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Original contribution

Expression of programmed cell death protein 1 (PD-1) and indoleamine 2,3-dioxygenase (IDO) in the tumor microenvironment and in tumor-draining lymph nodes of breast cancer☆,☆☆

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Summary Programmed cell death protein 1 (PD-1) and indoleamine 2,3-dioxygenase (IDO) are both immunosuppressive proteins. Here, we investigated the relationship between PD-1 and IDO in the tumor microenvironment (TME) and in tumor-draining lymph nodes (TDLNs) in breast cancer patients. First, the protein and mRNA expression levels of PD-1 and IDO in 20 frozen tissues were examined using Western blotting and real-time polymerase chain reaction. Second, 151 paraffin-embedded breast samples and 52 lymph node samples were analyzed by immunohistochemistry. Third, correlation and survival data for PD-1 and IDO in 963 breast tumor patients were mined using the cBio Cancer Genomics Portal. We found that the protein expression level of IDO was significantly increased in frozen tumor tissues ($P = .005$). From paraffin-embedded samples in the TME, PD-1$^+$ cells were only located in the stroma, while IDO was expressed in myoepithelial, stromal, and tumor cells. PD-1 and stromal IDO in the TME showed increased expression in tumors ($P < .001$ and $P < .001$, respectively). In TDLNs, PD-1$^+$ cells were primarily located in the germinal centers (GCs), and IDO$^+$ cells were primarily located in the paracortex. Normal lymph nodes expressed PD-1 and IDO at the same level as non-metastatic and metastatic lymph nodes ($P = .151$ and $P = .812$, respectively). According to cBioPortal, the correlation analysis showed that IDO and PD-1 had high correlation coefficients ($r = 0.83$). These findings suggest that there is a positive correlation between the expression of PD-1 and IDO and that blocking both PD-1 and IDO pathways may represent an attractive therapeutic strategy in breast cancer treatment.

Abbreviations: PD-1, programmed cell death protein 1; IDO, indoleamine 2,3-dioxygenase; TME, tumor microenvironment; TDLNs, tumor-draining lymph nodes; APCs, antigen-presenting cells; TNBC, triple-negative breast cancer; NAC, neoadjuvant chemotherapy; GCs, germinal centers.

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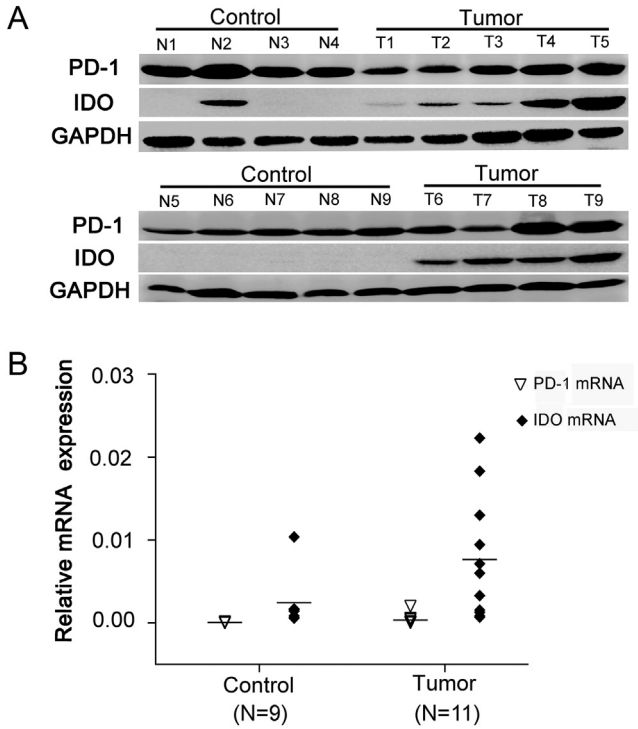

Fig. 1 The protein and mRNA levels of PD-1 and IDO in clinical frozen samples. A, The protein expression levels of PD-1 and IDO in tumor tissues (T) and normal tissues (N) were analyzed using Western blotting ($P = .458$ and $P = .005$, respectively). B, Scatter plots of the relative expression of PD-1 and IDO mRNA in tumor tissues and adjacent tissues ($P = .126$ and $P = .078$, respectively).

1. Introduction

Breast cancer is the most common malignancy in females [1]. Although the diagnosis and treatment of breast cancer have improved, effectively controlling tumor progression is still a problem. Cancer immunotherapy, following traditional treatments, such as surgery, radiation and chemotherapy, has shown promising results in cancer patients and was selected by *Science* as the most significant breakthrough of 2013 [2]. However, immunotherapy targeting a single checkpoint is limited, reflecting the rapid development of resistance. Therefore, developing combination therapies to improve the therapeutic effect of tumor control is a research topic with high priority.

Programmed cell death protein 1 (PD-1) is a cell surface receptor expressed on effector T cells [3], Tregs, activated B cells and natural killer cells [4]. Activated T cells express PD-1 on their surface, and PD-1 binding to its ligands limits effector T-cell activity [3]. Anti–PD-1 antibodies interfere with ligand binding and, thus, reactive T cells. Two PD-1 inhibitors, nivolumab and pembrolizumab [5], have been approved by the FDA to treat a variety of tumors, such as advanced melanoma, squamous non–small cell lung cancer [6]. However, targeting the PD-1 pathway alone does not result in the complete restoration of T-cell function, and in some cancers, targeting PD-1 does not restore T-cell function at all [7]. Some scientists speculated that PD-1 was expressed on T cells and that blocking these pathways can enhance T cells after activation but cannot affect the underlying nature of antigen-presenting cells (APCs), which initially present the antigen. If these tumor-associated APCs do not effectively cross-present tumor antigens, then T cells may never become activated [8]. Thus, identifying other molecules and inhibitory pathways involved in the immunosuppression of APCs is of great importance.

Indoleamine 2,3-dioxygenase (IDO) is a cytoplasmic enzyme that catabolizes tryptophan to kynurenine. A vital function of IDO is preventing the rejection of allogeneic fetuses during pregnancy [9]. A variety of cells express IDO, including dendritic cells, macrophages, NK cells [10] and certain tumor cells. Increasing the activity of IDO will decrease the concentration of tryptophan. T cells are highly sensitive to tryptophan shortage; therefore, the deprivation of tryptophan ultimately impairs T-cell proliferation. In addition, downstream toxic metabolites of IDO can bias dendritic cells (DCs) and macrophages toward an immunosuppressive phenotype [11]. In summary, IDO up-regulation not only deactivates the T-cell–mediated immune response but also inhibits the APC-mediated immune reaction by affecting the phenotype of APCs, ultimately changing the tumor milieu from immunogenic to tolerogenic. Thus, IDO has become a new target of immunotherapy in recent years.

Although numerous studies have shown that the strong expression of PD-1 or IDO alone is associated with a poor