INTRODUCTION

Indoleamine 2,3-dioxygenase (IDO) is an intracellular heme-containing enzyme that is highly expressed in myeloid cells (e.g., macrophages, dendritic cells) and catalyzes the initial step in tryptophan degradation by the kynurenine pathway. Tryptophan starvation by IDO activation inhibits T-cell function by several mechanisms. First, tryptophan depletion can directly lead to T-cell growth arrest in the G1 phase of the cell cycle. Second, alternative degradation of tryptophan produces metabolites shown to be toxic for CD8+ T cells and natural killer cells. Furthermore, IDO has the ability to convert naïve T cells to immunosuppressive regulatory T cells (1). IDO also triggers phosphorylation of the translation-initiating factor eIF2a and mobilizes translation of LIP, the inhibitory form of the immunoregulatory transcription factor LAP/NF-IL6/CEBPβ.

An increasing number of studies have linked overexpression of IDO to cancer progression. High levels of IDO expression are found in patients with ovarian carcinoma, hepatocellular carcinoma, invasive cervical carcinoma, non–small cell lung carcinoma, colon carcinoma, and endometrial carcinoma and are associated with poor prognosis (2). IDO localizes both in tumor sites, where depletion of tryptophan reduces the effector function of T cells, and in tumor-draining lymph nodes, where IDO-expressing plasmacytoid dendritic cells have a tolerogenic effect on T cells during their encounter with antigen-loading antigen-presenting cells. Increased IDO in tumor cells can be a part of the global changes involved in malignant transformation such as downregulation of BNI, which controls IDO expression (3), and may also be a component of the response to inflammatory cytokines, as IDO is known to be induced by IFN-γ (4). In vitro studies using human monocyte-derived macrophages showed that an increased level of IDO can suppress proliferation of T cells as well as monocyte-derived dendritic cells, thus reducing the immune response. Together with those terminally differentiated cells, fibroblasts and monocytes expressing IDO can suppress T-cell function (5).

Although genetic evidence for a direct role of IDO has thus far been lacking, these studies, in parallel with data concerning poor survival of patients whose tumors overexpress IDO, support the idea that blocking of IDO in clinical studies will have positive antitumor effects. Today, several compounds are reported to act as inhibitors of IDO function in vivo, among which 1-methyl-D,L-tryptophan (1-MT) is the most studied drug. IDO has 2 isoforms: IDO1 and IDO2, with IDO2 being the presumptive immunomodulatory isoform. IDO2 is expressed in a variety of antigen-presenting cells and is distinct from IDO1 in its sensitivity to 1-MT. Specifically, the levo (l)-stereoisofrom l-1-MT preferentially inhibits IDO1 activity, whereas the dextro (d)-stereoisofrom d-1-MT preferentially inhibits IDO2. Notably, in preclinical models, d-1-MT was shown to exhibit greater antitumor activity than l-1-MT (6).

STUDY

In this issue of Cancer Discovery, Smith and colleagues (7) present direct evidence of the importance of IDO in tumorigenesis. This study follows a previous study from the same group (8) in which the authors genetically deleted Ido1 for the first time and showed an essential role of this enzyme in tumor outgrowth in a model of inflammatory skin carcinogenesis. The present study provides additional information on the role of IDO in tumorigenesis and also complements previous results, which were tempered by the possibility that chemical exposure in the skin cancer model might produce anomalies irrelevant to the majority of spontaneous tumors. The study was designed to use LSL-KrasG12D transgenic mice, a well-known model of sporadic focal pulmonary adenocarcinomas induced by intranasal administration of Ad-Cre. RAS-induced adenocarcinomas elicit a robust inflammatory response, and the authors proposed that IDO may impact a protumorigenic skew. To test this hypothesis, the authors bred Ido1-null mice with the Kras mutant lung cancer model and also evaluated metastasis of orthotopically implanted 4T1 mammary carcinoma cells in IDO-deficient mice. Ido1 knockout mice with spontaneous Kras mutant lung tumor survived significantly longer than mice with intact
Ido1. Moreover, using micro-computed tomographic scans, the authors detected a significantly reduced tumor burden in the Ido1−/− Kras-mutant mice. Interestingly, these changes were not associated with reduced initiation of tumor growth, as Ido1−/− mice had a higher frequency of early precancerous lesions. Detailed analysis of angiogenesis in tumors showed diminished vessel density in Ido1−/− mice, and inflammatory cytokine profile analysis of Ido1−/− mice showed blunted interleukin (IL)-6 and MCP-1 levels during tumor progression compared with control mice. To exclude the possibility that Ido1−/− mice are resistant to the outgrowth of primary lung tumors, the authors engrafted the 4T1 carcinoma cell line to analyze the formation of pulmonary metastasis. Surprisingly, survival of Ido1−/− mice with 4T1 tumors was significantly increased, but there was no difference in tumor growth. The number of metastatic nodules in the lung was unambiguously lower in Ido1−/− mice, but the number of tumor cells in the peripheral blood and metastatic burden in the liver was unaffected. This indicates that IDO-mediated support of metastatic development in the lung is not dependent on the size of the primary tumor. However, in contrast to the Kras-mutant lung cancer model, in the 4T1 metastasis model, IDO1 protein and kynurenine levels were increased because of production of IDO by 4T1 cells. Therefore, metastasis development may be also associated with induction of an alternative mechanism of kynurenine production, such as IDO2 or TDO2.

In analyzing the immune compartment in tumor-bearing mice, the authors focused on myeloid-derived suppressor cells (MDSC) because their accumulation and function has been shown to be dependent on IL-6. Although there was no difference in the number and phenotype of MDSCs in Ido1−/− mice versus control mice, circulating MDSCs isolated from Ido1−/− hosts were functionally impaired in their ability to suppress T cells. To evaluate the involvement of IL-6 in MDSC defects observed in Ido1−/− mice, the authors used 4T1 cells constitutively expressing IL-6 and discovered that IL-6 supplementation not only rescued wild-type levels of MDSC suppressor function in 4T1 tumor-challenged Ido1−/− mice but also markedly restored their susceptibility to pulmonary metastasis development (findings summarized in Fig. 1).

**OPEN QUESTIONS**

These findings thus suggest that IDO supports tumor progression and metastasis and promotes tumor immune evasion through IL-6–stimulated MDSC activity. However, Mei and colleagues (9) recently found that inhibition of IDO1 also downregulates the expression of COX-2 and MMP9. Although this work was not directly related to tumorigenesis, these findings show that downregulation of IDO can have widespread inhibitory effects involving not only IL-6 and MCP-1 but also other important protumorigenic proteins such as COX-2 and MMP9. Furthermore, in the present study, Smith and colleagues (7) focused only on IL-6, although they also observed that deletion of Ido1 also blunted MCP-1 levels. It is known that MCP-1 (CCL2), together with CCR2, plays a role in the recruitment of immune cells to the tumor microenvironment and also increases the migration of tumor and stromal cells that express CCR2 (10). Moreover, CCL2 expression by tumor or stromal cells and CCR2+ macrophage infiltration correlates with poor prognosis and metastasis in human breast cancer (11).

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**Figure 1.** Schematic of the different protumorigenic mechanisms associated with IDO based on previously published data and on the work of Smith and colleagues (7). Tregs, regulatory T cells.
In the present work, the authors also only focused on MDSCs, which are known to suppress the function of T cells upon IL-6 action. However, other cell types located in tumors are likely to be affected by IDO downregulation. As IDO inhibits T-cell proliferation, the ratio of lymphoid/myeloid cells in $I_{dc}^{A-}$ mice should shift toward myeloid. Accordingly, an additional indirect mechanism of increased IL-6 secretion can be an increase in the number of macrophages, the main cell type producing IL-6, through upregulation of the macrophage chemocattractant MCP-1. One can speculate that $K_{ras}$-mutant lung tumors in control mice probably had more tumor-associated macrophages polarized to the M2 type with high secretion of IL-6 and pro-angiogenic properties, leading to increased vascularity. It would be very interesting to dissect out the role of macrophages in this model especially because the authors did not find differences in 4T1 tumor growth and the number of circulating MDSCs but reported dramatic differences in metastasis formation, leaving open the possibility of involvement of another cell type. In addition, in a recently published article by Levina and colleagues (12) where both IDO-deficient and IDO-overexpressing 4T1 cells were used, it was shown that IDO has both immunologic and nonimmunologic effects on tumor growth and spontaneous metastasis formation. However, global inactivation of IDO obscures the cell type(s) responsible for the antimetastatic effect of IDO downregulation. Further elucidation of the cell types involved could lead to a more specific way of targeting IDO in cancer therapeutics.

A number of published articles have indicated that blocking IDO pharmacologically reduces tumor growth. Smith and colleagues (7) extend our understanding of the IDO mechanism of action by showing that knocking out IDO promotes tumor progression and metastasis in an IL-6-dependent manner. This direct genetic evidence for a role of IDO in cancer provides a strong rationale for the further clinical development of IDO inhibitors. Currently, there are several ongoing clinical trials involving IDO inhibition: one trial uses IDO peptide vaccination and others are studying the effects of $I_{dc}^{A-}$MT alone or in combination with docetaxel or with an Ad.p53 dendritic cell vaccine. Although the trial results are not known yet, promising preclinical data, including this article by Smith and colleagues (7), provides hope that they will be successful and hence have the potential to prolong the lives of patients with cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Received July 3, 2012; accepted July 3, 2012; published online August 10, 2012.

REFERENCES