Review

Immunoregulatory Molecule B7-H1 (CD274) Contributes to Skin Carcinogenesis

Yujia Cao1, Lu Zhang1, Pacharee Ritprajak3, Fumihiko Tsushima2, Pornpan Yougnak-Piboonratanakit4, Yosuke Kamimura1, Masaaki Hashiguchi1, and Miyuki Azuma1

Abstract

B7-H1 (CD274), a member of the B7 family of coinhibitory molecules, is often induced in human tumors and its expression is closely correlated with a poor prognosis or higher malignancy grade. Tumor-associated B7-H1 is implicated in mechanisms of immune escape. Under inflammatory conditions, B7-H1 is also inducible in normal epithelial cells, but little is known about its involvement in the conversion of normal cells to tumor cells. We recently found that skin-specific expression of B7-H1 accelerates chemically induced carcinogenesis of squamous cell carcinoma (SCC), despite impaired skin inflammatory responses, in B7-H1 transgenic (B7-H1tg) mice. B7-H1tg-derived keratinocytes (KC) and SCCs exhibited a marked reduction of E-cadherin, and B7-H1tg–originated SCCs showed elevated expression of the transcription factors Slug and Twist, suggesting that B7-H1 overexpression in KCs promotes the epithelial–mesenchymal transition and accelerates carcinogenesis. This review discusses the diverse functions of B7-H1 in carcinogenesis and cancer progression, and considers future directions for developing cancer therapy targeting B7-H1.

Introduction

Antigen-specific T-cell responses are controlled by various cosignaling molecules that are responsible for T-cell activation and regulation (1, 2). In particular, 2 coinhibitory receptors, CTLA-4 (CD152) and PD-1 (CD279), act to induce and maintain peripheral tolerance. Two ligands of PD-1, B7-H1 (PD-L1, CD274) and B7-DC (PD-L2, CD273), have been identified. However, their functions are controversial, and both costimulatory and coinhibitory functions in T-cell responses have been reported (1, 2). Nevertheless, most reports strongly suggest that B7-H1 works as a dominant ligand in PD-1–dependent immune suppression. Ligation of PD-1 suppresses effector T-cell function by inhibiting cell proliferation and the production of cytokines such as interleukin 2 (IL-2) and IFN-γ, and by inducing apoptosis or exhaustion.

B7-H1 is expressed on various types of lymphoid cells and is further upregulated upon cell activation. B7-H1 is also found in cells of nonlymphoid tissues, including pancreatic islet cells; smooth muscle cells; endothelial cells in the heart and liver; epithelial cells in the cornea, colon, and skin; and trophoblasts in the placenta, where its expression is induced by inflammatory cytokines such as IFN-γ at local disease sites. Studies using B7-H1–deficient mice or treatment with antagonistic anti-B7-H1 monoclonal antibody (mAb) have suggested that tissue-associated B7-H1 interacts with PD-1 on effector/pathogenic T cells, resulting in T-cell regulation in allotransplantation and autoimmunity. These results suggest that nonlymphoid tissue-associated B7-H1 is involved in the maintenance and induction of peripheral tolerance at local disease sites.

The skin and type II mucosal surfaces, including those of the oral mucosa, cornea, and vagina, are covered by stratified squamous epithelial cells known as keratinocytes (KC), which are important for protection against foreign pathogens and internal and external stimuli (3). Various epithelial stimuli induce KC activation and initiate early local inflammatory responses. Antigen-specific responses are then primed and amplified in secondary lymphoid organs, resulting in the recruitment of effector T cells and subsequent inflammatory responses at the local sites. Persistent inflammation on skin or mucosal surfaces may induce abnormal proliferation and apoptosis of KCs, and, in some cases, atypical changes that promote carcinogenesis. We previously reported that B7-H1 is induced on KCs of the oral mucosa and skin in patients with lichen planus, a chronic inflammatory mucocutaneous disease characterized by massive T-cell infiltration under the epithelium (4), and on KCs of hapten-painted skin in a murine model of contact hypersensitivity (5, 6). The addition of IFN-γ upregulated the level of B7-H1 on cultured KCs, and B7-H1–expressing KCs directly inhibited the proliferative
response and IFN-γ production by cutaneous effector T cells (5, 6). These findings suggest the involvement of B7-H1 in the regulation of local inflammatory responses.

B7-H1 is also expressed in various human solid tumors, including squamous cell carcinomas (SCC) of the lung, esophagus, and head and neck; other types of carcinomas of the colon, ovaries, bladder, and breast; melanomas; and gliomas. Human SCC cell lines also express various levels of B7-H1, and its expression is further upregulated in responses to the common inflammatory cytokines IFN-γ, TNF-α, and IL-1 (5, 7). Tumor-associated B7-H1 has been shown to suppress T-cell effector functions such as cytotoxic activity, proliferation, and cytokine production, and to deplete T cells in tumor microenvironments (7–9). Furthermore, clinicopathologic analyses showed that B7-H1 expression in solid tumors was associated with poor survival, a higher malignancy grade, large tumor size, greater metastasis, a higher recurrence rate, and fewer tumor-infiltrating CD8+ T cells. In ovarian and esophageal cancers and renal cell carcinomas, B7-H1 status has been shown to be independent of prognostic factors. The absence of PD-1 or the blockade of PD-1 or B7-H1 in mice accelerated tumor eradication and inhibited tumor metastasis (7–9). Overall, tumor-associated B7-H1 appears to greatly contribute to tumor escape from immune surveillance.

Key Findings

We initially predicted that B7-H1–mediated immune regulation would have an influence on carcinogenesis because inflammatory mediators, growth factors, and inflammatory cells have been extensively implicated in tumor promotion, invasion, angiogenesis, and metastasis. Based on the aforementioned concept of inflammation-related carcinogenesis, we hypothesized that B7-H1 that is induced on normal epithelial cells in inflammatory conditions may control the transformation of normal cells to malignant cells. Surprisingly, we obtained the opposite result. B7-H1 transgenic (B7-H1tg) mice, in which epidermal KCs overexpress B7-H1 (6, 10), exhibited markedly increased tumor formation in a methylcholanthrene (MCA)-induced skin tumor model (10). B7-H1tg mice showed no skin or type II mucosal abnormalities without stimulation. The intradermal injection of an MCA/olive oil emulsion induced an initial inflammatory response characterized by a rapid proliferation of KCs, abundant infiltration of cells, and production of proinflammatory cytokines such as IL-1, IFN-γ, TNF-α, and IL-6. These inflammatory responses were clearly impaired in B7-H1tg mice, and the anti-inflammatory cytokine IL-10 was inversely enhanced, showing the inhibitory role of KC-associated B7-H1 in skin inflammatory responses. Skin inflammatory responses induced by topical 12-O-tetradecanoylphorbol-13-acetate (TPA) painting were clearly impaired in B7-H1tg skin, and this reduction was reversed by the blockade of B7-H1 or PD-1 using antagonistic mAbs (10). These results indicate that KC-associated B7-H1 lessens skin inflammatory responses in a B7-H1–PD-1 pathway-dependent manner.

Of interest, we found atypical changes consisting of disturbed alignment and chromatin condensation in the basal cells of B7-H1tg skin. This manifestation was observed at an early time point before the recruitment of inflammatory cells. Thus, these changes may be caused by intrinsic changes in B7-H1tg KCs. In support of this finding, the incidence of epidermal tumor formation (e.g., SCCs and basal cell tumors) at 7 weeks after MCA injection was markedly increased, by ~3-fold, in B7-H1tg mice compared with control mice. At this time point, the tumor cell colonies were very small, and no infiltration of T lymphocytes was seen in the tumor environment. An extended observation time revealed that the survival rate at 28 weeks after MCA injection was 3-fold less in B7-H1tg mice compared with control mice. Thus, the differences in the tumor incidence at 7 weeks and the final survival rate at 28 weeks were almost identical, suggesting that the poor survival in B7-H1tg mice was attributable to an accelerated rate of tumor formation.

Our findings of early atypical changes in B7-H1tg basal cells and accelerated SCC formation in B7-H1tg mice prompted us to focus on molecules involved in the epithelial–mesenchymal transition (EMT). Proinflammatory mediators and/or the Snail family of transcriptional repressors downregulate E-cadherin and upregulate N-cadherin, resulting in the promotion of EMT. The accelerated EMT promotes malignant transformation. We found that E-cadherin expression was constitutively downregulated in B7-H1tg KCs, and that the impaired expression was persistent even after cell transformation. The expression levels of the transcription factors Slug and Twist, which promote EMT, were markedly increased after SCC conversion occurred, but these levels were preferentially upregulated in B7-H1tg–derived SCCs (10). These results suggest that B7-H1 overexpression in KCs regulates EMT and promotes skin carcinogenesis by downregulating E-cadherin expression.

Implications

It is not yet understood how B7-H1 induction regulates EMT. The overexpression of B7-H1 may influence some signaling machinery within a cell and cause phenotypic changes before malignant conversion occurs. This process would seem to be PD-1 independent. Inflammatory mediators such as IL-1, cyclooxygenase-2 (COX-2), prostaglandin E2 (PGE2), and TGF-β and its related signaling pathways have been shown to contribute to the modulation of E-cadherin in SCCs (11–13). In a murine model of SCCs, upregulation of the transcription factors Snail and Slug was mediated by activation of the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling cascade (14), and the activation of this pathway is required for transcriptional repression of E-cadherin (15, 16).

Several signaling molecules related to the upregulation of B7-H1 have been reported. In a human lung tumor cell line, IFN-γ upregulated B7-H1 by initiating interferon regulatory factor-1 (IRF-1), a transcription factor with 2 binding sites on the B7-H1 promoter, via the Janus kinase (JAK)/signal transducers and activators or transcription (STAT) pathway (17). The mitogen-activated protein kinase kinase (MEK)/ERK and MyD88/TRAF6 pathways are involved in IFN-γ and
Toll-like receptor (TLR)-mediated expression of B7-H1 in multiple myeloma (18), and TLR4 induces B7-H1 through the MAPK pathways in bladder cancer cells (19). In breast cancer, the expression of B7-H1 is associated with the proliferation marker Ki-67 and cell-cycle progression (20). In the mouse mastocytoma cell line P815, B7-H1–mediated reverse signaling was shown to induce resistance to apoptosis (21). B7-H1 expression increased post-transcriptionally in human glioma after the loss of tumor suppressor gene PTEN and activation of the phosphatidylinositol-3-OH kinase (PI3K)/AKT/mTOR pathway (22). In human lymphomas, MEK/ERK, STAT3, and PI3K, which may play central roles in NPM/ALK-mediated oncogenesis, are differentially involved in B7-H1 expression; for example, activated STAT3 appears to bind to the B7-H1 promoter (23, 24). Consistently, STAT3 activation has been shown to be critical for induction of B7-H1 in human monocyte-derived tolerogenic dendritic cells (25). The ERK/PI3K and ERK/MAPK pathways differentially regulate B7-H1 expression in human dendritic cells (26). The aforementioned signaling molecules and transcription factors (IRF-1, Ki67, PI3K, AKT, mTOR, MEK, MAPK, ERK, and STAT3) have also been shown to be involved in carcinogenesis by regulating the proliferation/cell cycle, apoptosis/survival, and adhesion/migration. Thus, it is possible that B7-H1–inducing signals influence proteins related to EMT and carcinogenesis. Future studies are required to identify the exact link between signals for B7-H1 induction and E-cadherin repression, as well as the mechanisms of regulation of B7-H1 expression by complex interactions between environmental factors and intracellular signaling pathways.
pathways at both the transcriptional and post-transcriptional levels.

**Future Directions**

In healthy tissues, B7-H1 is transiently induced by inflammatory stimuli, and the induced B7-H1 functions as an anti-inflammatory molecule to calm the inflammation. B7-H1 and IFN-γ, which is a key factor in inducing B7-H1, are connected by a regulatory feedback loop (Fig. IA and B). However, continuous or repeated stimulation may cause intrinsic changes within a cell that is expressing B7-H1 (Fig. IC), thus promoting carcinogenesis. Once malignant conversion occurs, tumor-associated B7-H1 may play key roles in immune escape (Fig. ID). The direct involvement of B7-H1–expressing tumors in tumor invasion and metastasis formation will be examined in future studies. In our B7-H1tg mice models, we observed dysregulation of E-cadherin expression, but not PTEN, Cyclin D1, and Bcl-2 expression, between wild-type- and B7-H1tg–derived SCCs. Signaling molecules involved in B7-H1 expression may differ among tissue types and between humans and mice. Our experimental approach has certain limitations. In comparison with mouse tumor models, human tumor development requires much longer periods of time for malignant conversion and tumor progression to occur. During this period, B7-H1 expression and B7-H1–mediated signaling in each tissue are differentially affected by various factors in humans. The results obtained using transgenic expression of B7-H1 may not exactly reflect endogenous B7-H1 expression, because the level of transgenic expression is much higher than that of endogenous expression. Nevertheless, our findings will help to elucidate the induction and role of B7-H1 in malignant cells. Future simultaneous analyses of EMT-related proteins and B7-H1 protein expression at the invasive front of human tumors may reveal the clinical relevance of our findings.

Ongoing clinical trials are assessing the usefulness of agents that block interactions between PD-1 and B7-H1 as anticancer drugs (27, 28). An evaluation report for a phase I trial of a humanized monoclonal anti-PD-1 Ab (MDX-1106) involving 39 patients with advanced treatment-refractory solid tumors showed that immune-related adverse effects, which were often experienced with anti-CTLA-4 (CD152) Ab treatment, were relatively mild, and the treatment resulted in 1 complete response (colorectal cancer) and 2 partial responses (malignancy and renal cell carcinoma) (28). Furthermore, the efficacy of treatment seemed to correlate with tumor cell surface B7-H1 expression. Promising results were also obtained in a similar phase I trial involving 106 patients with metastatic solid tumors, and phase II and phase III trials are now under evaluation. A clinical trial of B7-H1 blockade by humanized anti-B7-H1 mAb (MGX-1105) in patients with advanced or recurrent solid tumors (ClinicalTrials.gov Identifier: NCT00729664) is also underway. These anticancer agents may effectively inhibit B7–H1–PD–1 interactions between PD–1–expressing T cells and B7–H1–expressing tumors or antigen-presenting cells. However, this strategy does not influence intratumoral signaling by B7–H1. The active regulation of B7-H1 expression may be required to prevent B7-H1–induced changes in cells and thereby reduce cellular malignancy. This would necessitate additional therapeutic strategies, such as the use of signaling inhibitors for B7-H1 expression or RNA interference targeting B7-H1. Further studies are needed to fully understand the roles of B7-H1 in carcinogenesis and tumor progression.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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