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Killing tumor cells via their surface β_2M or MHC class I molecules

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Abstract

Targeted antibody-based therapy has been successfully used to treat cancers. Recent studies have shown that tumor cells treated with antibodies specific forβ_2-microglobulin (β_2M) or major histocompatibility complex (MHC) class I undergo apoptosis in vitro and in vivo (mouse models). Antibodies against β_2M or MHC class I induce tumor cell apoptosis via 1) recruiting MHC class I molecules to lipid rafts and activating Lyn and the signal transducing enzyme phospholipase C-γ2–dependent JNK signaling pathway and 2) expelling IL-6 and IGF-1 receptors out of lipid rafts and inhibiting the growth and survival factor-induced activation of PI3K/Akt and ERK pathways. Consequently, mitochondrial integrity is compromised and the caspase-9–dependent cascade is activated in treated tumor cells. However, although β_2M and MHC class I are expressed on normal hematopoietic cells, which is a potential safety concern, the mAbs were selective to tumor cells and did not damage normal cells in vitro and in human-like mouse models. These findings suggest that targeting β_2M or MHC class I by antibodies or other agents offers a potential therapeutic approach for β_2M/MHC class I-expressing malignancies.

Keywords

β_2M; MHC class I; monoclonal antibodies; tumor cell apoptosis; signaling pathways

Introduction

MHC class I molecules consist of a 45-kDa α-chain that contains domains α1, α2, and Ig-like domain α3, and an 11.6-kDa light chain called β_2-microglobulin (β_2M). The α1 and α2 domains of the α-chain are polymorphic. Their polymorphisms frequently occur in three hypervariable regions that form the antigen-binding cleft or peptide-binding region, which is recognized by the T-cell receptor on $CD8^+$ T lymphocytes. Domain α3 contains a conserved seven-amino acid loop that binds with CD8 molecules [1, 2]. β_2M is a non-glycosylated polypeptide composed of 100 amino acids. Its best characterized function is to interact with and stabilize the tertiary structure of the α-chain [3]. Because it is non-covalently associated with the α-chain, it can be exchanged with the circulating form of β_2M, which is present at low levels in serum, urine, and other body fluids under physiological conditions [4].

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Conflict of Interest Disclosures

β_2M/MHC class I molecules are found on almost all normal nucleated cells and on most tumor cells, although the levels of expression may differ among different cells [5]. While some solid tumors express a low density of β_2M/MHC class I molecules on their surface [6, 7] to escape host immune surveillance [8, 9], overexpression of β_2M/MHC class I molecules has also been reported on other tumors, including hematological malignancies [10]. Thus, these molecules are potential targets of antibody-based therapy for β_2M/MHC class I-positive tumors [11, 12].

MHC class I as signaling molecules

MHC class I molecules are important signal-transducing molecules involved in the finely tuned regulation of immune responses. Ligation of MHC class I molecules on T and B cells by immobilized antibodies or secondary cross-linking triggers signal transduction, which is involved in responses ranging from anergy and apoptosis to cell proliferation and IL-2 production [13–17]. Cross-linking MHC class I activates several intracellular signaling pathways, including: 1) phosphorylation of tyrosine kinases leading to a rise in the intracellular free calcium concentration, 2) activation of the JAK/STAT pathway resulting in STAT3 activation, and 3) upregulation of PI3K leading to JNK activation [13–17]. However, it is yet unclear as to which part of MHC class I molecules transmits the signals. The cytoplasmic domain of MHC class I α-chain has a tyrosine 320 residue, which can be phosphorylated and forms a signaling motif. However, previous studies have shown that deletion of all but the four proximal amino acids from the cytoplasmic tail does not alter their signal transduction capabilities [18], and truncated molecules are still able to synergize with CD3, CD2, or CD28 to initiate IL-2 production [19, 20]. On the other hand, others have shown that MHC class I molecules are physically associated with some hormone or growth factor receptors, such as insulin receptor, insulin-like growth factor (IGF) receptor, epidermal growth factor receptor, IL-2 receptor, IL-4 receptor, and glucagon receptors on cell surfaces [21–26], suggesting that MHC class I-induced signaling may be transmitted through these receptors. Taken together, these findings indicate that, in addition to antigen presentation, MHC class I molecules or their components play an important role in the regulation of immune responses via MHC class I-mediated signaling.

MHC class I as an inducer of cell apoptosis

In the past decades, antibodies targeting surface MHC class I molecules on various cell types have been generated and investigated. Genestier reported that two monoclonal antibodies (mAbs; mouse mAb90 and rat YTH862), which bind epitopes of theα1 domain of the α-chain, induce apoptosis in activated, but not resting, T lymphocytes [27] and CD40-activated B lymphocytes [28]. Another mAb, a rat mAb RE2 that bound with α2 domain of the α-chain, induced apoptosis in activated murine lymphocytes that involved caspase cascade and PI3K activation [29]. Others reported that a murine mAb (5H7) specific for the α3 domain, but not other mAbs (TI2599, α3 domain-specific and W6/32, binding with both α2 and α3), could induce growth inhibition and apoptosis of B-cell–derived tumor cell lines [30, 31]. However, secondary cross-linking of the mAb was required, because only plastic-immobilized but not soluble 5H7 mAb were able to kill the cells [30, 31].

Antibodies against surface β_2M are therapeutic against hematological malignancies

Elevated levels of circulating β_2M, present in hematological malignancies such as multiple myeloma [32], lymphomas [33], and leukemias [34], are one of important predictive factors in these cancer patients for indication of aggressive diseases and poor survival prognosis [35]. Previous studies have shown that release of β_2M into surrounding tissues may contribute to

the induction of bone absorption in patients with myeloma [36] by inducing osteoclast formation. In addition, β_2M may induce osteoblast secretion of IL-6 [37] and promote fibroblast production of MMP-1 [38], both of which support tumor growth and bone destruction. These findings indicate that targeting β_2M may help control tumor growth and bone destruction in these malignancies.

Our recent studies showed that anti-β_2M mAbs induce programmed death of hematological malignant cells [39]. We used a commercially available anti-β_2M mAb B2 and mAbs D1 and E6 made in our own laboratory and showed that these mAbs exhibited potent in vitro tumoricidal activity against all 14 of β_2M/MHC class I-expressing multiple myeloma, Burkitt lymphoma, mantle cell lymphoma, T-cell and myelogenous leukemia cell lines, and primary tumor cells isolated from patients with myeloma that were tested. Tumor cell death occurred rapidly, without the need for exogenous immunological effector mechanisms or secondary cross-linking. Although the expression of β_2M on normal hematopoietic cells is a potential safety concern, the mAbs seemed to be selective to tumor-transformed cells and did not induce apoptosis of normal cells, including T and B lymphocytes and $CD34^+$ bone marrow stem cells. Furthermore, the mAbs were able to selectively kill myeloma cells without damaging normal stromal cells in their cocultures. After binding to cell surface, the mAbs mediated internalization and down-modulation of surface β_2M and MHC class I molecules. The mAbs induced cell death via upregulating Bad and Bax protein expression, inducing phosphorylation of Bcl-2, and decreasing phosphorylation of Bad, all of which compromised mitochondrial integrity, leading to cytochrome c release into cytosol and activation of the caspase-9-dependent cascade. Inhibitors to pan-caspases or caspase-9, but not to caspase-8, prevented anti-β_2M mAb-mediated tumor cell apoptosis. Moreover, knockdown of surface β_2M by β_2M-specific siRNAs decreased the expression of MHC class I molecules on tumor cells, and consequently abrogated apoptosis of tumor cells induced by the mAbs. Furthermore, our studies showed that anti-β_2M mAbs are also active and therapeutic in vivo. After subcutaneous or intraperitoneal injections, the mAbs significantly reduced tumor burdens and retarded tumor growth in xenografted SCID mice with human myeloma, lymphoma, and leukemia cell lines and in SCID-hu mice inoculated with primary myeloma cells isolated from patients. These findings indicate that use of anti-β_2M mAbs are a potential novel therapeutic approach to treating hematological malignancies that express surface β_2M/MHC class I molecules.

For future clinical application of the mAbs as a therapeutic agent, a major concern is whether anti-β_2M mAbs will be therapeutic and safe to treat cancer patients in whom every tissue expresses low densities of MHC class I molecules and elevated levels of soluble β_2M are present. To address this concern, we recently developed and used a myeloma-HLA-A2-transgenic NOD/SCID mouse model [40]. The mice are transgenic for HLA-A2 α-chain but not human β_2M. However, with established myeloma, all murine tissues express human HLA-A2 and β_2M, and high levels of circulating human β_2M, which are seen in most of myeloma patients, were detected, indicating that myeloma-derived human β_2M form mature MHC class I molecules with the HLA-A2 α-chain on murine cells. Moreover, the human MHC class I molecules on murine cells are functional. We found that anti-β_2M mAbs effectively suppress myeloma growth in these mice, activate caspase-9 and -3, and induce myeloma cell apoptosis in vivo. Although the mAbs can be detected on different organs including the heart, lung, spleen, liver, and kidney, no tissue damage or cell apoptosis or associated caspase activation was observed in the mice [40]. Moreover, treatment with anti-β_2M mAbs did not change the body weight of the mice or impair the implanted human bones in SCID-hu mice [39].

We also evaluated whether the therapeutic effects of the mAbs would be compromised by β_2M/MHC class I-expressing normal cells using in vitro cocultures, which contains

myeloma cells and 5-fold more numbers of β_2M/MHC class I-expressing normal peripheral blood mononuclear cells (PBMCs). We found that anti-β_2M mAbs killed myeloma cells with the same efficiency irrespectively whether they were surrounded and outnumbered by normal cells expressing human β_2M/MHC class I. In addition, although the bone marrow stromal cells such as osteoclasts promote tumor growth and protect myeloma cells from apoptosis in vitro, mAb-induced myeloma cell apoptosis was not protected in the cocultures with osteoclasts [39]. Furthermore, addition of higher molar concentrations of solubleβ_2M (50–100 μg/ml), which is 3- to 10-fold higher that those in patients with myeloma, in the culture of myeloma cells did not abrogate the apoptosis-inducing effects of the mAbs on myeloma [39]. These findings strongly suggest that anti-β_2M mAbs may be efficacious and that their therapeutic effects in cancer patients may not be compromised by tissue-expressed and soluble β_2M.

We have carefully examined the potential toxicity of anti-β_2M mAbs on normal hematopoietic cells. Normal PBMCs, resting and activated $CD3^+$ T cells and $CD19^+$ B cells, $CD16^+$ NK cells, and bone marrow $CD34^+$ stem cells were treated with the mAbs and were found to be resistant to the mAb-induced apoptosis [39]. However, Smith et al. reported that treatment with 5H7, a mAb specific for the α3 domain of MHC class I molecules, caused suppression of T cell-mediated cellular immunity in vivo [31]. To investigate the possibility that anti-β_2M mAbs would trigger the potential immunological consequences by binding to and blocking MHC class I on normal cells, we examined whether the mAbs could induce NK cells to kill normal cells coated with the mAbs in vitro and in vivo. The results showed that NK cells did not lyse PBMCs pretreated with or without the mAb or IgG1. However, these NK cells efficiently killed K562 cells. Furthermore, no cell apoptosis or tissue damage were observed in the human bone marrow tissue, after immunohistochemical examination of the human marrow cells from SCID-hu mice in which anti-β_2M mAb and subsequently purified human NK cells were injected directly into the implanted human bones [39]. Collectively, these in vitro and in vivo toxicity data using humanized mouse models provide strong evidence that targeting β_2M using monoclonal antibodies will have limited direct toxicity if this approach were translated into a therapeutic strategy despite the ubiquitous expression of β_2M and class I MHC on the majority of tissues. However, there is a possibility that by blocking β_2M or MHC class I molecules, these mAbs could inhibit MHC class I antigen presentation and induce immunosuppression. This possibility needs to be investigated.

Similar results are reported from other mAb-based therapy for cancers, such as anti-epidermal growth factor receptor (EGFR) mAbs, including cetuximab, panitummab, and matuzumab [41, 42]. Anti-EGFR mAbs effectively eradicate tumor cells and are currently used in the treatment of several solid cancers [41]. Like human β_2M/MHC class I molecules, EGFR is widely expressed by normal cells of epithelial, mesenchymal, and neuronal origin [41], and anti-EGFR mAbs still have low toxicity on these normal cells. The reasons might be partially due to overexpression of the antigens in tumor cells [39, 41], since our and other's studies showed that myeloma cells express significantly higher levels of surface β_2M than normal cells [39, 43]. In addition, the ability of mAbs to cross-link β_2M/MHC class I molecules and mAb-mediated intracellular signaling pathways might be different on normal cells, which may have contributed to normal cell-resistance to the mAb-induced apoptosis. Indeed, our studies showed that the mAbs did not recruit MHC class I molecules to lipid raft and activate the downstream apoptotic signaling pathways in normal B cells [39]. Nevertheless, as this is an important issue for future clinical application, further study will be needed to examine the potential toxicity in non-human primates using humanized mAbs against β_2M or MHC class I molecules.

In addition to naturally generated mAbs, Sekimoto and colleagues recently developed a recombinant single-chain Fv diabody (2D7-DB) specific to the α2 domain of HLA-A and showed that 2D7-DB specifically induced myeloma cell death in the bone marrow environment [44]. The diabody rapidly induced Rho-mediated actin aggregation in a caspase-independent death pathway in myeloma cells without damaging normal bone marrow cells. Furthermore, the diabody synergized with interferon-γ and chemotherapy drugs melphalan or bortezomib in killing myeloma cells [44]. Importantly, the single-chain diabody lacks Fc fragment so that it cannot bind or activate immune effector molecules or cells such as complements or NK cells, and will not be able to damage normal cells via mediating CDC or ADCC. Thus, targeting surface β_2M/MHC class I molecules may be a novel therapeutic approach against hematological malignancies.

Antibodies against surface β_2M induce apoptosis in solid tumors

Overexpression of surface β_2M/MHC class I was also detected in some types of solid tumors, including prostate cancer [43], renal cell carcinoma [45, 46], gastrointestinal [47], lung, and breast cancers [48, 49]. Previous studies have shown that increased β_2M expression linked to increased tumor growth and enhanced migration and invasion of breast cancer, lung cancer and renal cell carcinoma [45], and surface β_2M can be regarded as a signaling and growth-promoting factor for prostate cancer and cancer-associated bone metastasis [43,50]. Interestingly, both polyclonal antibodies and mAbs specific for surface β_2M had strong tumoricidal activity in prostate cancer and renal cell carcinoma [51]. Treatment of several renal cell carcinoma lines with anti-β_2M antibodies strongly suppressed the growth and induced apoptosis of these cells in vitro in a dose- and time-dependent manner [52]. In addition, Huang and colleagues demonstrated that the downstream signaling pathways of surface β_2M/MHC class I directly regulate the expression of androgen receptor, a prostate cancer survival factor, and its target gene prostate-specific antigen in prostate cancer [53]. Anti-β_2M antibodies inhibited the growth and induced apoptosis of prostate cancer cells via interrupting β_2M-mediated androgen receptor and prostate-specific antigen expression [53]. These studies indicate that targeting surface β_2M may be a novel and promising therapeutic agent for the treatment of β_2M/MHC class I-positive solid tumors as well.

Ligation of β_2M/MHC class I with antibodies activates apoptotic pathways via lipid rafts in tumor cells

Studies have shown that β_2M/MHC class I molecules possess the ability to regulate receptor-mediated transmembrane signal transduction [11, 54, 55]. Ligation of β_2M/MHC class I molecules with antibodies induced clustering, capping, and internalization of MHC class I molecules and activation of several intracellular signaling pathways in normal cells [13–17]. As a consequence, phosphorylation of tyrosine kinases led to a rise in the concentration of intracellular free calcium, activation of JAK/STAT3 pathway [13], and upregulated PI3K and JNK activities [17]. In tumor cells, ligation of MHC class I molecules with specific antibodies induced apoptotic signaling pathways via stimulating phosphorylation of kinases ASK, MLK3, and MEKK1; upregulating MKK4/7 activity; and activating JNK. In vitro pretreatment of cells with JNK inhibitor could completely abrogate anti-β_2M mAb-induced apoptosis, and injection of JNK inhibitor in vivo reduced mAb-mediated tumoricidal effects on established tumor cells in SCID mouse models. Moreover, anti-β_2M mAbs disrupted IL-6– or IGF-1–stimulated Ras/Raf/ERK1/2 and PI3K/Akt signaling pathways in myeloma cells [39, 55]. Different from Fas/Fas ligand-induced caspase-8 activation, treatment of tumor cells with anti-β_2M mAbs compromised mitochondrial integrity and activated caspase-9-dependent apoptotic cascades [39, 56, 57].

Our studies have also implicated lipid rafts as an important mediator for anti-β_2M mAb-induced apoptosis in tumor cells. Lipid rafts, cholesterol- and glycosphingolipid-enriched dynamic patches in the plasma membrane of cells, organize plasma membrane into functional units. Accumulating evidence has suggested that lipid rafts act as platforms to transduce signals into cells for various functions [58], and are involved in anti-MHC class II and anti-CD20 mAb-induced apoptosis of tumor cells [59, 60]. Under physiological conditions, some MHC class II molecules are found in lipid rafts, and more of the molecules are located within lipid rafts after treatment with anti-MHC class II mAbs. Such relocalization of MHC class II molecules seems to be critical for anti-HLA-DR mAb-mediated tumor cell apoptosis. Nagy and Mooney showed that MHC class II-specific mAb mediated signaling events and pathological changes on tumor cells by recruiting MHC class II molecules into lipid rafts and localizing actin and PKC to the rafts, which in turn activated PKC and its potential targets, leading to tumor cell apoptosis [61]. Different from MHC class II, MHC class I molecules are present outside lipid rafts under physiological situation. However, after treatment with anti-β_2M mAbs, MHC class I molecules on myeloma cells were recruited into lipid rafts, leading to activation of src family tyrosine kinase Lyn and PLC-γ2 [39]. In contrast, neither MHC class I molecules nor Lyn were associated with the lipid rafts in normal B cells treated with or without anti-β_2M mAbs, which could partially explain the selectivity of anti-β_2M mAbs against malignant but not normal cells. In addition, anti-β_2M mAbs also affected the raft distribution of growth factor receptors in tumor cells. It has been shown that growth and survival factors such as IL-6 and IGF-1 stimulate growth signaling and confer protection against chemotherapy drug-induced apoptosis due to translocation of their receptors into lipid rafts [62]. We observed that anti-β_2M mAb binding to surface β_2M/MHC class I molecules not only recruits MHC class I molecules into lipid rafts, but also expels growth factor (IL-6 and IGF-1) receptors out of lipid rafts [56], thereby disrupting their signaling pathways. Myeloma cells treated with methyl-β-cyclodextrin, an agent that disrupts the structure of lipid rafts in cell membrane, were no longer sensitive to anti-β_2M mAb-induced apoptosis. These observations further confirm the association and importance of MHC class I and lipid rafts in anti-β_2M mAb-induced apoptosis in myeloma cells.

Summary and conclusions

Taken together, antibodies against β_2M/MHC class I molecules exhibited tumoricidal activity against several tumor cell lines through surface β_2M/MHC class I molecules and their associated pro-apoptotic signaling pathways in preclinical studies. Targeting β_2M/MHC class I molecules has significant advantages in the treatment of β_2M/MHC class I-expressing tumors, because these mAbs have remarkably strong tumoricidal activities and are effective at killing all β_2M/MHC class I-expressing hematological malignant cells examined without the need for exogenous immunological effector mechanisms. The mAbs are able to kill chemotherapy-refractory myeloma in vitro and more importantly, lead to tumor regression in xenograft mouse models of myeloma and other hematological cancers, without damaging normal cells and tissues. Further, their therapeutic efficacy was not counteracted by the high concentrations of soluble β_2M and tissue-expressing β_2M/MHC class I. Although, the animal models reviewed here suggest that normal cells expressing MHC class I and soluble β_2M do not significantly impair the efficacy of anti-β_2M antibodies, this could still limit application of this therapeutic stragegy in patients that have high levels of circulating β_2M. The potential for immunosuppression due to blockade of surface β_2M or MHC class I molecules requires further study prior to clinical translation of this approach.

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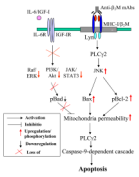

Figure 1.
Anti-β_2M mAb-mediated apoptotic signaling pathways in myeloma cells.