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COMMENTARY

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Targeting iNOS to increase efficacy of immunotherapies

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ABSTRACT

Inducible NO synthase (iNOS/NOS2) protein expression is a well-studied predictor of poor outcome in multiple cancers, and it has also been associated with inflammatory and immunosuppressive processes in the tumor microenvironment. Immunotherapies are becoming increasingly key components in cancer treatment, and iNOS is receiving more attention as a potential regulator of treatment resistance. As we have reported in pancreatic cancer, by modulation of effector T-cell activity, iNOS overexpression may allow the tumor to escape the immune response through creating a microenvironment which causes recalcitrance to immunotherapy. Based on studies describing its role in the immune environment of multiple cancers, strategies that include iNOS inhibitors as combination partners may enhance immunotherapy approaches. The expression and the function of iNOS both depend on the tumor type and microenvironment, as well as on the patient's treatment history. Thus, enhancing immunotherapies, including adoptive T-cell therapies and checkpoint blockade, will require tailored cancer-specific approaches and additional levels of microenvironment regulation.

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Introduction

Cancer immunotherapy has emerged as one of the most promising treatment modalities and made remarkable progress in the last decade. The main strategies to exploit the patient's immune system to fight cancer include cytokines, immune checkpoint blockade (e.g. CTLA-4, PD-1, PD-L1), cancer vaccines, and adoptive T-cell therapy approaches.[1] Although immunotherapies have shown impressive results in the clinic, most cancer patients are not cured completely, and many questions remain unanswered including how to select the patients who would benefit from these treatments. Combinations of immune and targeted therapies also show promise, especially when targeted therapies help modulate the immune system by increasing immune infiltration or immunogenicity of the tumor.[2] Immunogenic neoantigens arise from both mutated and non-mutated but tumor-specific proteins, and these are the main targets of currently available personalized cancer vaccine and T-cell immunotherapies.[3] Low mutation load often limits the availability of targetable neoepitopes, and loss of human leukocyte antigen (HLA) expression and/or active immunosuppressive mechanisms (e.g., inhibitory cytokines like TGF-β and IL-10; regulatory T cells - Tregs, myeloid-derived suppressor cells - MDSCs, and tumor-associated macrophages - TAMs) also help cancer cells to evade the immune response. We hypothesize that combination therapies may need to eliminate mediators of immune suppression to be able to evoke robust T-cell responses. We have recently identified inducible nitric oxide synthase (iNOS/NOS2) as a potential mediator of immune suppression in pancreatic ductal adenocarcinoma (PDAC).[4] Aberrant expression of iNOS/NOS2 has also been observed in several other tumor types, such as breast, colon and melanoma,[5-8] and its role in tumor progression appears to depend on the activity and localization of NOS isoforms, concentration and duration of nitric oxide (NO) exposure, and cellular sensitivity to NO. Although the role of NO and the protein iNOS, which is one of the enzymes that synthesize NO from L-arginine, in cancer development has been extensively studied in the last decades, we envisage that the need for effective combination (immuno-) therapeutics will renew interest in targeting this protein in clinical practice.

The dual role of iNOS in host defense and cancer development

It was first discovered that NO plays a critical role in various physiological processes including host defense by controlling replication or killing of intracellular microbial pathogens.[9] Increased expression of NO in response to cytokines or pathogen-derived molecules is an important component of host defense against a wide variety of intracellular microorganisms. In multiple tumor types, iNOS expression, which catalyzes the production of NO, is also high and has been reported to be expressed by various cell types, including M2 macrophages, MDSCs, dendritic cells, NK cells, tumor cells, endothelial cells, neuronal cells, and neutrophils; all of which are involved in inflammation and cancer. However, the role of iNOS in tumor

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development is complex, and it can promote tumor development and also inhibit immune response.[10] Among the numerous effects of NO in cancer, it is now evident that NO plays important roles in various stages of carcinogenesis such as DNA damage, oncogene activation, inhibition of DNA repair enzymes and tumor suppressor genes, and modulation of apoptosis.[10] Furthermore, augmented NO production promotes tumor progression and metastasis by increasing proliferation, migration, and angiogenesis.[10] Thus, we propose that inhibition of NO production may have a significant therapeutic potential to improve immunotherapies. It is postulated that the role of iNOS depends on the tumor type and the tumor microenvironment, therefore it is critical to identify exactly how and when targeting iNOS could be effective to treat cancer or increase efficacy of immunotherapies. It was recently shown that iNOS enhances disease aggressiveness in pancreatic cancer,[11] which, together with its potential in enhancing PDAC immunotherapies,[4] suggests that iNOS could be an effective target in this malignancy. In addition to PDAC, multiple studies point to that inhibiting iNOS could increase efficacy of immunotherapy of other cancers as well.

Enhancing immunotherapies by targeting iNOS

Expression of iNOS protein by tumor cells deleteriously influences the anti-tumor immune response primarily by mediation of immune suppression. Functional roles of iNOS in anti-tumor immunity include recruitment and/or activation of MDSCs, Tregs, tumor-associated macrophages, and Th2 lymphocytes.[12] Activated MDSCs also continuously produce NO in the tumor microenvironment, and this further increases the inhibition of anti-tumor T-cell activity.[13] Targeting NO production reverses MDSC-mediated immunosuppression by blocking MDSC recruitment to the tumor.[14] Similarly, in a lung metastatic model where IFN-γ production was stimulated using α-garactosylceramide, inhibition of iNOS expression enhanced therapeutic efficacy via suppression of MDSCs.[15] Although the beneficial effect of iNOS inhibition by suppressing MDSCs is clear, other immunosuppressive factors also need to be considered. MDSCs can deplete L-cysteine from the tumor microenvironment, resulting in decreased proliferation and activation of T cells.[16] In addition, L-arginine depletion by MDSCs may contribute to Treg expansion.[17] In a melanoma mouse model, MDSC levels were suppressed by iNOS inhibition, but FOXP3+ Treg levels were increased, and a combination treatment of iNOS inhibition and Treg depletion was necessary to control tumor growth.[18] A recent study also showed that the presence of N-AcetyL-cysteine (NAC) during ex vivo T-cell expansion improves the persistence of adoptively transferred cells, reduces tumor growth, and increases survival in a mouse model of melanoma.[19] NAC is a sulfhydryl donor molecule with antioxidant and anti-inflammatory effects. It attenuates NO generation by modulating iNOS expression, and it also inhibits NF-kB activity.[20] Thus, the addition of NAC to current therapeutic T-cell expansion protocols could improve adoptive T-cell therapies by directly inhibiting iNOS and NO production at the tumor site.

Although originally macrophages were identified as myeloid cells that infiltrate tissues to combat and eradicate invading pathogens and tumor cells, in recent years multiple studies have shown that subclasses exist and they may support tumor progression, growth, and metastasis as they produce growth factors, cytokines and chemokines which are necessary for these processes.[21,22] Accumulating evidence suggests that these tumor-associated macrophages actively promote all aspects of tumor initiation, growth, and development of a polarized M2 phenotype (alternatively activated type) instead of the M1 phenotype (classical activated type). It has been shown that iNOS-derived NO mediates nitration of tyrosine residues in multiple regulatory proteins, leading to the suppression of the M1 macrophage signature gene activation[23] and a pro-tumorigenic environment. TAM-infiltrated tumors are associated with worse clinical outcome and increased angiogenesis, local tumor progression, and metastasis. Through iNOS-expressing immune and inflammatory cells, cancer cells may acquire an additional immunosuppressed state, which may increase the barrier to effective cancer immunotherapies. Inhibition of iNOS also enhances the efficacy of Toll-like receptor (TLR) agonists by increasing Th1 immune response, and a combination of a TLR7 agonist and an iNOS inhibitor effectively inhibited tumor growth.[24] Increasing activity of Th1 immune cells may synergize with suppression of polarized M2 macrophages, therefore approaches that target iNOS in the tumor microenvironment could potentially strengthen anti-tumor immune responses.

iNOS and checkpoint blockade

Inhibition of iNOS may also enhance immune therapies that are based on checkpoint blockade. For instance, in a checkpoint blockade study of neuroblastoma, immunosuppression mediated by myeloid Gr1+ cells was rescued by blocking enzymatic activity of iNOS.[25] Furthermore, targeting the gamma isoform of phosphoinositide 3-kinase (PI3Kγ) in myeloid cells restored sensitivity to checkpoint blockade, however, expression of iNOS was also elevated after treatment.[26]

Similarly, CSF1/CSF1R blockade in a pancreatic ductal adenocarcinoma model improved response to checkpoint blockade-based immunotherapy (anti-CTLA4 and anti-PD1) by reprogramming tumor-associated macrophages, however, CSF1 blockade also upregulated NOS2/iNOS[27]. Another recent study has also shown that the checkpoint regulator VISTA is involved in suppression of B-cell response, and a combination of iNOS and VISTA inhibition was necessary to completely eliminate the MDSC-mediated suppression.[28] Although the literature on the role of iNOS in checkpoint blockade-related immunosuppressive mechanisms is new and sparse, we believe that further research in this area is warranted and may lead to effective iNOS-based interventions that enhance immune checkpoint therapies.

Conclusions

The multifaceted role of iNOS has been recognized in cancer, including potentially important functions in immune suppression. Evidence suggests that abrogation of immune suppression mediated by iNOS-produced NO may result in major improvements and combination therapies that include iNOS inhibition

could overcome some of the limitations of currently available T cell-based therapies. Furthermore, local production of NO alters the tumor microenvironment and may lead to resistance to checkpoint blockade, implying potentially effective iNOS-based therapeutic interventions. However, further research is needed to determine when and how iNOS inhibition can be applied to increase efficacy of immunotherapies.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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