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Myeloid-derived suppressor cells
Cellular missiles to target tumors

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While conventional anticancer therapies, including surgical resection, radiotherapy, and/or chemotherapy, are relatively efficient at eliminating primary tumors, these treatment modalities are largely ineffective against metastases. At least in part, this reflects the rather inefficient delivery of conventional anticancer agents to metastatic lesions. We have recently demonstrated that myeloid-derived suppressor cells (MDSCs) can be used as cellular missiles to selectively deliver a radioisotope-coupled attenuated variant of *Listeria monocytogenes* to both primary and metastatic neoplastic lesions in mice with pancreatic cancer. This novel immunotherapeutic intervention robustly inhibited tumor growth while promoting a dramatic decrease in the number of metastases.

Myeloid-Derived Suppressor Cells

Myeloid derived suppressor cells (MDSCs) are a heterogeneous population of myeloid progenitor cells, i.e., immature macrophages, granulocytes, and dendritic cells (DCs), that are endowed with a robust immunosuppressive activity.[1] In normal healthy individuals, immature myeloid cells differentiate into mature granulocytes, macrophages, and DCs. Conversely, in cancer patients, MDSCs respond to tumor-secreted factors including interleukin (IL)-6, granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-1β by emigrating from the bone marrow and accumulating within primary neoplastic lesions and metastases.[2] In the tumor microenvironment (TME), MDSCs are prevented from differentiation and are stimulated to express immunosuppressive enzymes like arginase I and inducible nitric oxide synthetase as well as to produce immunosuppressive mediators, including reactive oxygen species and various cytokines such as IL-6, IL-10, and transforming growth factor β1 (TGFβ1).[1,2] Altogether, these enzymes and factors are responsible for the suppression of T-cell and natural killer (NK)-cell responses in the TME.[1-3] While MDSCs are highly immunosuppressive,[1-3] we have recently shown that these cells can be used as a vehicle to deliver anticancer agents to the TME with help of *Listeria monocytogenes*.[4,5] MDSCs are abundant not only in the peripheral blood of tumor-bearing mice, but also in the circulation of patients affected by almost all types of cancer. Thus, MDSC/*Listeria*-based interventions might be suitable for the treatment of a wide panel of neoplasms.

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Listeria Monocytogenes

Listeria monocytogenes is Gram-positive facultative intracellular bacterium that causes food-poisoning. In contrast to wild type *Listeria*, attenuated non-virulent strains of *Listeria monocytogenes* are attractive vaccine vectors because of their unique ability to selectively deliver antigenic determinants to antigen-presenting cells (APCs) such as monocytes, macrophages, and DCs through phagocytosis, while activating strong innate and adaptive immune responses.[6] *Listeria*-based anticancer vaccines have been developed and tested by different groups, including ourselves, in animal models of various neoplasms including (but not limited to) breast, pancreatic, cervical, and colorectal carcinoma.[4,6,7]

We discovered that an attenuated strain of *L. monocytogenes* (*Listeria*^{at}) infects not only APCs but also cancer cells. Malignant cells are efficiently killed by *Listeria*^{at} upon the generation of high levels of reactive oxygen species.[7] Importantly, *Listeria*^{at} appears to multiply within primary neoplastic lesions as well as within metastases, and infected cancer cells become sensitive to the cytotoxic activity of *Listeria*^{at}-activated T and NK cells. The selective survival and replication of *Listeria*^{at} in malignant, but not in normal, tissues is attributed to the fact that *Listeria*^{at} is efficiently cleared by the immune system in non-transformed tissues but not in the heavily immunosuppressed TME.[4,5] This raised the question on how Listeria^{at} could safely reach the TME without being eliminated. It turned out that MDSCs play an important role in the delivery of *L. monocytogenes* to the TME.[4,5]

MDSCs Selectively Deliver *Listeria*^{at} to Primary Malignant Lesions and Metastases

MDSCs are well known as one of the major contributors to the establishment of an immunosuppressive TME, where they are recruited by chemoattractants produced by malignant cells.[2] We discovered a unique relationship between *Listeria*^{at} and MDSCs. *Listeria*^{at} infects MDSCs and can survive within MDSCs because of their immunosuppressive nature. Moreover, infected MDSCs appear to selectively deliver *Listeria*^{at} to primary malignant lesions as well as to metastases. Once in the TME, *Listeria*^{at} spreads first

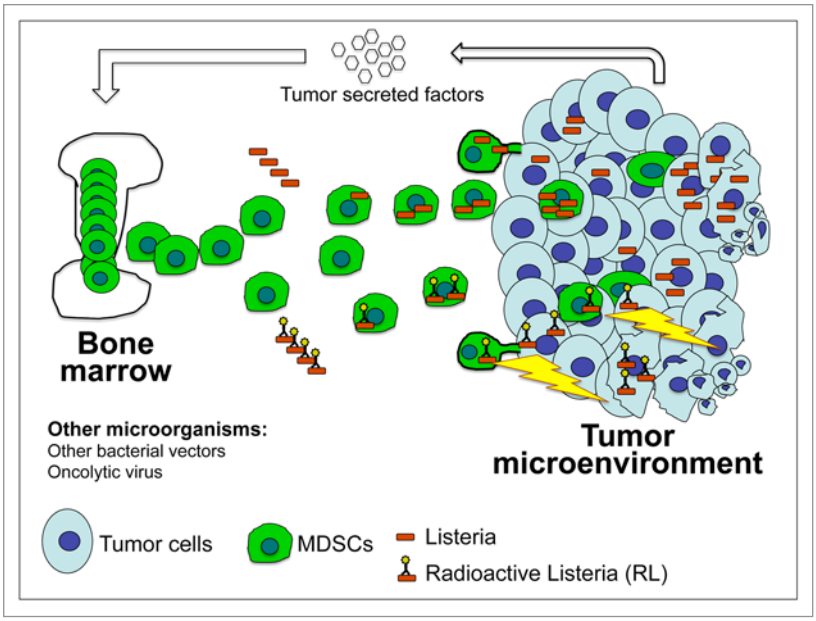

Figure 1. Myeloid-derived suppressor cells for the delivery of microorganisms or anticancer agents to the tumor microenvironment. Large numbers of myeloid-derived suppressor cells (MDSCs) are released from the bone marrow into the bloodstream of tumor-bearing hosts. MDSCs are attracted to the tumor microenvironment (TME), including primary neoplastic lesions and metastases, by cytokines and other chemoattractants. Upon infection, MDSCs can selectively deliver microorganisms such as an attenuated variant *Listeria monocytogenes* (Listeria), as such or coupled to a radionuclide (RL), to the TME, where these microorganisms can spread to tumor cells. In thus far, MDSCs attack cancer cells like bomb-loaded missiles. Malignant cells will also be killed through a "crossfire effect," i.e., the process whereby ^{188}Rhenium (^{188}Re) atoms taken up by one cell upon infection by RL also kill non-infected neighboring cells. With help of MDSCs, RL promotes the accumulation of radionuclides in primary tumors and metastases, promoting a significant inhibition of tumor growth as well as the near-to-complete elimination of metastases in a mouse model of pancreatic cancer. Also oncolytic viruses have been selectively delivered to the TME with the help of MDSCs, resulting in a reduction of tumor burden. Additional bacterial vectors are currently under investigation for the delivery of anticancer agents to the TME. Such novel immunotherapeutic regimens have great potential for the treatment of metastatic tumors.

from MDSCs to neighboring neoplastic cells, and then from cancer cell to cancer cell through a characteristic mechanism of dissemination.[8] These results suggest that MDSCs can be used as cellular missiles to deliver anticancer agents to primary tumors as well as to metastatic lesions.[4] We have recently demonstrated that radioisotope-labeled *Listeria*^{at} bacteria are selectively delivered by MDSCs to primary tumors and metastases in a mouse model of pancreatic cancer, resulting in a robust inhibition of tumor growth as well as in a significant decrease in the number of metastases.[5]

Radioactive *Listeria* (RL) for the Treatment of Pancreatic Cancer

Radioactive *Listeria*^{at} (RL) was developed by coupling the radioisotope ^{188}Rhenium (^{188}Re) with *Listeria*^{at} by means of anti-*Listeria* antibodies, a project that we ran in collaboration with the group of Ekaterina Dadachova.[5] Mice bearing pancreatic tumors received multiple treatments with low-dose RL, resulting in the nearly complete elimination of metastases and a significant reduction in tumor growth.[5] We provided experimental evidence that selectively *Listeria*^{at}-infected MDSCs delivered the radioactivity to the primary tumor and metastatic lesions, and that RL infected neoplastic cells. In this setting, cancer cells died upon the delivery of ^{188}Re to their cytoplasm as well as through a "crossfire effect," i.e., the process whereby ^{188}Re atoms taken up in one cell upon infection by RL also kill non-infected neighboring cells.[5,9] The amount of radioactivity (per gram of tissue) accumulated within metastases was 4–5-fold higher than that observed in all other organs, except the liver and kidneys. Extensive pathological studies revealed practically no side effects, not even in normal tissues exposed to comparatively higher amounts of radioactivity such as the liver and kidneys. Presumably, such a good safety profile reflects the fact that highly-proliferating cells, such as malignant cells, are preferentially sensitive to the DNA-damaging effects of radiation. Neither *Listeria*^{at} nor radioactivity was detected in non-malignant tissues one week after the last administration of RL. Both ^{188}Re and *Listeria*-based vaccines have already been tested in cancer patients separately, and only mild side effects were observed.[6,10,11] Overall, these observations suggest that RL may constitute a valuable treatment not only for pancreatic cancer, but perhaps also for other tumor types.

Other Microorganisms for Targeting Tumors

Additional studies have shown that MDSCs can be used for the delivery of microorganisms other than *Listeria* to the TME. For instance, it has been demonstrated that the intravenous administration of oncolytic virus-loaded MDSCs to tumor-bearing mice improves the delivery of viral particles to the TME as well as their local persistence as compared with the systemic injection of naked viruses.[12] This results in a significant decrease in tumor burden and increases the survival rate of mice treated with oncolytic virus-loaded MDSCs as compared with animals receiving oncolytic viruses as such. Other groups have demonstrated the potential of bacteria for the selective delivery of anticancer agents to malignant cells.[13,14]

Other Cellular Vehicles for the Delivery of Anticancer Agents to the TME

MDSCs are not the only type of myeloid cells that home to the TME and hence can be used for the delivery of anticancer agents to malignant cells. For instance, it has been shown that TIE2-expressing monocytes can deliver interferon α to the TME, promoting a near-to-complete inhibition of tumor growth coupled to a significant reduction in the amount of metastases in a xenograft model of human glioma as well as in a transgenic

model of mammary adenocarcinoma.[15] Mesenchymal stem cells, which normally provide stromal support to malignant lesions, have also been successfully used to deliver anticancer agents to the TME.[16] Taken together, these studies (including ours) highlight the great potential of immune cells that naturally home to the TME for selective delivery of anticancer agents.

Summary and Perspectives

While MDSCs are a major obstacle against the success of anticancer vaccines as they strongly suppress T-cell responses, we demonstrated that a highly attenuated strain of *L. monocytogenes* (*Listeria*^{at}) harnesses MDSCs for reaching the TME, where it infects and kills malignant cells. For the first time, we demonstrated that live *Listeria*^{at} bacteria can selectively deliver a radionuclide to the TME with help of MDSCs (**Fig. 1**). In thus far, MDSCs attack tumor cells like bomb-loaded missiles. Thus, immune cells that naturally home to the TME show great promise for the delivery of anticancer agents to primary neoplastic lesions as well as to metastases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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