellular doses estimates are crucial as well as dose dependent biological effects. Moreover, depending on the NP type, biological effects could be more pronounced in the extracellular versus the intracellular environment. In these cases extracellular dose would be of greater importance, like in case of the zinc oxide nanoparticles, which exert their cytotoxic activity through the release of Zn(II) in the extracellular environment [12]. The mechanisms by which engineered nanoparticles could induce toxicity after exposure include, but are not limited to [19]: generation of free radicals due to phagocytic cell response (oxidative stress), oxidation of DNA and proteins (biological damage), oxidative stress-induced upregulation of inflammatory factors (inflammation), cell components damage with release of reactive oxygen species (cell cycle arrest and apoptosis), generation of intermediate metabolic products (organotoxicity), interaction with immune system (immunotoxicity), interaction with blood components (haemolysis and thrombosis). Therefore cytotoxic, genotoxic/mutational and oxidative stress potential has to be established for every NP prior to their in-vivo application. Moreover, in a view of recently discovered Gadolinium MR contrast accumulation in the brain [20], at least some of the following additional questions have to be answered prior to clinical application of NPs [12]:

A. How long do NPs remain inside the cells?

B. Do NPs get eventually flushed out of a particular cell or stay there indefinetely?

C. Can the biological effects of NPs be predicted with any type of accuracy?

D. Could engineered NPs diminish the function of the immunological apparatus?

E. Do NPs pose any "antigenic challenges"?

Conclusion

Tumor associated macrophages are reported to play a crucial role in cancer growth and spread. In the sense of existing and upcoming TAM-targeted therapy strategies, adequate diagnostic imaging methods are vital for development of a coordinate system, which could be used for creating prediction, staging and treatment efficiency evaluation tools. Further diagnostic imaging research in this direction should aim at a) selective imaging of M1 and M2 macrophages in the tumor microenvironment, b) quantifying relation between the tumor macrophage infiltration density and disease prognosis, c) developing TAM-targeted treatment response criteria and assessing their correlation with disease control.

Engineered nanoparticles, being able to exploit intrinsic macrophage endocytosis mechanisms, have a very high potential of becoming a key imaging biomarker for TAMs. However further extensive research is needed to address the problems of optimal particle characteristics as well as associated questions of dosing, accumulation, toxicity, short- and long-term biological effects [21-25].

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