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Cytotoxic T cells – Stroma interactions

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Abstract. The tumor microenvironment is a complex system playing an important role in tumor development and progression. Besides tumor cells, the tumor microenvironment harbours a variety of host-derived cells, such as endothelial cells, fibroblasts, innate and adaptive immune cells, as well as extracellular matrix (ECM) fibers, cytokines, and other mediators. This review discusses the potential role of hypoxia and endothelial cells within tumor microenvironment and emphasizes their interaction with antigen specific killer cells. ▲

Key words: hypoxia, STAT3, HIF-1α, endothelial cells, tumor cells, CTLs

Introduction

Hypoxia is a common feature of solid tumors and one of the hallmarks of tumor microenvironment [1]. In a solid tumor microenvironment, a gradient of oxygen exists which extends from well-oxygenated areas to hypoxic areas and finally the necrotic areas where oxygen concentration falls to zero [2].

Tumor cells adapt to hypoxic microenvironment by the regulation of hypoxia inducible factor family of transcription factors (HIF's). This family is composed of three members namely HIF-1, HIF-2 and HIF-3. HIF-1 and HIF-2 has α and β subunits. HIF-1 is the major player in mediating tumor hypoxic response but HIF-2 is being implicated in participating in this response. HIF-3 is only a negative regulator of HIF-1 and HIF-2. It is well established that intratumoral hypoxia is the main inducer of HIF-1α but other factors like genetic alteration (VHL mutations, etc) may also contribute to the regulation of the stability of HIF-1α [2]. A large body of clinical data shows a positive correlation between increased hypoxic expression of HIF-1α and HIF-2α and patient's mortality [3]. Both HIF-1α and HIF-2α have common target genes as well as their respective target genes. The genes induced by hypoxia dependent HIF-1α and HIF-2α play important roles in regulating different aspects of tumor biology like angiogenesis, cell survival, chemo- and radioresistance, proliferation, invasion and metastasis, pH regulation and metabolism, resistance to immune system and maintenance of cancer stem cells [4, 5].

It is now well established that hypoxic tumor microenvironment favours the emergence of tumor variants with increased metastatic and invasive potential [6]. HIF-1α is believed to play a protective role under hypoxic conditions [7, 8]. Since these tumor variants are resistant to radiotherapy and chemotherapy, one might postulate that the exposure to low-levels of oxygen may lead to adaptive responses allowing tumor cells to escape from immune surveillance. Fink *et al.* reported inhibition of NK cytotoxicity toward liver cell lines under hypoxic conditions [9]. Siemens *et al.* have recently reported that hypoxia contributes to tumor cell shedding of MIC (MHC class I chain-related molecules) through a mechanism involving impaired nitric oxide (NO) signalling [10].

An active and bi-directional molecular cross talk between tumor cells and host cells has profound implications for immunological recognition of tumor cells and the formation of a microenvironment modulating tumor progression [11, 12]. Several reports [13] underscore the contribution of the microenvironment to tumor development and it has become clear that tumors are not merely masses of neoplastic cells, but instead, are complex tissues composed of both non-cellular (matrix proteins) and cellular components (tumor-associated fibroblasts, capillary-associated cells and inflammatory cells), in addition

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to the ever-evolving neoplastic cells. All of these components might be involved in shaping the interactive and migratory behavior of tumor-infiltrating T lymphocytes with and among tumor cells. In the context of microenvironment complexity and plasticity, tumor cells orchestrate the modification of the microenvironment by attracting or activating many non-tumoral cells, including blood and lymphatic endothelial cells, fibroblasts, bone marrow-derived cells, immune and inflammatory cells [13]. It is now acknowledged that tumor cells and their stroma co-evolve during tumorigenesis and progression. Besides, the critical importance of the recruitment of endothelial cells by a tumor to achieve tumor angiogenesis, leukocyte-endothelial interactions within tumor microvasculature are critical for mounting a host immune response against tumor tissue and for controlling tumor progression [14].

Recent evidences point at endothelial cell implication in the indirect rejection of the tumor by the immune system [15]. In fact, EC express the protein machinery for antigen processing, including proteasome subunits, TAP proteins, and both MHC classes I and II, and can present endogenous peptides to activated T cells. Several studies demonstrated the potential of endothelial cells to cross-present exogenous antigens from apoptotic tumor cells [16-18], or derived from proteins secreted by surrounding cells or donated by live cells interacting with the endothelium [19-21]. Recently, [22] it has been demonstrated that several natural epitopes from an endogenous protein constitutively expressed by endothelial cells, that is vascular endothelial growth factor receptor-2 (VEGFR-2), were presented in both HLA classes I and II [23].These observations clearly sustain the importance of endothelial cells as "target" for the immune system.

Results

Influence of hypoxic stress on antitumor cytotoxic response

Since hypoxia is a common feature of solid tumors and one of the hallmarks of tumor microenvironment, it is of interest to find out whether hypoxia confers tumor resistance to CTL-mediated killing. We have shown [24] that indeed hypoxic exposure of target tumor cells inhibits the CTL clone-induced autologous target cell lysis. Interestingly the observed lysis inhibition was not associated with an alteration of CTL reactivity and tumor cell recognition indicating that tumor-induced priming of the autologous CTL clone was not affected after exposure to hypoxia. We further demonstrated that HIF-1α induction and STAT3 activation are responsible for mediating hypoxia induced lysis inhibition [24]. Our results suggest a new role for hypoxia (HIF and STAT3) in tumor resistance to the immune system. Here, we discuss how these results add an important new facet to our traditional view of hypoxia and cancer.

Role of hypoxia activated STAT3 in modulating antitumor immune response

We show that hypoxia is directly implicated in the acquisition of tumor cell resistance to CTL-mediated lysis *via* HIF induction and STAT3 activation. Furthermore, STAT3 activation within tumor microenvironment is known to be associated with cytokine-induced proliferation, anti-apoptosis and transformation. Moreover, it is now well established that STAT3 modulates the cross talk between tumor and immune cells [25, 26]. More interestingly, we have shown that vascular endothelial growth factor (VEGF) neutralization resulted in the attenuation of hypoxic tumor target resistance to CTL-mediated killing [24]. We have also demonstrated that STAT3 phosphorylation can be stimulated by autocrine signalling through VEGF [24], suggesting that tumor microenvironment through hypoxia-induced VEGF may play a key role in the induction of active form of STAT3. In this regard, it is very likely that STAT3 activation is associated with the regulation of target gene expression potentially involved in the alteration of hypoxic tumor target-specific killing. Therefore, understanding how VEGF and other soluble factors may lead to STAT3 activation *via* the tumor microenvironment may provide a more effective cancer treatment strategy for hypoxic tumors with elevated P-STAT3 levels. This also suggests that reduction of VEGF release in tumor microenvironment may favour induction of a stronger antitumor CTL response against tumors expressing VEGFR. Our studies are in agreement with reports suggesting that inhibition of VEGF may be a valuable adjuvant in the immunotherapy of cancer [27] and indicating a synergy between tumor immunotherapy and anti-angiogenic therapy [28].

The consequence of hypoxic activation of STAT3 extends beyond its critical role in controlling cell survival and apoptosis. This emphasizes that a better