IN THE SPOTLIGHT

Repurposing Antiestrogens for Tumor Immunotherapy

Thomas Welte[1], Xiang H.-F. Zhang[1], and Jeffrey M. Rosen[2]

Summary: Svoronos and colleagues observed estrogen receptor alpha–positive cells in the tumor stroma of patients with ovarian cancer that appeared to be independent of both the tumor's estrogen receptor status and tumor type. These cells were identified as immunosuppressive myeloid-derived suppressor cells (MDSC) and could be targeted by antiestrogen therapy, thereby leading to the hypothesis that endocrine therapy when combined with immunotherapy may provide a potential therapeutic benefit by helping to reduce immunosuppressive MDSCs. *Cancer Discov; 7(1); 17–9. ©2017 AACR.*

See related article by Svoronos et al., 72 (4).

Endocrine therapies, which target the estrogen receptor (ER) in breast cancer, or the androgen receptor (AR) in prostate cancer, have been successfully used to treat hormone receptor–positive cancers and are the most effective treatment even for metastatic ER-positive breast cancer (1). However, estrogens do not only act directly on tumor cells but also regulate the development and function of certain immune cell lineages (for a more comprehensive review, see ref. 2). ERα, the major ER isoform, especially exhibits high expression in early hematopoietic progenitors in the bone marrow such as hematopoietic stem cells, common lymphoid progenitors (which have a role in B-cell development), and myeloid lineage progenitors.

The occurrence of several autoimmune diseases, such as systemic lupus erythematosus, multiple sclerosis, and Parkinson's disease, shows a sex bias. Estrogen is protective in multiple sclerosis, but harmful in systemic lupus erythematosus, whereas treatment with raloxifene, a selective estrogen receptor modulator (SERM), is beneficial in Parkinson's disease. These disparate effects of estrogen in different autoimmune disorders have been attributed to differential effects of estrogens on a variety of different cell types in the immune system, whereas triggering and pathogenic mechanisms could be disease specific.

Subsets of immature myeloid lineage cells frequently arise during tumor progression. These cells—the myeloid-derived suppressor cells (MDSC)—are in general protumorigenic and facilitate steps of metastatic progression (3). MDSCs also inhibit the adaptive immune response and accordingly interfere with immune checkpoint therapies. The recent study by Svoronos and colleagues (4) shows that MDSCs express ERα, and that estradiol signaling through ERα influences MDSC expansion in several different tumor models. Surprisingly, estrogen depletion slowed tumor progression by diminishing MDSC numbers and associated protumorigenic functions regardless of the actual ER status of the tumors. These results suggest a new opportunity to attack both ER-positive and ER-negative tumors by targeting MDSCs through estrogen depletion. Based on these observations, the authors suggest that endocrine therapy might provide a benefit when combined with immunotherapy, e.g., immune checkpoint therapies, by eliminating MDSCs that interfere with immunotherapy. Importantly, because this effect should not be dependent on the expression of ERα in tumors, this may provide a therapeutic opportunity not just in breast cancer, but also in other cancers, such as lung cancer and melanoma, where some, but not all, patients respond to immune checkpoint therapy (5).

MDSCs are thought to develop from hematopoietic progenitors and belong to the myeloid cell lineage, as they typically express CD11b in combination with myeloid markers in human cells and CD11b together with GR1 in mouse cells. These cells also express markers representative of immature developmental stages, such as CD33 in humans and Ly6C$^{lo/-}$ on Ly6G^{+}/CD11b^{+} cells in mice. Overall, the MDSC populations appear quite heterogeneous, raising important questions about how they might be specifically eradicated. The hallmark feature of MDSCs is their suppressive effect on T cells, natural killer cells, and other antitumor defenses. These functions are executed through production of reactive oxygen species (ROS), nitric oxide, arginase I, and other mechanisms. This activity is detrimental, as it helps the tumor to establish an immunocompromised environment and escape the adaptive immune response. MDSCs also have other less well–understood roles that promote metastatic progression, recurrence, and drug resistance.

There is considerable heterogeneity in the composition of the tumor microenvironment in different tumor types. However, MDSC expansion and recruitment is observed in a large proportion of cases regardless of tissue type. Tumor-derived factors play a major role in the induction of MDSCs. Growth factors involved in MDSC expansion include G-CSF, GM-CSF, IL6, M-CSF, and IL4. Additional extrinsic factors that control MDSC migration (CXCL5 and CCL3), differentiation (S100A8 and S100A9), and ROS and nitric oxide production are also important.

[1]Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, Texas. [2]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas.

Corresponding Author: Jeffrey M. Rosen, Baylor College of Medicine, 1 Baylor Plaza, Mail Stop CBM130, Room BCM-M638a, Houston, TX 77030. Phone: 713-798-6210; Fax: 713-798-8012; E-mail: jrosen@bcm.edu

doi: 10.1158/2159-8290.CD-16-1308

The study by Svoronos and colleagues showed that ERα deficiency (via genetic deletion or an ERα-specific inhibitor) abrogates the effects of estrogens on MDSCs. ERα signaling may be divided into two parts: (1) nuclear or "genomic" function as a transcription factor; upon ligand binding, AF1 and AF2 domain configuration changes such that ER can dimerize and bind to DNA sequences called estrogen response elements (ERE). ER itself recruits cofactors that can be either transcriptional coactivators or corepressors and positively or negatively regulate histone acetylation, respectively. (2) In addition, estrogens can act via a rapid, nonnuclear, or "nongenomic" function; this role is dependent on palmitoylation, which mediates attachment to the plasma membrane at lipid raft sites. This signaling pathway includes second messenger mobilization (e.g., cAMP), protein kinase pathways (PI3K/AKT, ERK), and nitric oxide production. Nitric oxide is a characteristic, functionally important product of MDSCs. However, it has not been determined whether there is a link between nongenomic ER function and nitric oxide in MDSCs.

Complex interactions of the ERα-regulated pathway with other signaling pathways have been described. For example, a role for ERα has been reported in Toll-like receptor–mediated induction of type I IFN, in the modulation of NFκB signaling through different mechanisms, and even in MDSCs through modulation of STAT3 activation (ref. 6; also reported in the study by Svoronos and colleagues). STAT3 is a transcription factor that is activated by tyrosine and serine phosphorylation following engagement of many cytokine and growth factor receptors. Tyrosine phosphorylation of STAT3 is regulated in part through receptor tyrosine kinases, such as the Janus kinase (JAK), and SRC family kinases. Tumor cell–derived factors may increase JAK2 and concomitant STAT3 phosphorylation in hematopoietic progenitor cells *in vitro* and cause expansion of these cells (reviewed in ref. 3). Tissue-specific *Stat3* deletion in hematopoietic progenitors has been shown to alter their cell fate and disrupts FLT3-L–induced dendritic cell differentiation (7). Furthermore, tissue-specific, Cre-mediated deletion of the *Stat3* gene in the myeloid cell lineages or treatment with specific inhibitors greatly reduces MDSC numbers in tumor-bearing mice. STAT3 is required for MDSC survival and proliferation and may be involved in maintaining an immature developmental status. STAT3 also regulates the expression of S100A8 and S100A9, important for MDSC immaturity/expansion and migration to tumor sites.

When bone marrow–derived MDSC cultures were treated with MPP, a specific ERα antagonist, reduced STAT3 tyrosine phosphorylation was observed. Furthermore, MDSCs of oophorectomized mice had low phospho-STAT3 levels, which were induced by estradiol treatment. Antiestrogen treatment appeared to reduce the level of active *Jak2* mRNA and *Src*. These effects were suggested to account for the reduced STAT3 phosphorylation observed following antiestrogen treatment. Although the precise mechanisms regulating these interactions are still not known, further results demonstrated a striking reduction of MDSC subsets after treatment with the JAK1/2 inhibitor ruxolitinib (4). Additionally, these results were supported by antiestrogen MPP treatment. Interestingly, there is indirect evidence that estrogen can induce ROS production, an important component of MDSC function.

It is noteworthy that in a mouse model of chronic myeloproliferative disease (myofibrosis), which may develop from similar early myeloid progenitors as MDSCs, modulation of estrogen signaling by tamoxifen was highly effective in inhibiting cell expansion (8). A link to STAT3 signaling is a likely possibility in this disease as well, as the cellular transformation caused by a *JAK2*^{V617F} gain-of-function mutant can be effectively treated with ruxolitinib. The cross-talk between ER and STAT3 signaling pathways is, however, highly cell-context dependent. Earlier studies reported that estrogens suppress IL6-induced osteoporosis and growth of multiple myeloma cells due to interference with IL6 signaling. Mechanistically, ER may directly interact with STAT3. Another study has reported that estradiol induced the expression of PIAS3, an inhibitor of STAT3, in multiple myeloma (9).

Historically, ER signaling pathways were initially targeted by SERMs such as tamoxifen, which influences the configuration of the AF2 domain and impairs ERα DNA binding. Subsequently, selective estrogen receptor degraders (SERD) such as fulvestrant were introduced. A third group of endocrine therapeutics, aromatase inhibitors, interfere with ER ligand synthesis. Because these classes of drugs differ in their mechanisms of action and agonistic/antagonistic effects in hematopoietic cells, their effectiveness on MDSC inhibition in specific disease states will need to be investigated.

How can we identify patients who will benefit from estrogen deprivation to deplete MDSCs? The prediction that MDSC-enriched tumors might respond to antiestrogen therapies even if the tumors are ER-negative can be tested in the clinic. However, the degree of MDSC accumulation varies widely across different patients. The first step, therefore, will be to identify appropriate patient populations, which will require validation of appropriate biomarkers to assess MDSC enrichment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors acknowledge the joint participation by the Diana Helis Henry Medical Research Foundation through its direct engagement in the continuous active conduct of medical research in conjunction with the Baylor College of Medicine.

Grant Support

The authors would like to acknowledge support from NIH grant CA16303-41 to J.M. Rosen; the Helis Foundation to T. Welte; and the Breast Cancer Research Foundation, US Department of Defense grant DAMD W81XWH-16-1-0073, and Susan G. Komen grant CCR14298445 to X.H.-F. Zhang.

Published online January 6, 2017.

REFERENCES

1. Osborne CK, Schiff R. Mechanisms of endocrine resistance in breast cancer. Annu Rev Med 2011;62:233–47.
2. Jiang X, Shapiro DJ. The immune system and inflammation in breast cancer. Mol Cell Endocrinol 2014;382:673–82.
3. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 2009;9:162–74.

4. Svoronos N, Perales-Puchalt A, Allegrezza MJ, Rutkowski MR, Payne KK, Tesone AJ, et al. Tumor cell–independent estrogen signaling drives disease progression through mobilization of myeloid-derived suppressor cells. Cancer Discov 2017;7:72–85.
5. Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. Cell 2015;161:205–14.
6. Pan T, Liu Y, Zhong LM, Shi MH, Duan XB, Wu K, et al. Myeloid-derived suppressor cells are essential for maintaining feto-maternal immunotolerance via STAT3 signaling in mice. J Leukoc Biol 2016;100:499–511.
7. Welte T, Zhang SS, Wang T, Zhang Z, Hesslein DG, Yin Z, et al. STAT3 deletion during hematopoiesis causes Crohn's disease-like pathogenesis and lethality: a critical role of STAT3 in innate immunity. Proc Natl Acad Sci U S A 2003;100:1879–84.
8. Sanchez-Aguilera A, Mendez-Ferrer S. Regulation of hematopoietic progenitors by estrogens as a basis for new antileukemic strategies. Mol Cell Oncol 2015;3:e1009728.
9. Wang LH, Yang XY, Mihalic K, Xiao W, Li D, Farrar WL. Activation of estrogen receptor blocks interleukin-6-inducible cell growth of human multiple myeloma involving molecular cross-talk between estrogen receptor and STAT3 mediated by co-regulator PIAS3. J Biol Chem 2001;276:31839–44.