HIF-2α/ITPR1 axis: A new saboteur of NK-mediated lysis

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We recently investigated the role of von Hippel-Lindau (VHL) mutation and the subsequent induction of hypoxia-inducible factor 2α (HIF-2α) in the regulation of renal cell carcinoma (RCC) susceptibility to natural killer (NK) cell-mediated killing. We demonstrated that the resistance of VHL-mutated RCC cell line 786-0 to NK-mediated lysis requires HIF-2α and ITPR1, a direct novel target of HIF-2α, through the activation of autophagy in target cells by NK-derived signals.

Introduction

The majority of renal cancers present with clear cell carcinoma histology usually associated with mutational or functional inactivation of the von Hippel-Lindau (VHL) gene.¹ The pVHL has many functions out of which the best recognized is the pVHL ability to target the hypoxia-inducible factor (HIF) family of transcription factors for ubiquitin-mediated degradation via the proteasome. VHL inactivation may result in stabilization of HIF-1α and HIF-2α or in a selective stabilization of HIF-2α, both involved in an unfavorable tumor microenvironment. Accumulating evidence indicates that HIF-2α is a critical factor in RCC progression.²,³

Recent studies suggest that immunotherapy may be an effective approach for patients with RCC and data from preclinical and clinical studies with donor-derived alloreactive NK cells has sparked interest in the possibility of exploiting the antitumor effect of NK cells.⁴ The activation status of NK cells has been reported as a reason for the lack of clinical response⁵ and evidence clearly indicates that NK cell activation is not sufficient to kill tumor cells due to complex interactions with the tumor and its inherent features. The major challenge for an effective NK-based immunotherapy is therefore to overcome the mechanisms of tumor cell resistance toward NK cells. In this regard, the molecular events involved in the susceptibility of RCC cells to natural cytotoxic effectors should be taken into consideration. Although several reports have established a link between hypoxia-induced HIF-1α and tumor cell resistance to immune effectors,⁶ the specific role of VHL mutations and selective activation of HIF-2α in modulating RCC susceptibility to cytotoxic immune effector cells remains unknown.

ITPR1, a novel target of HIF-2α, protects RCC cells from NK-mediated lysis by inducing NK-mediated autophagy

Using 786-0 cells, a VHL-mutated-RCC cell line stabilizing only HIF-2α, we first demonstrated that targeting HIF-2α in 786-0 cells attenuated their natural resistance to NK-mediated lysis, suggesting a critical role of HIF-2α associated with VHL mutations in the acquisition of resistance to cytotoxicity. Transcriptional analysis showed that among the differentially expressed genes in HIF-2α targeted 786-0 cells, inositol triphosphate receptor 1 (ITPR1) was found to be the most prominent gene involved in the regulation of RCC resistance to NK-mediated lysis. Our study indicated that ITPR1 is a novel target of HIF-2α. ITPR1 has been shown to be involved in the control of intracellular calcium signaling and the regulation of autophagy.⁷ Ca²⁺ accumulation was, however, found to be unchanged in 786-0 cells regardless of ITPR1 expression, ruling out a role for ITPR1 in global calcium accumulation in response to NK effectors. To check whether autophagy was involved in the acquisition of resistance to NK by 786-0 cells, we initially examined the effect of autophagy modulators including serum starvation and hydroxychloroquine on 786-0 cells transfected (or not) with anti-HIF-2α or anti-ITPR1 siRNAs. Irrespective of knockdown, no difference in the expression of autophagy markers was observed under these conditions.

Since it has been recently demonstrated that NK cells can induce autophagy in target cancer cells thereby promoting tumor cell survival and resistance,⁸ we evaluated whether NK cells induced autophagy in 786-0 cells. Interestingly, NK cells were able to induce the autophagic process in control 786-0 cells but not in ITPR1 knockdown 786-0 cells. This suggests an involvement of ITPR1 in NK-induced autophagy in VHL-mutated tumor target cells. Our results also strongly support that NK-mediated and ITPR1-dependent autophagy was associated with a decreased...
activity of NK-derived granzyme B. In vivo data using mouse renal carcinoma cells (Renca cells) revealed that ITPR1 targeting in tumor cells combined with NK depletion significantly enhanced tumor growth, strengthening the involvement of ITPR1 in regulating the in vivo susceptibility of renal carcinoma cells to NK activity. Taken together, these data support a putative role for ITPR1 in tumor progression and immune resistance and suggest that the HIF-2α/ITPR1 axis, triggered by VHL mutations in RCC, may play a critical role in controlling the switch from antitumor immunity to tumor growth and immune escape (Fig. 1).

Renal cell carcinomas: from biology to successful immunotherapy
Renal cell carcinomas are characterized by their important angiogenic potential and resistance to conventional therapies. They are usually associated with VHL inactivation leading to an increased level of hypoxia inducible factors and their target genes. In this study, HIF-2α appears to play a major role in enhancement of tumor escape from NK cells surveillance through the regulation of ITPR1 expression. A better understanding of the molecular basis of hypoxia and angiogenesis in renal carcinoma have permitted the development of targeted therapies, inhibiting multiple HIF-related pathways, such as mTOR and VEGF signaling pathways.4 Targeted therapies have undoubtedly improved clinical outcome but most patients eventually relapse and develop progressive disease. More recently, the identification of PD-L1 (CD274) as an immune modulator expressed in RCC has led to the development of a number of promising cancer immunotherapies that target the interaction between PD-L1 and its receptor programmed cell death 1 (PDCD1, better known as PD-1).10 It is conceivable that these therapies could be expected to be more effective if they were associated with HIF factor targeting. In this regard, future protocols of immunotherapy should integrate the intrinsic features of tumor cells (i.e., HIF-2α triggered by inactivating VHL mutations) and their cross-talk with tumor microenvironment to achieve complete remission and durable responses in RCC patients.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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