Inflammatory programming and immune modulation in cancer by IDO

Courtney Smith, George C Prendergast

Lankenau Institute for Medical Research (LIMR), Wynnewood PA USA (CS), Department of Pathology, Anatomy and Cell Biology, Jefferson Medical School and Kimmel Cancer Center, Thomas Jefferson University, Philadelphia PA USA (GCP)

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Abstract

Immune dysregulation is one of the hallmarks of tumor growth and progression, a key event that allows for tumor evasion of the host immune system. More recent cancer modalities are embracing combinations incorporating immunotherapy with more traditional chemotherapy and radiotherapy. Traditional approaches are difficult to tolerate for the patient and become less effective as tumors evolve to survive these treatments. Immunotherapy has the benefit of reduced toxicity as it utilizes the patient's own immune system to identify and eliminate tumor cells. One mechanism manipulated by tumors is upregulation of the immunoregulatory enzyme indoleamine 2,3-dioxygenase (IDO). In this review, we focus on the mechanism by which tumors use IDO to evade detection by T cell immunity, as well as on novel small molecules that inhibit it as a cancer therapeutic strategy.

Diversity of IDO-related immunoregulatory enzymes

Identification of the non-hepatic tryptophan catabolizing enzyme, indoleamine 2,3-dioxygenase (IDO; EC 1.13.11.42; originally D-tryptophan pyrrolase) was first reported in 1963 (Higuchi and Hayaishi, 1967; Higuchi et al., 1963). IDO, also known as IDO1, catalyzes the first and rate-limiting step that converts tryptophan to N-formyl-kynurenine, a process that utilizes oxidative cleavage of the 2,3 double bond in the indole ring resulting in the biosynthesis of nicotinamide adenine dinucleotide (NAD) (Takikawa, 2005). The catabolism of tryptophan can also occur through the enzyme tryptophan 2,3-dioxygenase (TDO2), though studies have shown that the enzymes are not redundant. While both IDO1 and TDO2 catalyze the same reaction, beyond that the two enzymes are structurally distinct and do not share any significant sequence similarity. Structurally, IDO1 exists as a 41kD monomeric enzyme whereas TDO2 is a 320kD homotetramer (Watanabe et al., 1981). Furthermore, TDO2 and IDO1 are differentially localized. Unlike TDO2, IDO1 is not involved in the dietary homeostasis of tryptophan degradation. Instead IDO1 is found in various cells including immune cells, endothelial cells, fibroblast and some tumor cells (Serafini, et al., 2006; Friberg et al., 2002; Uyttenhove et al., 2003; Munn et al., 2004).

IDO1 is relatively well conserved between species suggesting evolutionary importance. The primary sequence of IDO1 is 63% identical between mouse and human. The crystal structure of IDO1 and the resulting site-directed mutagenesis show that both substrate binding and the precise relationship between the substrate and iron-bound dioxygen are necessary for activity (Sugimoto et al., 2006). More recently, a homolog to IDO1, termed IDO2, has been identified. Initially, a missannotation in the human genome database prevented the identification of IDO2. The correction of this error revealed the 420 amino acid open-reading frame that shares 44% sequence homology with IDO1 (Ball et al., 2007; Metz et al., 2007). Importantly, IDO2 contains the conserved residues that are identified as critical for tryptophan binding and catabolism (Sugimoto et al., 2006). Between mouse and human, IDO2 is 73% identical at the primary sequence. Due to the recent discovery of IDO2, there is still much to learn about this enzyme. While both human and murine IDO2 enzyme have been
shown to catabolize tryptophan to kynurenine, IDO2 has a distinct pattern of expression that differs from both IDO1 and TDO2 (Metz et al., 2012). Using total RNAs from human tissues, full-length IDO2 was found expressed in the placenta and brain. Interestingly, primers located in exon 10 showed IDO2 mRNA in a greater number of tissues including liver, small intestine, spleen, placental, thymus, lung, brain, kidney and colon supporting the possible existence of additional splice isoforms and perhaps transcriptional start or polyadenylation sites. To further complicate its study, transcripts of IDO2 in murine tissues are localized to liver and kidney which differs from human tissues somewhat. Interestingly, IDO2 mRNA is expressed in murine pre-dendritic cells and following stimulation with IFNγ, IL-10 or lipopolysaccharide (LPS). IDO2 protein can be detected (Metz et al., 2012), suggesting in these specialized antigen-presenting cells of the immune system.

**IDO in the immune system**

The first evidence for a role of IDO in immune regulation was observed when IDO expression was induced following viral infection or treatment with interferon (IFNγ), an important inflammatory cytokine (Yoshida et al., 1979; Yoshida et al., 1981). A prior observation that IDO is present in the urine of cancer patients then re-surfaced and its importance re-evaluated (Rose, 1967). A groundbreaking study from Munn and Mellor in 1999 directly established IDO as an important immune regulator (Munn et al., 1998). This study showed that pregnant female mice treated with 1-methyl-tryptophan (1MT), an IDO inhibitor, caused rejection of allogeneic concepti but not syngeneic concepti. Further studies showed that this occurs through an MHC-restricted T cell-mediated rejection of the allogeneic mouse concepti (Mellor et al., 2001). These findings have been widely interpreted to mean that the normally high levels of IDO in the placenta are important in preventing the maternal immune system from attacking the "foreign" fetus.

Once IDO was established as an immune modulator, studies have focused on its role both in disease states as well as in normal immune surveillance. The immunosuppressive function of IDO1 manifests in several manners. Collectively, IDO1 and its metabolites can directly suppress T cells (Fallarino et al., 2002; Frumento et al., 2002; Terness et al., 2002; Weber et al., 2006) and NK cells (Della Chiesa et al., 2006) as well as enhance local Tregs (Fallarino et al., 2003). The protumorigenic capabilities of myeloid derived suppressor cells (MDSCs) (Smith et al., 2012) suggest that this population is also affected by IDO1. Furthermore, IDO1 is produced in response to IFN-γ in endothelial cells, fibroblasts and the immune cells including dendritic cells and myeloid derived cells (Taylor and Feng, 1991; Burke et al., 1995; Varga et al., 1996; Munn et al., 1999; Hwu et al., 2000). It has therefore been hypothesized that IDO1 not only regulates immunity at the level of T cells but is regulated by or and regulated by cytokine production in the host that is associated with the generation of a protumorigenic microenvironment. A role for IDO1 in cancer is further suggested by the fact that many human tumor cells themselves express IDO1 (Uyttenhove et al., 2003; Taylor and Feng, 1991).

**IDO in cancer**

Treatment of cancer commonly entails surgical resection followed by chemotherapy and radiotherapy. The standard regimens show highly variable degrees of success in the longer term, because of the ability of tumor cells to escape these treatments to regenerate primary tumor growth and more importantly seed distant metastasis. The production of IDO in the tumor microenvironment appears to aid in tumor growth and metastasis. It is logical then to target IDO as a means of slowing tumor progression. This has been the premise of several recent studies.

Studies have revealed a pathophysiological link between IDO1 and cancer, with increased levels of IDO1 activity associated with a variety of different tumors (Brandacher et al., 2006; Okamoto et al., 2005). In a case study of ovarian cancer, overexpression of IDO correlated with poorer survival. Immunohistochemical staining on tumor sections were categorized as negative or positive, with the latter further defined as sporadic, focal or diffusely staining. While patients with no IDO expression had greater than 5-year survival following surgery, the three subcategories of positive IDO staining showed a 50% survival of patients to 41, 17 and 11 months, inversely correlated with the amount of staining (Okamoto et al., 2005). Two mechanisms by which IDO suppresses the local immune system are inhibition of effector T cells or activation of Tregs (Fallarino et al., 2006; Munn and Mellor, 2007). In the colorectal study, high IDO expression was associated with few tumor infiltrating CD3+ T cells. In addition to affecting the local environment, tumor biopsies with high expression of IDO in both colorectal and hepatocellular carcinomas have shown greater metastasis in patients (Brandacher et al., 2006; Pan et al., 2008). While these studies showed IDO expressed by the tumor itself, other clinical studies have found both stromal cells and surrounding immune cells to be the source of IDO overexpression. Poor survival correlated with IDO-positive eosinophils in small cell lung cancer (Astigiano et al., 2005) while a study of melanoma patients showed poor prognosis in patients with detectable IDO in the dendritic cells (DC) from the tumor draining lymph nodes (Lee et al., 2003; Munn et al., 2004). These clinical cancer studies are supported and enhanced by studies in the mouse model. As shown in the clinical studies, tumors may induce IDO1 production in neighboring cells such as antigen presenting dendritic cells located in the tumor-draining
lymph nodes (TDLNs). 4T1 is a highly malignant breast carcinoma-derived cell line that, following orthotopic engraftment into the murine mammary fatpad, forms tumors of the latter variety in which no IDO1 expression is detectable in the primary tumor but is found expressed at high levels in the TDLNs. The IDO1-expressing cells in the TDLNs appear morphologically to be plasmacytoid dendritic cells. A similar pattern of IDO1 expression has previously been observed in a mouse model of melanoma (Munn et al., 2004). Importantly D-1MT treatment of 4T1-tumor bearing mice cooperated with chemotherapy to suppress primary tumor growth, ascribing an immunosuppressive role of IDO1 in the TDLNs (Hou et al., 2007).

The use of an immunogenic tumor cell line transfected to overexpress IDO demonstrated that IDO prevents immune surveillance from rejecting these tumors in preimmunized mice. There was also a reduction in tumor associated T cells. Furthermore the use of 1MT resulted in a slowed progression of the tumor, further implicating IDO as a tumor evasion mechanism (Uyttenhove et al., 2003). In the MMTV-neu mouse model of breast cancer, the synergistic benefit of combining chemotherapy with the indoleamine-2,3-dioxygenase (IDO1) inhibitor 1-methyl-D-tryptophan was observed (Muller et al., 2005). It was shown that the effects of D-1MT were greatly enhanced when given in conjunction with the commonly used chemotherapeutic agent paclitaxel. Depletion of either CD4+ or CD8+ T-cells in these mice abolished the benefit provided by D-1MT, indicating the importance of T cell immunity to the antitumor response. These studies have all led to the initiation of phase I clinical trials testing the efficacy of 1-MT as a cancer vaccine adjuvant.

**Signaling mechanisms upstream and downstream of IDO**

The NF-κB signaling pathway has been implicated in IDO1 signaling through the initial observation that INDO can be induced by interferon-γ (IFN-γ) treatment (Ozaki et al., 1988). A more detailed report showed that BAR adapter proteins encoded by the Bin1 gene are important mediators of NF-κB signaling to IDO. Bin1 is a suppressor of tumor growth that is poorly expressed in tumor cells (Ge et al., 1999; Ge et al., 2000; Tajiri et al., 2003). Using a knockout mouse for Bin1 it was shown that there was an increase in STAT1 and NF-κB signaling leading to increased IDO (Muller et al., 2005; Muller et al., 2004). This suggested that under normal conditions, Bin1 acts to suppress tumor growth by keeping levels of IDO under control. However, loss of this regulatory gene led to increased tumor growth as Bin1 supported T-cell mediated immune surveillance was impaired (Muller et al., 2005). The engraftment of c-myc-ras-transformed skin epithelial cells in syngeneic mice resulted in limited tumor growth if Bin1 was present than if it was deleted, mimicking the effects of Bin1 attenuation in human tumor cells. Notably, the beneficial effects of Bin1 deletion to tumor growth were lost in immune deficient or T-cell depleted mice, revealing the importance of the immune system in mediating the primary effects of Bin1 on tumor growth. Studies in Bin1-deficient cells established that IDO expression was upregulated and that the inhibitor 1MT could phenocopy the effect of Bin1 competency. This work also indicated that Bin1 limits IDO transcription by limiting the activity of NF-κB and STAT which are sufficient to support IDO expression (Muller et al., 2004; Bild et al., 2002).

GCN2 signaling is another mechanism by which IDO may regulate immune cell function. GCN2 is a kinase that acts as a sensor for amino acid starvation. The depletion of tryptophan from the microenvironment can trip GCN2 signaling resulting in apoptosis, cell cycle arrest and differentiation. GCN2 rapidly responds to amino acid deprivation through the phosphorylation of eIF-2α resulting in inhibition of translation (Bild et al., 2006). GCN2 is activated by IDO as a result of the tryptophan deprivation it creates. GCN2 is also a signaling component of T cells which may be a mechanism by which IDO regulates the immune system and produces biological effects. Notably, GCN2-deficient T cells are resistant to the immune suppressive effects of IDO (Munn and Mellor, 2007; Munn et al., 2005). GCN2 signaling switches on the expression of stress-activated proteins that trigger growth arrest and apoptosis as well as differentiation. ATF4, ATF3 and CHOP/GADD153 (Harding et al., 2000; Jiang et al., 2004; Vattem and Wek, 2004; Lu et al., 2004; Hai et al., 1999; Wang et al., 1996; Fan et al., 2002) have been implicated as three critical targets of GCN2 in responding to amino acid deprivation. Cytokines are critical for immune recognition of tumor cells, but when they are hijacked by the tumor they may provide a mechanism of immune escape for both the primary tumor and distant metastases. This was seen in Ido1-nullizygous mice that exhibited both reduced lung tumor burden in the oncogenic KRAS-induced model as well as in the metastatic 4T1 orthotopic breast cancer model. Both models showed reduction of lung tumor burden that was directly correlated with improved survival of Ido1-/- mice (Smith et al., 2012). Further investigation into the immune regulatory role revealed a reduction in the levels of the inflammatory cytokine IL-6 in IDO-deficient mice. However, when IL-6 was restored in these mice, the rate of metastasis was also restored to levels of wild-type mice. Ex vivo studies of myeloid derived suppressor cells (MDSC) from IDO-deficient mice with 4T1 primary tumors showed an impairment in the ability of MDSC to suppress T cell function, compared to MDSC derived from 4T1 tumor-bearing wild-type mice. The attenuation of IL-6 levels in IDO-deficient mice was associated with an impairment in MDSC function, and as before restoring IL-6 overcame the MDSC defect, allowed metastatic disease to...
progress at the rate observed in wild-type mice (Smith et al., 2012). The implication of these results was that IL-6 serves as a key regulator of tumor growth downstream of IDO, a connection of potential therapeutic value since IL-6 levels are increased in patients with recurring tumors (Kita et al., 2011).

The mechanisms through which IDO affects tumor growth remain only partly elucidated. One intriguing effector pathway appears to involve the aryl hydrocarbon receptor (AHR), discovered to be a target receptor for dioxin (Opitz et al., 2011). AHR was discovered originally as the receptor for dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin [TCDD]). Upon binding to AHR in dendritic cell cultures, TCDD can induce expression of both IDO1 and IDO2 suggesting the presence of a feed-forward regulatory loop (Vogel et al., 2008). It is postulated that induction of IDO1 through TCDD requires a combination of signaling by AHR and RelB, the non-canonical NF-κB signaling molecule that may work through IL-8 and AHR following CD40 ligation (Vogel et al., 2007a; Tas et al., 2007; Vogel et al., 2007b). Furthermore, it was shown that TCDD treatment of mouse splenic T-cells resulted in increased levels of FoxP3, an effect that was abrogated in Ahr-null mice signifying that Ahr is important in the development of Tregs (Vogel et al., 2008). Further mechanistic connections are suggested by observations that kynurenic acid, a byproduct of tryptophan catabolism, also induces AHR activity and results in IL-6 signaling (DiNatale et al., 2010).

Interestingly, IDO1-nullizygous mice show a diminished IL-6 levels in primary lung tumors and pulmonary metastases (Smith et al., 2012). Taken together, studies identify AHR and IL-6 (a target of the GCN2 pathway activated by IDO (Metz et al., 2007)) as key players in IDO signaling in cancer.

**Clinical trials of IDO inhibitors**

IDO is an appealing therapeutic target for cancer treatment for several reasons. Structurally, it is well-defined, allowing for easier discovery of molecular inhibitors. Furthermore, it is both structurally and spatially distinct from the tryptophan catabolic enzymes IDO2 and TDO2. From a clinical viewpoint, pharmodynamic evaluations are eased by measuring serum tryptophan and kynurenine levels. Additionally, while immunotherapies are gaining clinical use, they are often restricted by being either tailored to each patient or expensive. An enzymatic inhibitor provides a more generic and less expensive method to alter immune recognition and elimination of tumor cells.

The potential value of targeting IDO in cancer was further given credence by the addition of 1MT onto a select list of the 12 immunotherapeutic agents identified by an NCI workshop panel as having high potential for use in cancer therapy (Koblish et al., 2008). 1MT is a tryptophan analog that entered early stage clinical trials in 2008 and results are expected by the conclusion of 2013. While 1MT may provide antitumor effects, recent preclinical studies in mice have suggested that 1MT does not act directly on IDO but rather downstream in an effector pathway leading to mTOR control (Metz et al., 2012).

An enzymatic inhibitor of IDO termed INCB024360 has entered clinical trials. INCB024360 is a hydroxyamide that competitively blocks the degradation of tryptophan to kynurenine through IDO with an IC50 of approximately 72 nM (Liu et al., 2010). Similarly to 1MT, treatment with INCB024360 showed attenuated tumor growth in wild-type mice but not in immune-deficient mice (Liu et al., 2010; Koblish et al., 2010). In both mice and dogs, INCB024360 was given orally, resulting in a reduction of kynurenine in the tumors, tumor draining lymph nodes and also plasma (Koblish et al., 2010).

There was no apparent maximum tolerated dose determined in the Phase I trials allowing it to move into Phase II trials, where it will be tested as a monotherapy in ovarian cancer and as a combination therapy with ipilimumab for metastatic melanoma.

One interesting aspect of IDO inhibition is that it may already be occurring with other cancer drugs. For example, the paradigm targeted cancer drug Gleevec has been found to suppress IDO expression in GIST cells as a result of Kit inhibition (Balachandran et al., 2011). Another recent study has revealed that the cytotoxic agent β-lapachone is a direct inhibitor of IDO, postulating that this cytotoxic agent may benefit from the additional immunological effects that derive from its potent uncompetitive inhibitory effects on IDO1 activity.

Other drugs in use include NSAIDs that indirectly block IDO activity as a result of COX2 inhibition (Sayama et al., 1981). Another effective IDO inhibitor in mouse studies is the anti-inflammatory ethyl pyruvate, which by inhibiting NF-kB activity blocks IDO expression and produces robust anti-tumor responses that are both T cell and IDO dependent (Muller et al., 2010). Taken together, these findings point to a promising future for IDO inhibitors as new tools for immunotherapy and immunochemotherapy of cancer.

**References**


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