Review

Cancer Research

Tryptophan Catabolism in Cancer: Beyond IDO and Tryptophan Depletion

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Abstract

Tryptophan catabolism in cancer is increasingly being recognized as an important microenvironmental factor that suppresses antitumor immune responses. It has been proposed that the essential amino acid tryptophan is catabolized in the tumor tissue by the rate-limiting enzyme indoleamine-2,3-dioxygenase (IDO) expressed in tumor cells or antigen-presenting cells. This metabolic pathway creates an immunosuppressive milieu in tumors and in tumor-draining lymph nodes by inducing T-cell anergy and apoptosis through depletion of tryptophan and accumulation of immunosuppressive tryptophan catabolites. Competitive inhibitors of IDO are currently being tested in clinical trials in patients with solid cancer, with the aim of enhancing the efficacy of conventional chemotherapy. There are, however, certain tumor types that are capable of catabolizing tryptophan but are largely IDO-negative. Recent evidence from studies in malignant gliomas and other types of cancers points to alternative enzymatic pathways of tryptophan catabolism involving tryptophan-2,3-dioxygenase (TDO). TDO, which is considered responsible for regulating systemic tryptophan levels in the liver, is constitutively expressed in some cancers and is equally capable of suppressing antitumor immune responses. Depletion of tryptophan induces signaling events in T cells, leading to anergy and apoptosis; however, active immunomodulation by accumulating tryptophan catabolites, most notably kynurenine, appears to play an equally important role. These immunomodulatory effects of kynurenine are mediated by the aryl hydrocarbon receptor. This intracellular transcription factor has classically been viewed as a receptor for environmental toxins, such as dioxin, and its important role in influencing immune responses, especially in epithelial barriers, is only beginning to emerge. This review summarizes the exciting developments in our understanding of tryptophan catabolism as a key factor in the immunobiology of cancer. *Cancer Res; 72(21); 5435–40. ©2012 AACR.*

Introduction

Two decades after the importance of tryptophan catabolism for maintaining the immune privilege of the placenta was discovered (1), increasing evidence is extending its biological relevance beyond immune tolerance to non-self. According to the generally accepted concept, tryptophan, an essential amino acid, is catabolized in the local microenvironment of tumors, immune-privileged sites, or sites of inflammation (2). In these tissues, cancer cells, immune cells, or specialized epithelial cells (e.g., syncytiotrophoblasts in the placenta) create an environment that suppresses antigen-specific T-cell responses both by depletion of tryptophan and by accumulation of immunosuppressive tryptophan catabolites. Because tryptophan catabolism is induced by inflammatory mediators, notably IFN-γ, it is thought to represent an endogenous mechanism that restricts excessive immune responses, thereby preventing immunopathology. In the context of cancer, this feedback loop may not be beneficial, as tryptophan catabolism has been implicated in inflammation-driven cancers such as colon cancer (3). There is strong evidence that suppression of antitumor immune responses in precancerous lesions and established cancers by tryptophan catabolism promotes tumor growth, which would make such catabolism an attractive target for therapeutic intervention (4). Hence, a considerable effort is being made to identify selective and efficient inhibitors of tryptophan catabolism, and methylated tryptophan, which enhances the efficacy of conventional chemotherapy in preclinical models, is currently being tested in clinical trials. In addition, the extent of tryptophan catabolism measured in serum and other biological fluids may serve as a biomarker for monitoring disease activity and response to therapy in cancer patients.

Despite considerable advances in our understanding of this metabolic pathway, a number of questions remain unresolved, revolving mainly around (i) the redundancy of indoleamine-2,3-dioxygenase (IDO) as the key and rate-limiting enzyme of tryptophan catabolism, and (ii) the molecular targets and

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mechanisms responsible for the biological effects of both tryptophan depletion and the accumulation of tryptophan metabolites. This review addresses some of these questions by summarizing recent studies that have shed some light on the immunobiology of tryptophan catabolism.

Tryptophan Catabolism by Dioxygenases—the Rate-Limiting Step

Most of the research to date has focused on IDO as the central and immunobiologically relevant enzyme that catalyzes the conversion of tryptophan to kynurenine. In addition to IDO, 2 other enzymes are known to catalyze the same enzymatic step: indoleamine-2,3-dioxygenase 2 (IDO2) and tryptophan-2,3-dioxygenase (TDO). IDO2 was recently identified as an IDO-related enzyme with a slightly different expression pattern and molecular regulation. However, its physiological relevance remains unclear due to its very low activity, the presence of common polymorphisms that inactivate its enzymatic activity in approximately half of all Caucasians, and the presence of multiple splice variants (5–7). Although the clinically used D-stereoisomer of the IDO inhibitor 1-methyltryptophan was reported to preferentially target IDO2 (5), the L-stereoisomer displayed inhibitory activity in cellular assays as well (8). TDO has long been known as a tryptophan dioxygenase that is expressed at high levels in the liver and is responsible for regulating systemic tryptophan levels. More recently, TDO was also found to be expressed in the brain, where it may regulate the production of neuroactive tryptophan metabolites such as kynurenic acid and quinolinic acid (9). Two recent studies (10, 11) point to the relevance of TDO in some cancers where it is expressed constitutively (particularly malignant glioma, hepatocellular carcinoma, melanoma, and bladder cancer). Functional studies in human tumors indicate that constitutive TDO enzymatic activity is sufficient to sustain biologically relevant tryptophan catabolism that is equally capable of suppressing antitumor immune responses (10, 11). Although many studies have identified IDO as an enzyme that is silent in most tissues but can be induced by proinflammatory stimuli, the signaling events that drive constitutive expression in cancer cells are far less clear. Even more obscure are the signaling events that drive TDO expression and activity. Posttranslational regulation may also take place, and phosphorylation of IDO was described and found to regulate its turnover and signaling activity (12, 13).

A puzzling discovery in the field was the phenotypical unremarkability of IDO-deficient mice (14). Given all the previous evidence from preclinical disease models using IDO inhibitors that IDO plays a central role in maintaining immune tolerance in general, it was expected that such mice would display a general inflammatory phenotype and would be capable of rejecting experimental tumors. This is clearly not the case, although IDO-deficient mice may be slightly more prone to induction of autoimmunity via autoreactive peptides and stimulation of the innate immune system (15, 16); however, this is controversial. The function of IDO may be subtler than revealed by the animal model studies conducted thus far. Indeed, although IDO may be dispensable for restricting acute inflammation, it appears to be important for restraining chronic inflammation, which may, depending on the model and the microenvironment, promote or suppress neoplastic transformation. Thus, IDO may play a dual role in tumorigenesis by restricting the development of cancers that are driven by chronic inflammation, such as colon cancer (15), while promoting the development of tumors that are controlled by inflammation, such as certain types of skin tumors (3). In this respect, the general natural function of IDO may be to restrict chronic inflammation rather than to limit acute inflammatory processes, which are usually induced in animal models mimicking chronic inflammatory diseases. The use of subtle chronic inflammatory disease models, as described in a recent study (15), may reveal more central aspects of IDO as an endogenous anti-inflammatory molecule.

Although there are no published data on IDO2 ablation in mice, the TDO knockout mouse seems to be phenotypically normal, except for alterations in anxiety behavior (9). To our knowledge, no study to date has analyzed whether these mice display immune alterations constitutively or after manipulation. Although the discrepancy between the effects achieved by pharmacologic IDO inhibition and genetic IDO ablation suggests that IDO2, TDO, and/or other, as yet unidentified tryptophan dioxygenases may compensate for IDO deficiency, the evidence for this notion is sparse. Future studies using combined ablation of IDO, IDO2, and TDO will be necessary to determine the endogenous function of tryptophan catabolism as a suppressor of immune activation.

Numerous studies have linked cancer with activation of systemic tryptophan metabolism. In most of these studies, the ratio of kynurenine to tryptophan was measured in patient plasma as a measure of IDO and TDO activity (10, 17). The resulting data indicate that tryptophan catabolism may serve as a biomarker to monitor disease activity and response to therapy in cancer patients. When taking the kynurenine/tryptophan ratio as a measure of tryptophan catabolism, however, one has to keep in mind that this pathway is also responsive to unspecific inflammation and is induced in all states of chronic immune activation, because *IDO* is a very sensitive IFN-γ response gene. In addition, other metabolic pathways and drugs may interfere with the conversion of tryptophan to kynurenine, such as steroids, which are known inducers of TDO. Finally, the shifts in tryptophan catabolites that result from changes in IDO, IDO2, and/or TDO enzymatic activity may not be easy to predict, because this pathway is regulated on multiple levels, as exemplified by numerous and distinct cellular transport systems identified for tryptophan and its downstream metabolites (18). For instance, in mice, TDO deficiency results as expected in an increase in plasma tryptophan levels. However, kynurenine is not reduced but is increased as well in these mice, possibly by a compensatory upregulation of IDO and/or IDO2 in response to high tryptophan concentrations (9). When exploring the relevance of tryptophan catabolism as a biomarker in cancer patients, investigators in future studies will have to not only analyze the full spectrum of tryptophan metabolites (the "tryptophanome") but also take into account confounding effects on its

activation, such as concurrent medication, nutritional state, and infection.

Molecular Targets: Tryptophan Depletion versus Kynurenine Accumulation

Initially, it was proposed that immunoregulation by tryptophan catabolism is mediated by depletion of the essential amino acid tryptophan from the local microenvironment. According to the "death by starvation" paradigm, effector T cells are particularly susceptible to low tryptophan concentrations in the extracellular space, resulting in anergy and apoptosis. Although cells may respond to nutrient limitation through multiple pathways, an integrated stress response controlled by the nonderepressible kinase GCN2 was identified as an important mechanism that mediates the suppression of T-cell function by tryptophan depletion (19). T cells lacking GCN2 in mice are not susceptible to IDO-mediated anergy by myeloid cells, including dendritic cells in tumor-draining lymph nodes (19).

On the other hand, numerous studies have shown that tryptophan metabolites such as kynurenine, kynurenic acid, 3-hydroxy-kynurenine, and 3-hydroxy-anthranilic acid suppress T-cell function and are capable of inducing T-cell apoptosis (20). Although some studies have identified potential receptors for individual tryptophan catabolites, recent studies have delineated an important role for the aryl hydrocarbon receptor (AHR) as a direct target of kynurenine (10, 21, 22). The AHR is a basic helix-loop-helix Per-Arnt-Sim (PAS) family transcription factor that is activated by xenobiotics such as benzo[*a*]pyrene and 2,3,7,8-tetrachlordibenzo-*p*-dioxin (TCDD), and whose function extends beyond binding and eliminating xenobiotics from the body. Endogenously produced metabolites such as arachidonic acid metabolites, bilirubin, and cAMP, as well as tryptophan metabolites such as tryptamine, kynurenic acid, and 6-formylindolo[3,2-b]carbazole (FICZ) are all ligands of the AHR. Of interest, the AHR is enriched in interleukin 17 (IL-17)-producing CD4+ T cells (T_H17 cells) and controls the differentiation of naïve CD4+ T cells (23). Its function in the context of T-helper-cell differentiation is ligand dependent; for example, FICZ promotes T_H17 differentiation, whereas kynurenine results in the generation of regulatory T cells (T_{reg}). Given its selective expression in T_H17 cells, investigators have explored a prominent role for the AHR in models of T_H17-mediated autoimmune diseases, chiefly multiple sclerosis, where the AHR appears to restrict autoimmunity by favoring the generation of T_{reg}, although AHR-deficiency does not lead to an alteration of T_{reg} number and/or function (24, 25). Although the role of T_H17 cells in cancer immunity is increasingly being recognized, the role of AHR in tumor immunity remains uncertain. Our studies indicate that the immunosuppressive effects of kynurenine are mediated by the AHR and also affect CD8+ T cells (10). Whether the AHR-mediated alteration of CD8+ T-cell function is a direct effect or is mediated through T helper cells is currently under investigation. Because some studies indicate that tryptophan depletion cooperates with kynurenine to exert suppression of antitumor immune responses [e.g., by inducing T_{reg} (26)], it is tempting to speculate that the effects are somehow linked beyond GCN2 and AHR. A possible link is the transport of tryptophan and its metabolites through the cell membrane. The large amino acid transport system, although promiscuous, appears to function as an antiport system that replenishes intracellular tryptophan pools while depleting detrimental kynurenine from the cytosolic compartment (18). It will be important to elucidate the molecular targets of tryptophan catabolism and their interaction because this will not only help us understand the compartmentalized immunological consequences on the cellular level, it will also help us identify novel and possibly more specific immunotherapeutic targets to reverse cancer-associated immunosuppression mediated by tryptophan catabolism.

Nonimmune Effects of Tryptophan Catabolism

Another possible explanation for the discrepancy between the strong cancer-suppressive effects achieved by pharmacological IDO inhibition versus the limited efficacy of genetic IDO ablation in controlling experimental (transplanted) cancer is that tryptophan catabolism extends its biological effects beyond the immune system, driving tumor growth in a direct autocrine fashion. Transplanted tumor models using cancers with forced expression of TDO and IDO or knockdown of the IDO suppressor Bin-1 argue against a dominant nonimmune role of tryptophan catabolism in tumor growth, because the tumor-suppressive effect of IDO or TDO inhibition is abrogated in immunodeficient hosts (11, 27, 28). In principle, tryptophan depletion by IDO or TDO in tumor tissue should result in activation of the integrated stress response not only in T cells but also in the tumor cells themselves (although the thresholds may be different), resulting in growth arrest. Whether tryptophan depletion in tumor tissue is sufficient to induce integrated stress response in tumor cells remains to be determined, possibly by using molecular biosensors (18). On the other hand, biologically relevant levels of kynurenine accumulate in tumors that constitutively express TDO (10, 11). As opposed to immune cells, kynurenine and possibly other tryptophan metabolites are beneficial for tumor cells because they promote clonogenic survival and enhance the cell motility of malignant glioma cells. Consequently, gliomas that constitutively express TDO grow faster in immunodeficient mice (10). It is tempting to speculate that the autocrine effects of kynurenine on the clonogenic survival of glioma cells represent a unique feature of astrocytic brain tumors, which may have adapted to use the ability of kynurenine to activate the AHR target pathway while lacking the ability to further degrade kynurenine.

The AHR is certainly capable of mediating direct, nonimmune effects in cancer cells, because TCDD promotes cancer in an AHR-dependent manner (29) and transgenic mice with a constitutively active AHR spontaneously develop tumors (30). AHRR, the repressor of the AHR, is a tumor suppressor in multiple human tumors (31). Collectively, these data indicate that activation of the AHR is sufficient to promote tumorigenesis independently of its ligands' identities. In tryptophan-catabolizing cancer cells, the AHR is constitutively active, and ablation of tryptophan catabolism either by ablation of TDO

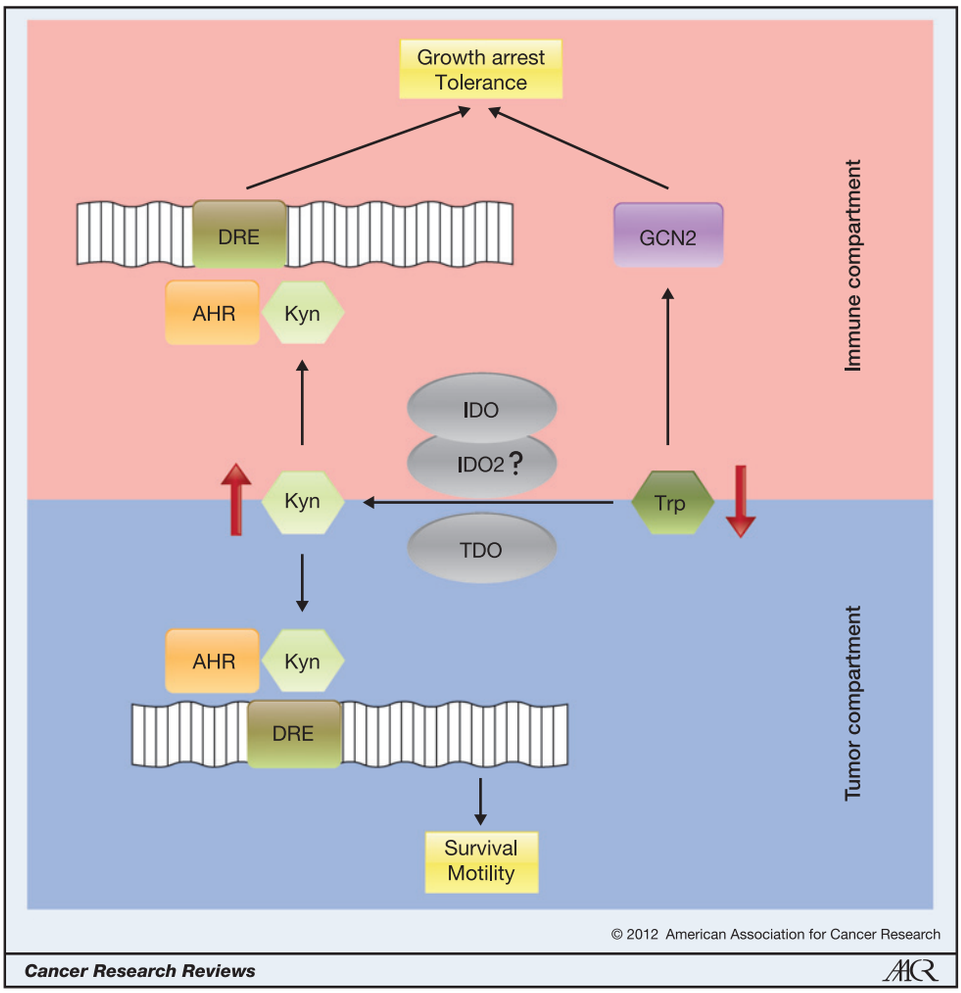

Figure 1. Conversion of Trp to Kyn takes place in tumor cells or at the interface of the tumor compartment (blue) and in the immune compartment through the activity of the tryptophan-metabolizing enzymes IDO, TDO, and potentially IDO2. Depletion of Trp in the local microenvironment results in activation of the GCN2 kinase and subsequent growth arrest and tolerance of T cells. Accumulation of Kyn binds the AHR in tumor cells and T cells, where it translocates to the nucleus and activates transcription of target genes regulated by DRE. In T cells this results in tolerance, whereas in tumor cells it leads to increased survival and motility. DRE, dioxin-responsive elements; Kyn, kynurenine; Trp, tryptophan.

and/or IDO or by depriving the cells of tryptophan leads to suppression of constitutive AHR activity, lending support to the hypothesis that endogenous kynurenine drives AHR activity in cancer cells (10). As opposed to its effect in immune cells, the net effect of AHR activation by kynurenine in tumor cells is protumorigenic, with increased cell motility and clonogenic survival *in vitro* and *in vivo* in immunocompromised hosts, although tryptophan catabolism is probably not the sole determinant for the constitutive AHR activity in cancer (10). The dichotomy between prosurvival signals in cancer cells and suppressive effects in immune cells is well established. Because the AHR function is critically ligand dependent, it also seems to be tightly controlled by the cell type and the local microenvironment, leading to the activation of many signaling pathways that interfere with AHR signaling, such as the hypoxia pathway. Hypoxia-inducible factor 1β (HIF-1β) not only associates with HIF-1α to activate hypoxia-driven gene expression, it also binds the AHR to mediate nuclear translocation [hence it is also referred to as AHR nuclear translocator (ARNT)] (32). However, whether these endogenous AHR ligands are produced in the tissue microenvironment in amounts sufficient to modulate immune responses has long been a matter of debate. Although kynurenine is not a high-affinity AHR ligand, its mean concentrations in TDO-expressing tumors and the culture supernatant of cancer cells are within a relevant range to activate the AHR (10). In summary, whereas tryptophan depletion does not seem to exert direct, nonimmune-mediated effects on cancer cells, kynurenine seems to have direct nonimmune-mediated effects on tumor cells via the AHR.

Inhibitors of Tryptophan Metabolism

Because dioxygenases catalyze the rate-limiting step in tryptophan catabolism, and the differential accumulation and biological effects of downstream tryptophan metabolites in cancer biology are largely unexplored, IDO has been the focus

of pharmacological targeting for drug development. The L-isomer of methylated tryptophan (1-L-MT) is a competitive inhibitor of IDO1 that was shown to be efficient in combination with preventive vaccination in a preclinical model (27). Although the D-isomer 1-D-MT does not block IDO1 activity, it showed IDO-dependent efficacy in preclinical models when combined with chemotherapy, and therefore has entered a clinical phase of testing (33). As shown in a recent study (34), the off-target effects of 1-D-MT may weaken its immunostimulatory effects in humans through transcriptional activation of IDO while supporting the intrinsic efficacy of conventional chemotherapy by targeting resistance pathways such as mitogen-activated protein kinase and c-*jun* N-terminal kinases. Several hypothesis-driven and screening approaches have identified compounds with IDO inhibitory activity at much lower concentrations than methylated tryptophan, and some of these compounds have also started clinical trials (35). Of note, most of these inhibitors do not actively inhibit TDO, indicating that although IDO and TDO catalyze the same enzymatic step, they are structurally distinct (36). Although novel IDO inhibitors are currently undergoing preclinical and early clinical testing, the search for TDO inhibitors has only just begun with the discovery of its relevance in cancer biology. A derivative of the known TDO inhibitor 3-(2-(pyridyl) ethenyl)indoles is biologically active and nontoxic in mice (11, 37). Although the approach to target TDO systemically seems to be feasible in mice, it remains to be seen whether perturbation of liver TDO activity provokes unwanted side effects, because TDO-deficient mice display elevated systemic levels of kynurenine (9). Preclinical studies, however, indicate that pharmacological inhibition of TDO with an orally active drug does not result in relevant toxicity, especially with respect to liver function (11). It is also conceivable that blocking TDO with a blood-brain-barrier–permeable inhibitor could lead to side effects in the central nervous system. Nevertheless, current studies clearly show that IDO and TDO are druggable with compounds that are tolerated after systemic application, and that combining anti-IDO and anti-TDO therapies may increase the efficacy of reversing cancer-induced immunosuppression (11).

Conclusions

Despite considerable advances in our understanding of the biological role of tryptophan catabolism in cancer, many open questions remain to be explored before its full therapeutic potential in the immunotherapy of cancer can be unleashed. IDO and TDO in tumor cells and antigen-presenting cells are capable of depleting tryptophan from the local microenvironment while resulting in the accumulation of kynurenine. Tryptophan depletion leads to T-cell anergy and apoptosis via the GCN2 pathway, whereas kynurenine suppresses T-cell differentiation and function through the AHR. Thus, tryptophan depletion and kynurenine accumulation cooperate to mediate immunosuppression in tumors and tumor-draining lymph nodes (Fig. 1). Studies in preclinical models and cancer patients thus far indicate that tryptophan catabolism (i) is a potential biomarker of disease activity in cancer patients, (ii) contributes to local and possibly systemic immune paralysis in cancer, and (iii) is a druggable cancer target for which readily available, systemically applicable compounds are at hand. While we await the development of numerous compounds derived from hypothesis-driven and library screening approaches, clinically approved drugs may exert antitumor functions in part through modulation of tryptophan catabolism, as recently described for the tyrosine kinase inhibitor imatinib (38). With the identification of the chief molecular targets of immunosuppressive tryptophan catabolism, particularly GCN2 and AHR, we have novel downstream targets at hand to modulate this pathway more specifically. Future research will focus on elucidating the cooperation of the 3 known dioxygenases, the specific immunoregulatory effectors downstream of GCN2 and AHR, and the molecular mechanisms that lead to constitutive tryptophan catabolism in tumor cells.

Disclosure of Potential Conflicts of Interest

B.J. Van den Eynde is a cofounder of and received consultancy fees from iTeos Therapeutics. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: M. Platten
Development of methodology: M. Platten
Writing, review, and/or revision of the manuscript: M. Platten, W. Wick, B.J. Van den Eynde

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