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B7-H3 and its relevance in cancer; immunological and non-immunological perspectives

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1. ABSTRACT

B7-H3 is a transmembrane glycoprotein and a member of the B7 family of proteins. It was previously known as an immunoregulatory molecule, shown in recent years to be of clinical significance in several different types of cancer. In some tumor types high expression of B7-H3 has been linked to a poor prognosis, whereas in other cancers the opposite effect has been observed. Taken together, the precise role of B7-H3 in tumor immunity is unclear and further investigations are needed. Another aspect of B7-H3 that so far has received little interest is its role in non-immunological systems. We have demonstrated that knockdown of B7-H3 in melanoma and breast cancer cells results in both increased chemosensitivity to several different chemotherapeutic compounds and decreased metastatic potential. This has been observed in both *in vitro* and *in vivo* experiments. Several different signaling pathways seems to be involved, as B7-H3 knockdown can be linked to both higher expression of apoptotic markers and increased phosphorylation of Stat3. Increased knowledge of also the non-immunological role of B7-H3 protein is therefore of great biological and putative therapeutic interest.

2. INTRODUCTION

The B7 family of proteins consists of activating and inhibitory co-stimulatory molecules that positively and negatively regulate immune responses. In this review, we focus on the role of the B7 family member B7-H3, and its function and clinical relevance in cancer. In addition, we demonstrate that B7-H3 has an important role in chemosensitivity and metastasis, independently of the immune system.

3. THE B7 FAMILY MEMBERS

The identification of the B7-CD28-cytotoxic T lymphocyte associated-4 (CTLA-4) pathway provided the basic for understanding costimulatory and coinhibitory signals during T-cell activation (1). During the past decade, seven members of the B7 family have been identified: B7-1, B7-2, B7-H1, B7-DC, B7-H2, B7-H3 and B7-H4. Each member displays distinct, although overlapping, functions controlling the priming, proliferation and maturation of T-cells (2).

Members of the family have been characterized predominantly in humans and mice, but some members are

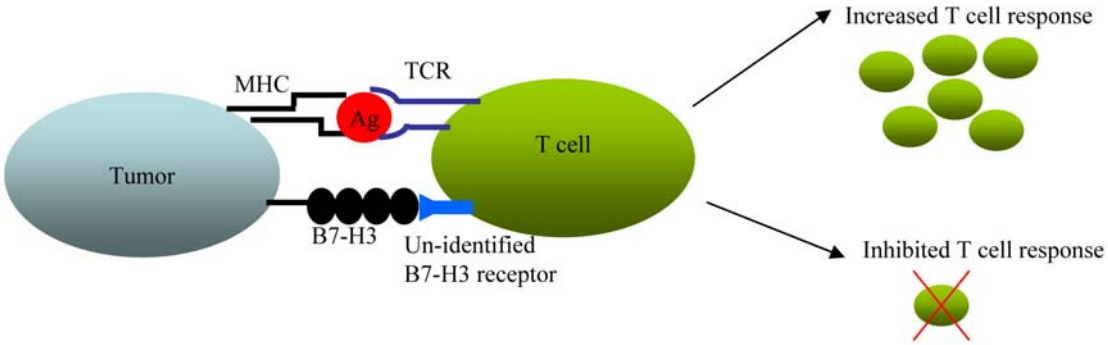

Figure 1. B7-H3 binds to a putative receptor to deliver an inhibitory signal, in addition to binding to its co-stimulatory receptor. The receptor(s) for B7-H3 is unknown.

also found in birds. They share 20-40% amino-acid identity and are structurally related, with the extracellular domain containing tandem domains related to variable and constant immunoglobulin domains (3).

3.1 Characteristics of the B7 family members

The B7 family members are classified into three groups according to their function. Group I include B7-1, B7-2 and B7-H2. B7-1 (CD80) and B7-2 (CD86) are expressed on professional antigen-presenting cells (APCs) and bind to both the costimulatory CD28 and the coinhibitory CTLA-4 molecule on T-cells (1). B7-H2 is expressed on B-cells, macrophages and non-lymphoid tissue. B7-H2 mediates costimulatory signals upon binding to another member of the CD28 family, the inducible costimulator (ICOS) (4-6).

The group II B7 molecules include B7-H1 (PD-L1) and B7-DC (PD-L2) (1). These two molecules are ligands of the programmed cell death-1 (PD-1) receptor (7). B7-H1 is abundantly expressed by various cells and tissue (8-11), while the B7-DC expression is more restricted. The interaction of B7-H1 and B7-DC with PD-1 modulates T cell receptor (TCR) and B cell receptor (BCR) signaling (12).

Group III B7 members include B7-H3 and B7-H4. In mice, the B7-H3 protein has one extracellular IgV-IgC domain, whereas in humans B7-H3 contains tandemly duplicated IgV-IgC-IgV-IgC domains because of exon duplication (13). No functional differences have been observed between these two forms. B7-H3 mRNA expression has been found in many lymphoid and non-lymphoid cells and peripheral organs, like prostate, testis, heart, placenta, liver, pancreas and spleen (14). At the protein level, B7-H3 is found in human liver, bladder, testis, prostate, breast, placenta and lymphoid organs (14). B7-H4 mRNA transcription occurs in several peripheral tissue and in most stromal and haematopoietic cells (15).

4. THE DUAL ROLE OF B7-H3 IN T-CELL ACTIVATION

B7-H3 was initially identified as a T-cell activator (14). Several recent studies in mice, however, indicate that B7-H3 might also have inhibitory functions (Figure 1) (16-18).

4.1 B7-H3 may act as both a T-cell activator and inhibitor

A study from 2001 demonstrated that B7-H3 has an activating effect on human T-cells. In the presence of anti-CD3 antibody, B7-H3 was able to increase proliferation of both the CD4+ and CD8+ T cell populations and increase IFNγ production (14). Consistent with this finding, it has been shown in several mouse cancer models that ectopic expression of B7-H3 leads to activation of tumor specific CTLs that are able to slow tumor growth or even completely eradicate tumors (19-21).

However, most data published so far support the notion that B7-H3 inhibits T cell activation and proliferation (16-18). A study from 2003 showed that T cell responses developing in conditions favouring T_H1 differentiation were enhanced in B7-H3-deficient mice compared with that of wild-type mice (18). This study was supported by another study which demonstrated that B7-H3 regulates not only T_H1-mediated but also T_H2-mediated immune reactions (16). This demonstrates that at least in mice, B7-H3 serves as a negative regulator of T cell activation and function.

5. B7-H3 AND ITS CLINICAL RELEVANCE IN CANCER; AN IMMUNOLOGICAL PERSPECTIVE

In tumor immunity, the precise role of B7-H3 remains unclear. In 2006, a study reported that high expression of B7-H3 in gastric carcinoma correlated to survival time, infiltration depth and tumor histology. The B7-H3 expression was reported to be higher in the patients who survived more than 5 years than in those who survived less than 2 years (22). Also in patients with pancreatic cancer, high tumor B7-H3 levels had significantly better postoperative prognosis than patients with low tumor B7-H3 levels (23).

In sharp contrast, several other studies have reported the negative role of B7-H3 in tumor immunity, and subsequently a poor prognosis (24-29). In non-small-cell lung cancer (NSCLC), the number of T infiltrating

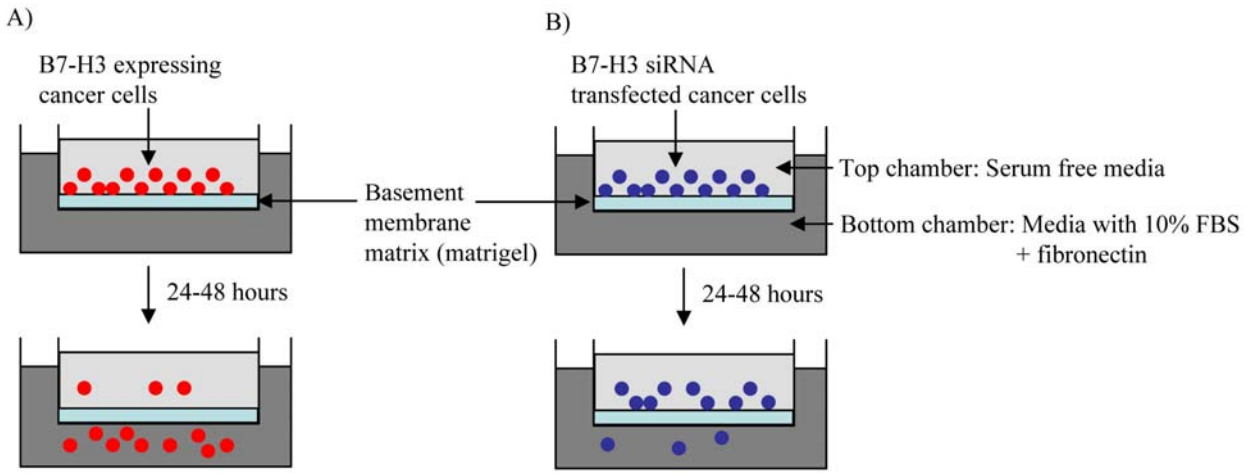

Figure 2. Illustration of a cell invasion assay. Cells migrate from the top chamber through the matrigel to the bottom chamber. siRNA B7-H3 transfected cells (B) demonstrate reduced invasion compared to control cells (A).

lymphoid cells (TILs) in the tumor tissues that express B7-H3 is much lower than those who do not (26). In addition, serum B7-H3 levels in patients with NSCLC was significantly higher than in healthy volunteers, and B7-H3 was believed to contribute to tumor progression (28).

Taken together, the precise role of B7-H3 in tumor immunity is not clear and needs further investigation.

6. B7-H3 AND ITS NON-IMMUNOLOGICAL ROLE IN CANCER

Various tumor-associated antigens (TAAs) are known to regulate cell proliferation, apoptosis, adhesion and tumor metastasis (30-33). The monoclonal antibody (mAb) 376.96 has been used for detection of a wide range of tumor cells from melanoma, glioma, neuroblastoma, osteosarcoma and breast cancer cells (34). We have been using the 376.96 mAb to isolate micrometastatic tumor cells from clinical samples of blood, bone marrow, ascitic and pleural fluid, as well as from disaggregated solid tumor biopsies (34). Although it was indicted that the TAA recognized by 376.96 was a membrane–bound glycoprotein with the size 89-100 kDa, the target antigen was not known. In 2008, we demonstrated through immunoprecipitation and mass spectrometry, that the 376.96 antigen is human 4Ig-B7-H3 (35). Immunoblots of whole cell lysates, subcellular fractionation and tunicamycin treatment of human tumor cells indicated that the 4Ig-B7-H3 is a 100 kDa N-linked glycosylated membrane protein (35).

Since this antigen clearly is expressed in micrometastatic cells, we wanted to study its role in metastasis in general. In 2008, we demonstrated for the first time that downregulation of B7-H3 by siRNA resulted in significant inhibition of cell migration and invasion of cancer cells (Figure 2). These studies were performed in melanoma and breast cancer cells cultured without any lymphoid cells present. This indicates that B7-H3 is involved in cancer progression via non-immunological, but so far unknown mechanisms (35).

Recently, we have been focusing on putative roles of B7-H3 in the sensitivity of cancer cells to chemotherapeutic compounds and the possible underlying mechanisms. We have created stable B7-H3 knock down cancer cell lines from different cancer origins, such as breast, melanoma and colorectal cancer. In *in vitro* assays, we demonstrate that silencing of B7-H3 increases the sensitivity of the cancer cells to different chemotherapeutics. This has also been observed in corresponding *in vivo* models. In the search for the mechanism of action, we have found that the cells lacking B7-H3 are more prone to undergo apoptosis after chemotherapeutic treatment (Liu H *et al.*, submitted). Furthermore, we have seen that B7-H3 seems to be involved in different cell signaling pathways. One of which is the Jak/Stat-3 pathway. Signal transducer and activator of transcription-3 (Stat3) is a point of convergence for numerous signaling pathways, and is known to be activated by numerous cytokines, growth factors and oncogenic proteins. Its target genes are involved in multiple steps of metastasis, including invasion, cell survival, self-renewal, angiogenesis and tumor-cell immune evasion (36). Hence, Stat3 is a target for therapeutic intervention, and a possible link between Stat3 and B7-H3 was therefore of great interest. Our results showed that the level of Stat3 Tyr 705 phosphorylation and its downstream targets Mcl-1 and survivin were sharply decreased in B7-H3 knockdown cells. The phosphorylation of Jak2 was also significantly decreased with B7-H3 knockdown (Liu H *et al.*, submitted).

The metastatic potential of melanoma cells with and without B7-H3 expression is currently under investigation. Our present results demonstrate that mice and rats injected intracardially with the B7-H3 knock down cells survive longer than those injected with control cells.

We are now examining the expression of several genes involved in metastasis in both B7-H3 expressing and non expressing melanoma cells. Hopefully, we will find several interesting candidate genes that at least partly can explain why B7-H3 expressing cells are more metastatic.

If we can demonstrate that B7-H3 is important for both chemoresistance and metastasis in several cell lines of different tumor types both *in vitro* and *in vivo*, it will further strengthen its potential as a possible therapeutic target in cancer.

7. PERSPECTIVES

B7-H3 is a transmembrane glycoprotein and a member of the B7 family of proteins. Several reports demonstrate its importance and its clinical significance in cancer progression, both in immunological and non-immunological systems. So far, most studies claim its role as an immunoregulatory protein. However, B7-H3 is also affecting chemosensitivity and cancer metastasis by interfering with signaling pathways activated in non-immunological systems. Precise understanding of the molecular and biochemical processes of B7-H3 might open up an exciting area for the development of new treatment options for human cancer.

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