

B7-H3 in cancer - beyond immune regulation

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Abstract

B7-H3, a member of the B7 family of immunoregulatory proteins, is overexpressed in cancers and promotes tumor growth, metastasis and drug resistance. Here, we discuss the involvement of B7-H3 in cancer that goes beyond its immune regulatory function, and discuss the potential of B7-H3 as a biomarker and therapeutic target.

With the successful introduction of checkpoint inhibitors in cancer treatment, the B7 family of proteins is receiving increasing attention. B7 proteins bind to members of the CD28/CTLA-4 family which operate as the costimulatory signal in the activation of T cells. Without this additional stimulation signal, T cell activation may lead to cell death. B7 family members are classified as i) co-stimulatory, ii) co-inhibitory, or dually as iii) co-stimulatory and co-inhibitory, depending on the effects they induce in T-cell activation through CD28 signaling. For instance, B7-H3 related protein, PD-L1/B7-H1, is the inhibitory ligand of PD-1 death receptor in immune cells, leading to reduced T cell proliferation, and this provides the rationale for targeting both proteins in cancer. B7-H3 was initially characterized as a T-cell stimulating protein, but the majority of current studies describe B7-H3 as a T-cell inhibitor that promotes tumor aggressiveness and proliferation. This suggests that B7-H3 may be an important immunological target in cancer [1], and underlies a need for studies that elucidate further its role in immune regulation, including the identification of B7-H3 binding partners. Importantly, B7-H3 is also emerging as a protein that regulates tumor growth, metastasis and drug sensitivity independently of the immune system.

Expression and regulation of B7-H3 in cancer

Expression of B7-H3 mRNA is found in a wide range of normal human tissues, but B7-H3 protein is expressed at low levels, suggesting a tight post-transcriptional regulation. In contrast, the B7-H3 protein is overexpressed in many types of malignancies, and is linked to poor prognosis, increased tumor grade and metastasis, resistance to therapy, and decreased overall survival.

The human B7-H3 protein exists either as a transmembrane or soluble isoform. Transmembrane B7-H3 is mainly found at the surface of tumor cells, but also in

cytoplasmic vesicles and the nucleus. The nuclear localization is associated with poor outcome in colon cancer [2]. High-levels of soluble isoform are detected in the serum of cancer patients [3], which suggests soluble B7-H3 as a potential non-invasive biomarker. Finally, B7-H3 protein has also been found in the secretome, including exosomes and other extracellular vesicles [4].

B7-H3 is also expressed in tumor-associated endothelial cells, and high expression levels are associated with advanced tumor grade. Expression of B7-H3 in the tumor vasculature was recently exploited to improve the accuracy of a breast cancer diagnostic [5]. Preclinical radioimmunotherapy in ovarian cancer using B7-H3 antibodies radiolabeled with ^{212}Pb α -particles target tumor cells and vasculature and showed promising effects with low toxicity [6]. In addition, an antibody targeting B7-H3 conjugated with the DNA alkylating compound Pyrrolbenzodiazepine was shown to target both B7-H3 expressing tumor cells and vasculature [7]. High expression of B7-H3 in tumor vasculature may also contribute to the formation of pre-metastatic niches and facilitate metastasis. Expression of VEGF (vascular endothelial growth factor) in tumor cells was found mediated by sB7-H3 [3], thus combination of anti-B7-H3 and anti-VEGF drugs might be a valuable combinatorial therapeutic approach.

B7-H3 induces pro-oncogenic traits such as cell growth and metastasis

Knocking down expression or inhibiting B7-H3 decreases the growth of many types of cancer cells *in vitro* and *in vivo*. Also, the potential of adhesion, migration, invasion and metastasis of cancer cells is affected by B7-H3 expression levels [3, 6-8]. An increasing number of studies support a pro-oncogenic role for B7-H3 in various types of cancer that is independent of its immune function.

How B7-H3 promotes tumors is not independently of the immune system completely understood, but B7-H3 seems to act upstream to activate signaling routes, such as JAK/STAT and PI3K/Akt pathways, to induce anti-apoptotic and proliferative mechanisms [9, 10]. B7-H3 modulates the expression of cytokines and metalloproteinases involved in metastasis, such as IL-8, MMP-2, TIMP-1 and TIMP-2 through the PI3K/Akt and NF-kappa B pathways (Figure 1). B7-H3 expression was also recently shown to inhibit the transcription factor NRF2, leading to increased reactive oxygenated species (ROS) and HIF1 α levels, thus inducing aerobic glycolysis

leading to tumor growth [11]. If these tumor promoting effects of B7-H3 are due to signaling triggered from the extracellular domain of B7-H3 is yet to be determined.

Since exosomes can travel and fuse to distant cells, cancer-derived exosomal B7-H3 may be transferred to stromal or tumors cells. Increasing B7-H3 signaling in the recipient cells by exosomes could activate the downstream signaling pathways of B7-H3 in the exosome-receiving cell, for example the aforementioned JAK/STAT and PI3K/Akt pathways, and thus induce proliferation, metastasis or resistance to therapy. In this manner, soluble B7-H3 isoforms can also contribute to increased invasion and metastasis capacity.

B7-H3 also promotes resistance to cancer drugs. A growing number of studies show that inhibition or reduced expression of B7-H3 increases the response of tumor cells to drugs that target DNA replication, alkylating agents, and inhibitors of PI3K/Akt/mTOR and Ras/Raf/MEK signaling [4, 6, 9, 10]. This further supports B7-H3 as a target in anticancer therapy, alone or in combination with other existing therapeutic modalities.

Concluding remarks

The differential expression of B7-H3 in tumors *versus* healthy tissues makes targeting of B7-H3 particularly attractive, potentially with limited side effects. The efficacy of inhibiting B7-H3 activity was evaluated in preclinical studies with short hairpin RNAs, RNA interference, or anti-B7-H3 monoclonal antibodies. Inhibition or reduction of B7-H3 protein expression decreased proliferation and glycolysis and increased drug sensitivity in tumor cells. Currently, there are several clinical trials targeting B7-H3 (Table 1). The first results from a clinical trial with enoblituzumab (MGA271; anti-B7-H3 Ab) show, antitumor-properties and increased T cell repertoire with no dose-limiting toxicity and no severe immune-related side effects [12]. Clinical studies combining anti-B7-H3 antibodies with chemotherapy, small molecule inhibitors of PI3K/Akt/mTOR and Raf/MEK pathways, or with immune checkpoint inhibitors, may establish B7-H3 as a therapeutic target in cancer treatment as a synergistic antitumor approach or to enhance immune response respectively.

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Figure legend

FIGURE 1. Schematic overview of B7-H3 signaling in the tumor cell. Much is still unknown of how B7-H3 exerts signaling that induce cell survival and proliferation. However, the displayed pathways have been shown to be involved. B7-H3, present on antigen presenting cells or tumor cells, is activated by soluble or exosomal B7-H3 or by an unknown protein present on T cells or other tumor cells. This activation will, indirectly, activate NF κ B, PI3K/Akt and JAK/STAT3 pathways to induce cell survival and proliferation. B7-H3 activation will also inhibit NRF2, leading to increased ROS and HIF1 α levels, leading to increased glycolysis. sB7-H3: soluble B7-H3. ROS: reactive oxygen species. Green arrow: activation, blue arrow: translocation. Red arrow: inhibition.

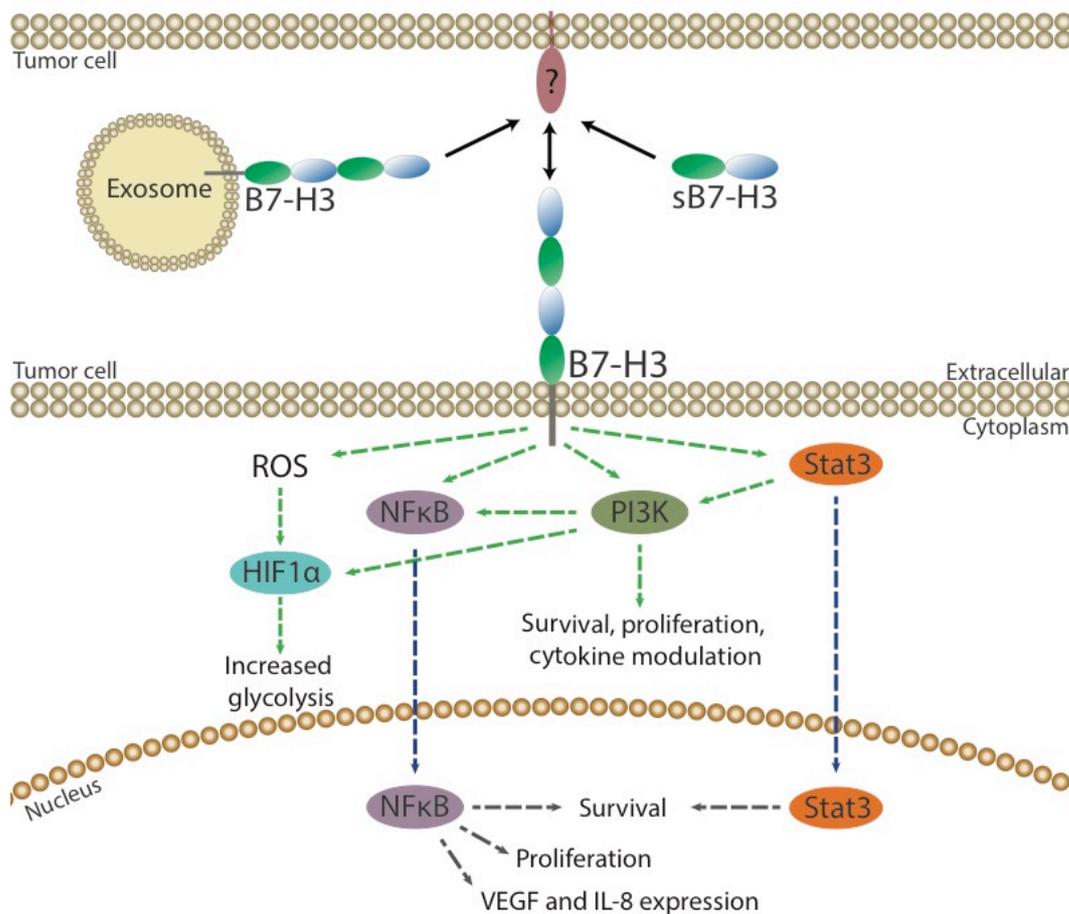


TABLE 1. B7-H3 RELATED PHASE 1 CLINICAL TRIALS			
Identifier #	Agent/drug	Brief trial description	Status and/or Estimated Study Completion Date
NCT02628535	MGD009	B7-H3-expressing tumors	<i>Recruiting</i> December 2020
NCT02982941	Enoblituzumab ¹	Children With B7-H3-expressing Solid Tumors	<i>Recruiting</i> February 2022
NCT01391143	Enoblituzumab	B7-H3-expressing refractory cancer	<i>Recruiting</i> June 2018
NCT02475213	Enoblituzumab +	B7-H3-expressing refractory cancer	<i>Recruiting</i> August 2020
NCT02381314	Enoblituzumab + Ipilimumab	B7-H3-expressing refractory cancer	<i>Recruiting</i> September
NCT0191893	Enoblituzumab	Melanoma	<i>Terminated</i>
NCT02923180	Enoblituzumab	Localized Intermediate and High-Risk Prostate Cancer	<i>Recruiting</i> October 2019
NCT01099644	131 I-8H9	Intraperitoneal Radioimmunotherapy	<i>Recruiting</i> April 2018
NCT00089245	131 I-8H9	Refractory, Recurrent, or Advanced CNS or Leptomeningeal	<i>Recruiting</i> July 2018
NCT01502917	131 I-8H9	Non-Progressive Diffuse Pontine Gliomas Previously Treated With External Beam Radiation Therapy	<i>Recruiting</i> December 2018
NCT00582608	131 I-8H9	CNS cancer, neuroblastoma and Sarcoma. Detection of the cancer.	Terminated Completed
1) Enoblituzumab also known as MGA271 131 I-8H9: Radioactive iodine-labeled monoclonal antibody 8H9			