Flipping the script on macrophages in leiomyosarcoma

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Abbreviations: CSF1, colony stimulating factor 1; LMS, leiomyosarcoma; mAb, monoclonal antibody; SIRPα, signal regulatory protein α; TAM, tumor-associated macrophage

Macrophages promote the growth of leiomyosarcoma (LMS), a malignant soft-tissue tumor. CD47 on tumor cells binds to the macrophagic receptor signal regulatory protein α (SIRPα) and prevents phagocytosis. We showed that anti-CD47 monoclonal antibodies (mAbs) allow macrophages to engulf LMS cells and prevent tumor growth and metastases. Therefore, anti-CD47 mAbs represent a promising targeted immunotherapy for LMS.

Macrophages are monocyte-derived cells that play important functions in adaptive and innate immunity through their ability to perform phagocytosis. In recent years, experimental and clinical evidence indicates that macrophages play a prominent role in tumor progression (reviewed in ref. 1). In most tumor types, tumor-associated macrophages (TAMs) are able to potentiate the malignancy of tumor cells by promoting angiogenesis, suppressing antitumor immune responses, and augmenting the invasiveness of cancer cells. The tumor-promoting of TAMs activities appear to result from changes that polarize macrophages from a “classically activated” M1 phenotype (which is believed to possess antitumor functions, including tumor cell phagocytosis) into an “alternatively activated” M2 phenotype.

Colony-stimulating factor 1 (CSF1) is a cytokine involved in the differentiation, growth, chemotaxis and M2-polarization of macrophages.2 Leiomyosarcoma (LMS) is a malignant neoplasm of smooth muscle cells that can occur in the uterus as well as in soft tissue throughout the body. Work in our laboratory revealed that LMSs that express high levels of CSF1 exhibit high levels of macrophagic infiltration.3,4 Consistent with the pro-tumorigenic role ascribed to TAMs in many cancer types, we found that patients whose LMSs were densely infiltrated by macrophages had highly vascularized tumors and significantly worse clinical outcomes.5 This suggests that one therapeutic approach for the treatment of LMS could be the inhibition of tumor-secreted CSF1, resulting in reduced TAM infiltration and perhaps inhibited cancer growth. This hypothesis has previously been put forward and successfully tested in a mouse model of osteosarcoma.6

An alternative to inhibiting CSF1-mediated TAM infiltration into LMS is to harness the presence of macrophages and to educate them to perform antitumor (rather than pro-tumor) functions. One molecule with the potential to activate this switch is CD47, a widely expressed transmembrane protein that serves as a ligand to signal regulatory protein α (SIRPα), a receptor expressed on the surface of macrophages. The interaction between CD47 and SIRPα results in the inhibition of phagocytosis, highlighting the role of CD47 in preventing phagocytosis of normal cells by macrophages (Fig. 1) (reviewed in ref. 7). In studies using leukemia, lymphoma and solid tumor cells, we demonstrated that the expression of CD47 is a mechanism co-opted by tumor cells to evade macrophage phagocytosis, and that blocking the CD47-SIRPα interaction using monoclonal antibodies (mAbs) can lead to macrophage-mediated tumor clearance.6 Given the prognostic importance of TAMs in LMS, the lack of effective therapies for LMS patients, and the fatality associated with the LMS cases that present as metastatic (approximately 40% of call cases), we sought to investigate whether an anti-CD47 therapy would be efficacious in LMS. The results of these experiments have recently been published in reference 8.

First, we demonstrated that CD47 is highly expressed on LMSs (using immunofluorescence and mRNA microarrays), while benign smooth-muscle tumors exhibit significantly lower levels of expression. CD47 was also abundant in two patient-derived human LMS cell lines, which we used for subsequent investigations. Using mAbs against CD47 that interfere with CD47-SIRPα interaction, we showed a dramatic increase in phagocytosis of LMS cells by both human and mouse macrophages in vitro. When we tested the ability of anti-CD47 mAbs to exert antitumor effects in vivo, we found striking reductions in the growth of LMS

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Xenografts growing in immunodeficient mice. Importantly, we found that one of the LMS cell lines, LMS04, metastasized to the lungs, and that anti-CD47 mAbs were able to eliminate pulmonary metastases almost completely.

In humans, primary tumors can often be controlled using surgery, with or without a radiotherapeutic/chemotherapeutic intervention. However, in LMS (as in other cancers), once metastasis occurs, the disease burden is often fatal, owing to the inadequacy of adjuvant therapies. We sought to mimic this disease progression in mice by generating a new model for neo-adjuvant treatment. As in primary tumor growth experiments, LMS04 cells were grown subcutaneously in mice, but they were allowed to reach a size that coincided with the onset of pulmonary metastasis.

Then, to mimic the clinical scenario of a patient presenting with an established primary tumor that has already metastasized, we began treatment with anti-CD47 mAbs and resected the primary tumors. Using this approach, we found a striking decrease in the size and number of metastatic tumor cells at the end of the experiment. We not only found the metastatic disease burden to be quantitatively different in anti-CD47 vs. control mice, but also that residual metastatic deposits in mice receiving anti-CD47 mAbs were associated with inflammatory infiltrates and showed morphologic features of cell degradation. In contrast, the lungs of control-treated mice showed large tumor cell clusters.

These experiments demonstrate that anti-CD47 mAbs can be used to harness TAMs as anti-cancer cellular tools against LMS upon the reversal of their tumor-promoting functions. Future work will be necessary to investigate the relationship between CSF1 secretion by LMS cells and anti-CD47 therapies, and to better understand whether these mechanisms could be used in concert to control LMS tumor growth. Also, as anti-CD47 mAbs have shown synergistic anti-tumor effects when used in conjunction with other mAbs (such as the anti-CD20 mAb rituximab in lymphoma), combinatory treatments could be developed once LMS-specific antigens are identified. We hope that experiments such as those we performed in LMS and in other solid tumors will help to pave the way for clinically efficacious therapies that harness the body’s natural immunity to fight cancer.

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