

Indoleamine 2,3-Dioxygenase Expression in Human Cancers: Clinical and Immunologic Perspectives

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Abstract

Indoleamine 2,3-dioxygenase (IDO) is a tryptophan-catabolizing enzyme with immune-regulating activities in many contexts, such as fetal protection, allograft protection, and cancer progression. Clinical trials are currently evaluating IDO inhibition with 1-methyltryptophan in cancer immunotherapy. However, the exact role of tryptophan catabolism by IDO in human cancers remains poorly understood. Here, we review several studies that correlate IDO expression in human cancer samples and tumor-draining lymph nodes, with relevant clinical or immunologic parameters. IDO expression in various histologic cancer types seems to decrease tumor infiltration of immune cells and to increase the proportion of regulatory T lymphocytes in the infiltrate. The impact of IDO on different immune cell infiltration leads to the conclusion that IDO negatively regulates the recruitment of antitumor immune cells. In addition, increased IDO expression correlates with diverse tumor progression parameters and shorter patient survival. In summary, in the vast majority of the reported studies, IDO expression is correlated with a less favorable prognosis. As we may see results from the first clinical trials with 1-methyltryptophan in years to come, this review brings together IDO studies from human studies and aims to help appreciate outcomes from current and future trials. Consequently, IDO inhibition seems a promising approach for cancer immunotherapy. *Clin Cancer Res*; 17(22); 6985–91. ©2011 AACR.

Introduction

Indoleamine 2,3-dioxygenase (IDO) is the key metabolic enzyme implicated in tryptophan catabolism. Its immunomodulating function was first described by Munn and colleagues in a study highlighting the crucial role played by IDO in fetal protection from maternal T lymphocytes during pregnancy (1). Since its discovery, IDO has been studied in many immunologic contexts. For instance, IDO can suppress T-cell-mediated graft rejection in a pancreatic islet allograft model (2). IDO effects on immune suppression are due to decreased tryptophan availability and the generation of tryptophan metabolites, culminating in multipronged negative effects on T lymphocytes in proximity to IDO-expressing cells, notably on proliferation, function,

and survival. More specifically, tryptophan degradation by IDO directly affects T-cell proliferation via the activation of the GCN2 kinase pathway (3). Some tryptophan metabolites also have the potential to induce apoptosis in lymphocytes (4). Moreover, low tryptophan concentration combined with the presence of kynurenin, a downstream metabolite, decreases the expression of the T-cell receptor ζ chain, resulting in reversible T-cell anergy (5). Finally, IDO activity seems to favor a regulatory phenotype in CD4⁺ T cells (6–8).

Functional IDO also emerges in tumors, making it a good candidate for cancer therapy (9). Its expression seems to contribute to the negative immune regulation arising in the tumor microenvironment. IDO seems to act as one of the multiple immunoregulatory mechanisms modulated by the tumor to evade immune surveillance, in addition to the downregulation of MHC class I, the expression of other immunosuppressive molecules, and the recruitment of regulatory immune cells. Blockade of IDO activity by 1-methyltryptophan (1-MT) results in decreased tumor proliferation dependent on specific T-lymphocyte response (10). Furthermore, IDO expression has been described in 25 different human cancer types (10). In addition, expression by tumor-draining lymph node (TDLN) dendritic cells is a second mode of action by which IDO can favor cancer progression (11). Expression of IDO in the tumor microenvironment negatively affects the immune response within the tumor and, by extension, in the TDLN. On the other hand, tryptophan catabolism by IDO can act as a negative modulator of tumor growth. One group has reported an

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Translational Relevance

Indoleamine 2,3-dioxygenase (IDO) inhibitors, such as 1-methyltryptophan, were recently identified as a priority in translational research by the Immune Response Modifier Pathway Prioritization Working Group from the NIH. Although the causal link between IDO and tumor growth is confirmed in many mouse models, the exact role of IDO in human tumors needs further confirmation. In this review, we correlate IDO expression with different prognosis factors and immunologic consequences in multiple human cancers. The aim of this review is to help better appreciate multiple clinical trials that are currently evaluating 1-methyltryptophan benefits in different solid and hematopoietic cancers. Furthermore, studies presented in this review tend to link IDO expression with different negative prognosis markers. Modulation of IDO functions is, accordingly, a future target for large-scale cancer treatment.

inhibitory effect of IFN- γ on cancer cell proliferation mediated by tryptophan deprivation via IDO enzymatic activity (12). In a different context, Muller and colleagues observed the regression of established tumors when 1-MT was administered to T-cell-competent mice in combination with chemotherapy (13).

IDO's role and mechanism of action on immune evasion and tumor growth are still poorly understood in human cancer. Many studies have examined a possible correlation between IDO expression and diverse clinical or immunologic parameters. This review discusses the potential implication of IDO in tumor progression by analyzing its expression in human cancer samples correlated with different immunologic and clinical parameters.

Development

The link between clinical parameters and IDO expression in tumors has been investigated in many cancer types. This research has revealed an association between IDO expression and a change in the density of different immune cell populations infiltrating tumors and an effect on cancer progression and on patient survival (Fig. 1; Table 1).

Tumor-infiltrating immune cell decrease

Different immune cell populations found at the tumor site or in TDLNs can be affected by the presence of IDO in their environment. In patients with colorectal cancer, high expression of IDO has been associated with lower CD3⁺ T-lymphocyte infiltration in tumor specimens (14). Similarly, CD8⁺ T lymphocytes were found in reduced numbers in ovarian cancer tissue in which high IDO expression was detected (15). Also, CD3⁺ and CD8⁺ T-lymphocyte as well as CD57⁺ natural killer cell infiltration was diminished in endometrial cancers with elevated IDO levels (16). Because of the importance of immune infiltration for antitumor response, this reduction of some beneficial immune populations suggests that IDO expression in human tumors can facilitate immune evasion.

Surprisingly, association between the presence of IDO and CD4⁺ T-lymphocyte infiltration has never been evaluated, despite the defined negative effect of IDO on this cellular population and, especially, on the Th1 subtype (17). Only 1 study has reported a significant CD8⁺ T-lymphocyte decrease without any difference in total CD3⁺ T-lymphocyte population (15). These results suggest that the proportion of CD4⁺ T lymphocytes is either unchanged or increased. However, because of the absence of a marker specific to CD4 in this study, the abundance of the CD4⁺ population could not be directly quantified. These observations could, however, lead to the hypothesis that the differentiation from naïve T lymphocyte to CD4⁺CD25⁺Foxp3⁺ regulatory T cells

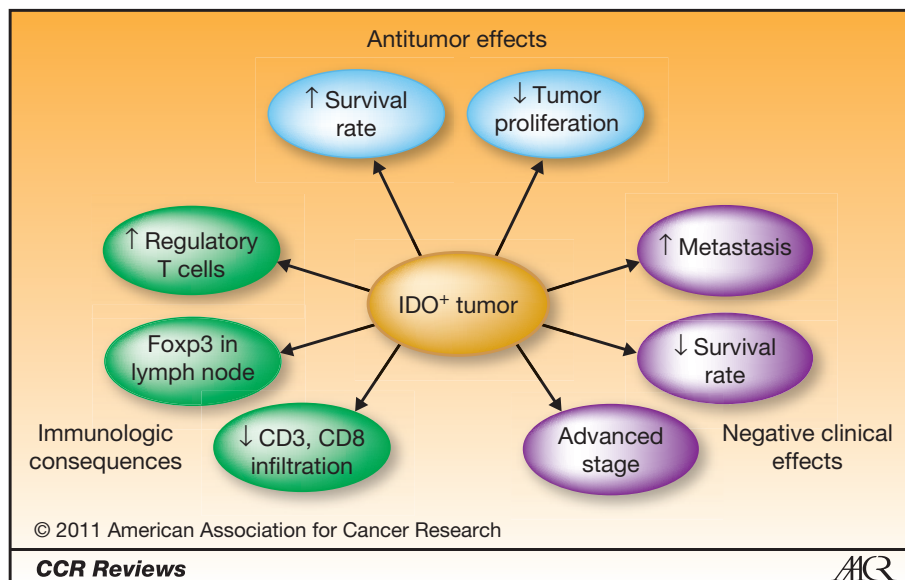


Figure 1. Schematic of the different clinical parameters associated with tumors expressing IDO. The different aspects are divided based on their impact on the immune system, tumor progression, or tumor regression.

Table 1. Correlation of clinical parameters with IDO expression in different cancer types

Cancer type	Detection methods	IDO correlates with: ^a	Reference
Colorectal	IHC (high expression 56/143 patients)	↑ Hepatic metastasis ↓ CD3 infiltration	14
	Serum tryptophan (66 patients) IHC in tumor (high expression in 9/71 patients) and dLN (high density in 21/60 patients)	↑ Hepatic metastasis ↓ 5-year survival rate	39 33
Uterine cervical	IHC (100% of microinvasive or invasive cancer)	Invasion by tumors	29
Endometrial	IHC (high expression in 37/80 patients) and Western blot (4/7)	Advanced stage LN metastasis ↓ Global and progression-free survival	26
Hepatocellular	IHC (high expression in 32/65 tissues)	↓ CD3, CD8, CD57 infiltration ↓ Progression-free survival	16
	RT-PCR (12/21 patients) and IHC (for IDO ⁺ in mRNA)	↑ Progression-free survival	36
Leukemia (AML)	RT-PCR (40/76 patients) and ICC (40/76 patients)	↑ IFN- γ ; TNF- α ; IL-1 β	20
	IHC (overexpression in 49/138 tissues)	Metastasis ↓ Global survival	25
Breast	IHC (SLN of 47 patients)	↑ CD4 ⁺ CD25 ⁺ Foxp3 ⁺ frequency LN metastasis with Foxp3 coexpression	24
Melanoma	IHC (SLN of 40 patients)	↓ Long-term survival	31
	IHC and RT-PCR (15 patients)	Advanced stage	28
Ovarian	IHC (high expression in 34/60 patients)	Advanced stage ↓ CD8 infiltration ↓ Global and disease-free survival	15
	Microarray and IHC (expression in 17/24 serous type patients)	Paclitaxel resistance	34
Pancreatic	IHC (expression in 67/122 patients)	↓ Global survival	35
	IHC (3%–50% expression in 17/17 tumors)	T _{reg} in metastatic LN	22
Lung (non–small cell)	IHC (25 patients with IDO in eosinophils)	↓ Survival	32
Renal	Quantitative RT-PCR (55 primary tumor + 52 metastasis)	↓ Tumor proliferation	37
		Western blot and IHC (expression in microvessels)	
Nasopharyngeal	IHC (9 patients + immortalized cell line)	Advanced histologic stage	27

Abbreviations: AML, acute myelogenous leukemia; dLN, draining lymph node; ICC, immunocytochemistry; IHC, immunohistochemistry; IL, interleukin; LN, lymph node; RT-PCR, reverse-transcription PCR; SLN, skin lymph node.

^aUp arrow indicates increase, and down arrow indicates decrease.

(T_{reg}), which is known to be favored by an IDO-rich environment (8), can be responsible for this apparent increase in CD3⁺ and CD4⁺ populations. In addition, IDO activity seems to inhibit natural killer, CD8⁺ T-, and Th1 CD4⁺ T-cell proliferation without affecting the Th2 population (18), and it could even favor a Th2 polarization (19). These observations could be another explanation for the lack of CD4⁺ T-lymphocyte decrease in IDO-expressing tumor.

Regulatory T-cell infiltration increase

As mentioned earlier, T_{reg} and IDO have been shown to have closely related immunosuppressive features. Thus, an

association between IDO expression by tumor cells and T_{reg} frequency has been a matter of interest in different studies. Specifically, higher T_{reg} CD4⁺CD25⁺Foxp3⁺ frequency has been observed in patients with acute myeloid leukemia when tumor cells express IDO (20, 21). Moreover, patients with pancreatic adenocarcinoma were more densely infiltrated by T_{reg} in metastatic lymph nodes compared with nonmetastatic lymph nodes (22). Also, in all the cases, those metastatic lymph nodes were expressing IDO at different levels. The authors proposed that IDO expression in lymph nodes from patients with pancreatic adenocarcinoma could lead to the recruitment of regulatory T

lymphocytes. However, it is not clear whether IDO allows the specific recruitment of T_{reg} or the conversion from naïve $CD4^+$ T lymphocytes, because this phenomenon can occur, at least *in vitro* (23). On the other hand, increase in T_{reg} proportion could be attributed to an inhibition of $Foxp3^+$ T_{reg} reprogramming in Th1 by IDO, as shown by Sharma and colleagues (8). Following CpG vaccination, IDO was expressed in mice. Conversely, the expression of IDO may be a consequence of T_{reg} infiltration in metastatic lymph nodes. Finally, simultaneous expression of IDO and $Foxp3$ in TDLNs has been measured in many patients with breast cancer, and this has been correlated with the presence of metastases in those TDLNs (24). From all these studies, we can conclude that IDO favors T_{reg} in tumors by recruitment or by enhancing conversion of naïve T lymphocytes in T_{reg} .

Link with cancer progression

Many factors associated with tumor progression have been positively correlated with high expression of IDO in cancer cells. On the one hand, frequency of hepatic metastasis has been found to be significantly increased in patients with colorectal cancer presenting high IDO expression in their tumor (14). Strong IDO expression in hepatocellular tumors has also been associated with the presence of distant metastases (25). In addition, metastases in TDLNs have been found preferentially in patients with endometrial tumors expressing IDO at a high level (26). For patients with this tumor type or for patients with ovarian carcinoma and nasopharyngeal carcinoma, high levels of IDO have been detected in advanced stages of the disease (15, 26, 27). Polak and colleagues investigated many immunosuppressive molecules in cutaneous melanoma, and IDO expression in positive or negative skin lymph nodes seems to increase with melanoma progression (28). Also, invasive and microinvasive forms of uterine cancer are correlated with high expression of IDO (29). Finally, IDO expression has been found in all microinvasive and invasive uterine cervical cancer, whereas noninvasive tumors presented a much lower expression (30). However, the mechanistic link between cancer progression and IDO expression cannot be explained by these correlative observations. Further studies are needed to better understand whether IDO expression is mechanistically responsible for tumor progression or whether tumors evolve to express IDO during their development to maintain an immune-suppressive environment in response to antitumor T cells.

Association with patient survival

In the majority of the studies that have focused on IDO expression in human cancer, patients with high expression of IDO had decreased global and progression-free survival. Notably, this correlation has been reported for patients with endometrial cancer. In 2 studies, IDO expression evaluated by immunohistochemistry (IHC) and enzymatic activity has been inversely correlated with progression-free survival (16, 26, 31). Also, IDO measurement by IHC in hepatocellular carcinoma samples from 138 patients has established that low IDO expression correlates with an improved

overall survival (25). For patients with invasive cervical cancer treated with radical hysterectomy, it has been observed that focal and diffuse IDO expression in cancer tissue correlates with decreased overall and progression-free survival, whereas patients with no IDO expression in their tumor had a better survival (30). In non-small-cell lung carcinoma, eosinophils were also positive for IDO and correlated with lower patient survival (32). In a Chinese cohort of patients with colon cancer, IDO and Bin1 were analyzed in tumor tissues and TDLNs with or without metastasis. A relatively low percentage of tumors was found to express IDO (12%). However, 21 out of 60 TDLNs analyzed had a high density of IDO⁺ cells, and these have been associated with a decreased 5-year survival rate (33). Finally, it has been observed that patients with ovarian carcinoma presenting high IDO expression, as evaluated by IHC (15) or global gene expression profiles (34, 35), have a shorter postsurgery life expectancy.

Only 2 studies have reported results contradictory to those discussed above. First, increased relapse-free survival has been correlated with the presence of IDO mRNA in hepatocellular carcinomas (36). However, protein expression was not investigated in this study. On the other hand, Kaplan–Meier curves could not show a significant difference between low and high IDO expression in patients with renal carcinoma. Interestingly, this study was the only one to report IDO expression almost exclusive to newly formed microvessels in the tumor. This IDO expression has been inversely correlated with Ki67⁺ tumor cells, a proliferation marker. From these observations, it has been assumed that endothelial cells expressing IDO were responsible for the decreased tumor cell proliferation (37). These results suggest that IDO can have a noxious effect on tumor growth when an inefficient antitumor immune response is taking place in a host.

Association between IDO expression by tumors and the presence of metastases or various clinical parameters strongly suggests that IDO contributes to tumor progression. Moreover, IDO expression has been identified as an independent prognostic marker in many cancer types. Thus, this immunosuppressive molecule could possibly be used as a classifying marker to identify patients with higher relapse susceptibility or a less favorable prognosis. In addition, IDO expression could even serve as a marker capable of identifying patients for whom adjuvant therapies would be more beneficial, thus preventing unnecessary administration in a proportion of patients. In the era of personalized care, TDLNs or tumor tissue biopsies could be done prior to IDO inhibitor administration to select patients expressing IDO in their tumors who would better respond to the treatment.

Discussion

Globally, studies reviewed herein tend to associate IDO expression by human tumors with a less favorable prognosis for patients. In the majority of these studies, IDO expression in tumor tissues was evaluated by IHC. However, until now no attention has been paid to the recently

identified IDO2 isoform. Furthermore, IDO enzymatic activity in cancer has rarely been investigated. Interestingly, researchers in an ovarian epithelial cancer study have reported IDO expression in 43% of analyzed tissues (83/192), and this finding was shown to be correlated with a lower tryptophan-to-kynurenin ratio in the tumor environment (38). Similar results were obtained in a study from Huang and colleagues in which they observed that lower serum tryptophan in patients with colorectal carcinoma was correlated with reduced quality of life (39). Also, the tryptophan serum level and the tryptophan-to-kynurenin ratio have been found to be significantly lower in patients with ovarian carcinoma compared with healthy women. This level correlated with different serum immune activation markers (40). Together, these results point out that the IDO expressed in tumor tissue can be enzymatically active, but no mechanistic link has been established as a causative factor. Further studies must be conducted to completely elucidate the relationship between IDO expression and tumor growth in humans.

As previously mentioned, the studies presented above have associated IDO with a less favorable clinical outcome. However, mechanistic studies aimed at establishing how IDO can affect tumor progression are still rare. Yoshida and colleagues have shown an *in vivo* role of IDO in tumor progression, using a xenograft model of human ovarian tumor cells transfected with IDO (41). Also, Muller and colleagues have proposed that the downregulation of *Bin1*, a tumor suppressor gene, promotes IDO expression in tumors via STAT1/NF- κ B-dependent mechanisms (13). Furthermore, IDO production resulting from these molecular events has been found to mediate the immune escape of tumor cells lacking *Bin1*. The latter finding is interesting given that the expression of *Bin1* is frequently altered in human breast, prostate, brain, colon, skin, and lung cancers (reviewed in ref. 42). However, IDO and *Bin1* expression in colon cancer tissue was stained by IHC and failed to show any correlation (33).

Considering the prevailing expression of IDO in a large proportion of tumors, this enzyme could be an interesting target in cancer immunotherapy. Moreover, the induction of this immuno-regulating molecule has been reported as a result of immune system manipulation aimed at inducing an antitumor response. On the basis of the findings in the studies discussed above, this IDO induction in response to immune system stimulation could hamper the generation of an effective antitumor response. We can speculate that the efficiency of anticancer vaccines could be improved when the vaccines are administered in combination with an IDO-inhibiting agent. This hypothesis has been corroborated in a mouse model. In fact, Ou and colleagues have observed that the administration of a vaccine made of dendritic cells fused with pulmonary carcinoma cells induced IDO expression in TDLNs and splenocytes (43). Considering that CpG oligonucleotides are known to elicit IDO expression in dendritic (44) and B cells (45), it is not surprising that a vaccine containing CpG elements induces IDO in various immune cells. CpG elements incorporated

in DNA vaccines could stimulate IDO expression in the spleen and lymph nodes (46). Apart from small-molecule inhibitors, other ways of targeting IDO in a clinical setting have been proposed. Sørensen and colleagues have patented the use of IDO-specific cytotoxic T cells for cancer treatment (47). These specific clones combined with viral antigen immunity have been shown to enhance the specific immune response (48). Although promising, this alternative option presents some potential disadvantages, such as the potential recognition of antigen-presenting cells expressing IDO. Also, there is no evidence that IDO-specific CTLs have the potential to recognize and kill tumor cells *in vivo*. Together, these findings suggest that the inhibition of IDO enzymatic activity could improve attempts to generate an efficient antitumor immune response.

IDO enzymatic activity can be inhibited by small molecules analogous to tryptophan. The most studied inhibitor to date is the 1-MT. Both isomers (D-1-MT and L-1-MT) have been used for their IDO inhibition capacity. Depending on the experimental context, one or the other is more efficient in inhibiting tryptophan catabolism or immunomodulatory properties of IDO. In mice, the administration of 1-MT as a single therapeutic agent was sufficient to delay the apparition of tumors (10, 49) but could not lead to the complete rejection of established tumors (13). However, chemotherapeutic drugs have shown superior efficacy to inhibit tumor progression when administered in combination with 1-MT (13), suggesting that IDO inhibitors combined with other agents could be more beneficial than their administration as a single agent.

Information obtained from murine studies has led to the initiation of clinical studies aimed at investigating the antitumor efficacy of D-1-MT in patients with cancer at the end of 2007. Currently, National Cancer Institute clinical trials are aimed at evaluating safety, pharmacodynamics, and clinical responses in patients with breast, non-small-cell lung, and other refractory solid cancers (50). However, the use of this isomer is still controversial in humans because of its relative specificity to the IDO2 isoform. Even if some effects have been shown in mouse models, D-1-MT has been found to preferentially target the IDO2 isoform that could be inactive in proportions reaching 50% of the population, because of 2 polymorphisms affecting its enzymatic activity (51). Recent studies established that L-1-MT would be preferable to inhibit IDO activity in both dendritic cells and human cancer cells. These studies also showed that both IDO and IDO2 were expressed in monocyte-derived dendritic cells, but only IDO could participate in tryptophan degradation and only L-1-MT could abolish this activity (52). Moreover, *in vitro* studies with T lymphocytes from blood, ascites, or tumor tissue of patients with ovarian epithelial cancer showed that their proliferation could be inhibited by ovarian IDO-expressing cancer cells. Only L-1-MT or a mix of D and L-1-MT, but not D-1-MT, was able to restore T-cell proliferation (38). Currently, further investigations are needed to confirm the potential use of IDO inhibitors in a clinical context to treat cancer. However, with the results of the

studies reviewed here, IDO seems to be a potential target to enhance antitumor response in patients with cancer. Although 1-MT may bring interesting effects, the ideal inhibitor for clinical use may have yet to be discovered.

Conclusions

All of the observations described earlier seem to implicate IDO as a key regulator of tumor immune evasion. Almost all of the correlation analyses show an association between IDO and characteristics generally related to poor prognosis.

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