INTRODUCTION

Multiple myeloma (MM) is a plasma cell neoplasm, characterized as malignant plasma cell infiltrating and growing in the bone marrow (BM) and development of a progressive osteolytic bone disease [1]. This disease is one of the most common hematological malignancies among people older than 65 years in the United States and is more prevalent than lymphocytic leukemia, myelocytic leukemia or Hodgkin disease [2]. Estimated by the American Cancer Society, approximately 20,580 new cases were diagnosed and about 10,580 patients died from this disease in 2009 [3]. Although advances in the treatment of MM by new therapeutic agents, such as thalidomide, lenalidomide, and the proteasome inhibitor bortezomib, has been reported to prolong patient survival to 5-7 years over the past decades [4], this disease still remains a largely incurable and fetal, and patients are prone to quickly relapse after high-dose chemotherapy, stem cell transplantation and other novel therapies [4]. Therefore, development of a novel therapeutic approach to eradicate tumor cells is necessary, and will be helpful to improve outcomes of patients with MM.

Application of monoclonal antibodies (mAbs) is one of the successful approaches and has been utilized in current cancer therapy. Although the mechanism of mAb action to initiate and induce tumor cell death is not entirely known so far, it has been proposed that mAbs are able to bind to and cross-link target molecules and subsequently, elicit antibody-dependent cell-mediated cytotoxicity (ADCC) and activate complement-dependent cytotoxicity (CDC), and/or directly induce tumor cell apoptosis [5]. For induction of mAb-mediated ADCC, binding of the Fc portion of mAbs to Fcγ receptors on immune cells is necessary. The immune cells including monocytes, natural killer cells, and granulocytes can destruct mAb-bound tumor cells either by...
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phagocytosis or by release of cytotoxic granules contained in immune effector cells. To induce antibody-mediated CDC, cross-linking of mAbs activates complement cascades, which trigger assembly of membrane attack complex and subsequently, osmotic cell lysis. Moreover, a few of mAbs can directly induce tumor cell apoptosis through transduction of an apoptotic signal to cells, which triggers intracellular apoptotic signaling pathways and cleaves caspase and poly (ADP-ribose) polymerase (PARP), leading to tumor cell apoptosis [5].

Thus far, several mAbs have been successfully used in solid tumors, such as trastuzumab for breast cancer [6]; bevacizumab for renal cell carcinoma and colorectal cancer [7, 8] and cetuximab for squamous-cell carcinoma of the head and neck [9, 10]. Because therapeutic efficacy of mAbs can be achieved at low doses and response can be achieved rapidly, mAbs also have been extensively used in hematological malignances. One successful example is rituximab, a chimeric human-mouse mAb specific for CD20, a cell surface glycoprotein expressed on the majority of B cells. This mAb so far has been used as a frontline therapy for diffuse large B-cell lymphoma and other B-cell tumors [11-13] [14], even though its therapeutic efficacy may vary in individual patients. Derived from rituximab, several novel anti-CD20 mAbs have been developed, such as ofatumumab, ocrelizumab, veltuzumab, GA101, AME-133v and PRO131921 [5, 15]. The potential of their therapeutic efficacy is currently under investigation in preclinical and early clinical studies. Unfortunately, the majority of myeloma patients are not sensitive to anti-CD20 mAb treatment, because only 20% of malignant plasma cells from patients with MM express CD20 [15]. To develop specific and potential therapeutic mAbs for MM, several novel mAbs have been generated recently. In this review, we will focus on mAbs that have been developed in the past years and may become potential therapeutic agents in MM in the near future.

MABS CURRENTLY UNDER INVESTIGATION FOR THEIR THERAPEUTIC ACTIVITY IN MM

Several novel mAbs with strong anti-myeloma activity have been developed over the past years, including but not limited to mAbs specific for CD38 [16, 17], CS1 [18], CD40 [19], CD74 [20, 21], CD70 [22, 23], HM1.24 [24], interleukin (IL)-6 [25, 26], [27], and β2M [28]. The targets of these potential therapeutic mAbs include not only tumor cells alone but also tumor microenvironment, such as the interaction of tumor-BM stromal cells (BMSCs), and the components of BM milieu, including cytokines or chemokines that support myeloma cell growth and survival.

1. MAbs Targeting Tumor Cells

**Anti-CD38 mAbs**

CD38 is a 46-KDa type II transmembrane glycoprotein with a short 20-amino acid N-terminal cytoplasmic tail and a long 256-amino acid extracellular domain. The biological function of CD38 includes receptor-mediated adhesion [29] and signaling transduction and regulation of intracellular calcium mobilization [30]. In MM, malignant plasma cells express relatively high levels of CD38 [31]. Currently, Daratumumab, a human mAb specific for CD38, is in a phase I/II safety and dose finding study for MM therapy [16]. In vitro studies show that Daratumumab is able to effectively kill tumor cells isolated from patients with MM and myeloma-derived cell lines by ADCC and/or CDC [16]. The effects of Daratumumab-mediated ADCC and/or CDC are not affected by the presence of BMSCs. Moreover, Daratumumab has in vivo therapeutic effects in a myeloma xenograft SCID mouse model at low doses [32]. These results indicate that Daratumumab may be a therapeutic mAb with potential for the treatment of CD38-positive MM.

**Anti-CD70 mAbs**

CD70 is a member of tumor necrosis factor (TNF) family. Interaction of CD70 and its ligand CD27 regulates the expansion and differentiation of effector and memory T-cell populations [33] and promotes B-cell expansion, germinal center formation and plasma cell differentiation [34]. CD70 is only transiently expressed on activated B cells, T cells, mature dendritic cells and thymic medulla stromal cells, but it is not expressed in other normal, non-hematopoietic tissues [33]. However, aberrant CD70 expression in tumor cells has been reported in diffuse large B-cell lymphoma, follicular lymphomas, B-cell lymphocytic leukemias, Burkitt and mantle cell lymphomas, Waldenstrom macroglobulinemia, Hodgkin disease Teed-Sternberg cells, and
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MM [22]. SGN-70, a humanized mAb specific for CD70, has been developed recently [23]. SGN-70 exhibits potent anti-myeloma activity in vitro and significantly prolonged the survival of tumor-bearing mice in vivo. This mAb induces Fc-mediated effector functions, such as ADCC, complement fixation and CDC [22].

Anti-CD74 mAbs

CD74 is a HLA-DR (MHC class II) invariant chain, associated with the α and β chains of HLA-DR and plays a role in antigen presentation [35]. It may interact with macrophage migration inhibitory factors that are critical mediators of the host defense and is involved in both acute and chronic response [36]. Recent studies have shown that CD74 is frequently expressed in MM [37]. Malignant plasma cells from 80% of MM patients and from majority of myeloma cell lines express CD74 mRNA and protein [37]. Anti-CD74 mAb, Milatuzumab (hLL1), has been developed and is currently in clinical evaluation for MM therapy [20]. Preclinical studies showed that Milatuzumab has in vitro growth inhibitory effects on myeloma cell lines and in vivo therapeutic effects on established myeloma in SCID mouse models [20]. Preclinical studies showed that Milatuzumab has in vitro growth inhibitory effects on myeloma cell lines and in vivo therapeutic effects on established myeloma in SCID mouse models [20].

2. MAbs Targeting Tumor-BMSC Interaction

Anti-CS1 mAbs

CS1 (CD2 subset 1, CRACC, SLAMF7, CD319 or 19A24) is a cell surface glycoprotein belonging to the immunoglobulin gene superfamily [38]. CS1 mRNA and protein are highly expressed in tumor cells from majority of myeloma cell lines and from more than 97% of patients with MM [39]. Moreover, low levels of circulating CS1 are present in myeloma patient sera [39]. Except activated B, natural killer (NK), CD8+ T cells, and mature dendritic cells with low expression levels, it is not expressed in normal tissues or stem cells [39]. Recent studies have shown that CS1 mediates tumor cell adhesion and supports tumor growth and proliferation via c-maf-mediated interaction of tumor cells with BMSCs in MM [39, 40]. Moreover, it regulates NK cell cytolysis activity via recruiting EWS-activated transcript-2 and activating the PI3K/PLCγ signaling pathways [41, 42]. Currently, a humanized anti-CS1 mAb, Elotuzumab (HuLuc63), has been developed [18]. The studies showed that Elotuzumab significantly inhibits myeloma cell binding to BMSCs and induces ADCC in vitro in a dose-dependent fashion. Administration of Elotuzumab markedly induces tumor regression in myeloma xenograft mouse models [18, 43]. Furthermore, combination of Elotuzumab with bortezomib significantly enhances the in vivo therapeutic efficacy to eradicate patient-derived myeloma cells in a SCID-hu mouse model [44]. This mAb is currently in a phase I clinical trial in relapsed/refractory myeloma.

Anti-CD40 mAbs

CD40 is a transmembrane protein belonging to TNF superfamily [45]. Recent studies have shown that CD40 is essential to mediate a broad variety of immune and inflammatory responses, such as T cell-dependent immunoglobulin class switching, memory B cell development and germinal center formation [46]. CD40 is expressed in many resting cell types but is highly expressed in B and dendritic cells [46]. In MM, CD40 is highly expressed on malignant plasma cells [47]. It binds to its ligand CD154 and induces myeloma cell proliferation and migration via PI3K/Akt/NF-κB signaling pathways [48]. CD40 is also expressed on BMSCs [49]. The interaction of CD40–CD154 increases myeloma growth factor secretion from BMSCs, such as IL-6 and VEGF [50]. Blockade of CD40–CD154 interaction by anti-CD40 mAbs inhibits intracellular signaling pathways in both tumor cells and BMSCs and displays remarkable anti-myeloma activity. Preclinical studies observed that Dacetuzumab (SGN-40), a recently generated humanized anti-CD40 mAb, induces strong tumor cell death in MM via breaking CD40–CD40L interaction and activating NK cell-mediated ADCC [19, 51, 52]. This mAb is currently in a phase I study in patients with advanced MM [51].

Anti-HM1.24 mAbs

HM1.24 (BST2/CD317) was originally identified as a plasma cell-specific antigen [53, 54]. Currently it has been proposed that HM1.24 serves as a target molecule for myeloma therapy [54]. A humanized mAb specific to HM1.24 antigen
has been developed. Injection of the mAb significantly reduces M-protein levels in sera and tumor cell numbers in BM, and prolongs survival of myeloma-bearing mice in myeloma xenograft mouse models [55]. However, its anti-myeloma activity is diminished when the mice are pre-treated with anti-Fc receptor III/II antibodies, indicating that anti-HM1.24 mAb kills myeloma cells via ADCC and/or CDC [56]. In vitro studies validated the mAb action mechanism via NK- and monocyte/macrophage-mediated ADCC [56, 57]. These results suggest the potential application of anti-HM1.24 mAb in MM therapy.

3. MAbs Targeting Components of Bone Marrow Milieu

Anti-IL-6 mAbs

IL-6, mainly secreted from BMSCs, is a major cytokine for myeloma growth, survival, and drug resistance [58]. IL-6 activates several signaling pathways, such as JAK/STAT3, MEK/ERK and PI3K/Akt signaling pathways [59]. In MM, elevated levels of IL-6 and soluble IL-6 receptor are frequently present in the BM plasma and sera of MM patients [60], and are considered as an indicator of poor prognosis [61]. Thus far, several anti-IL-6 mAbs have been generated for MM therapy. Siltuximab (CNTO 328) [25], a chimeric human-mouse neutralizing mAb against IL-6, has demonstrated promising antimyeloma activity in combination with bortezomib [26], dexamethasone [62] and melphalan [25] in preclinical studies. Combination of Siltuximab with chemotherapy drugs enhances the levels of tumor cell apoptosis [25,26,62]. Siltuximab inhibits IL-6-mediated phosphorylation of ERK1/2, STAT1 and STAT3 in myeloma cells [25]. In addition, Siltuximab also inhibits bortezomib-induced increase of myeloid cell leukae mia-1 and heat shock protein-70 [26], both of which may be involved in inducible drug resistance. Other anti-IL-6 mAbs, such as single-chain fragment NRI [63] and humanized mAb 1339 [27], have also demonstrated therapeutic effects on myeloma.

ANTI-β2M MABS AS A NOVEL THERAPEUTIC AGENT IN MM

1. Human β2M and MM

Human β2M, an 11.6-kDa nonglycosylated polypeptide, interacts with and stabilizes the tertiary structure of the MHC class I α-chain [64]. Because it is non-covalently associated with the α-chain and has no direct attachment to the cell membrane, β2M on the cell surface can be exchanged with free β2M present in body fluids under physiologic conditions and in serum-containing medium [65, 66]. β2M is almost exclusively catabolized in the kidney, and 95% to 100% of circulating β2M is eliminated via glomerular filtration, reabsorbed in the tubes, and degraded by proteolytic enzymes. In healthy individuals, the serum concentration of β2M is usually < 2 mg/L and urinary excretion < 400 µg/24 h [67]. Increased synthesis and release of β2M, as indicated by an elevation of serum β2M concentration, occurs in inflammatory, autoimmune and infectious diseases [67]. The stimulation of T- and B-cell antigen or cytokines, such as TNF-α, IL-2, interferon (IFN)-α and IFN-γ, increases cellular β2M release [68].

There is a close association of free β2M with many hematological malignancies, such as MM [69, 70], leukemias [71, 72] and lymphomas [73]. Elevated levels of β2M in serum are present and correlate with a poor patient outcome in MM [70, 74, 75]. In fact, the serum levels of β2M are one of the most important prognostic factors for patients with MM, giving a reliable survival prediction [70, 76]. Moreover, in long-term survivors of MM, an elevated level of β2M is still an unfavorable factor [77, 78]. In vitro studies have shown that primary myeloma cells and myeloma cell lines produce β2M [79]. Myeloma cells are presumed to contribute to the high levels of serum β2M, which is considered a surrogate marker for tumor burdens in myeloma patients. In addition, β2M is also involved in negative regulation of immune system [80] and in activation of bone resorption [81]. These results point to an important yet unidentified role of β2M in the pathogenesis of MM.

2. Mechanism of Anti-β2M mAb Action in Myeloma Cells

Anti-β2M mAbs have been developed recently [28]. The studies showed that anti-β2M mAbs may be a novel agent for MM therapy, because the mAbs have: (1) remarkably strong tumoricidal activities to kill all examined myeloma, including tumor cell lines and primary CD138+ malignant plasma cells isolated from patients with MM, and β2M+/HLA-ABC+ hematological malignant cells; (2) direct induction of tumor
cell death without the need for exogenous immunological effector cells and/or molecules; (3) ability to kill chemotherapy-refractory myeloma; (4) therapeutic efficacy in vivo in xenograft mouse models of myeloma; and (5) effectively kill myeloma cells in the presence of high concentrations of soluble β2M or myeloma cell-surface survival cytokines, such as IL-6 and insulin-like growth factor (IGF)-I, or BMSCs. Although the mechanisms of its action in myeloma cell death need to be further investigated, evidence has shown that anti-β2M mAbs directly induce tumor cell apoptosis without immunological effector mechanisms [28].

Anti-β2M mAb-induced apoptotic signaling pathways in myeloma cells

β2M/MHC class I complex has been shown to serve as an important signal-transducing molecule, which is involved in responses ranging from anergy and apoptosis to cell proliferation and IL-2 production [82, 83]. Earlier studies showed that cross-linking of β2M/MHC class I leads to a rise of intracellular free calcium concentration and activation of STAT3 and/or JNK through phosphorylation of a signaling motif at tyrosine 320 residue in the cytoplasmic domain of MHC class I-α chain [84, 85]. However, previous studies also demonstrate that deletion of all but the four proximal amino acids from the cytoplasmic tail does not alter their signal transduction capabilities [84]. Nevertheless, our studies demonstrate that binding of anti-β2M mAbs to myeloma cells results in internalization and down-modulation of surface β2M/MHC class I molecules, and induction of myeloma cell apoptosis [28]. Knockdown of surface β2M by specific small interference RNAs (siRNAs) significantly abrogates the mAb-induced tumor cell apoptosis. As a result, cross-linking of β2M/MHC class I molecules by the mAbs activates JNK and inhibits PI3K/Akt and ERK, leading to compromised mitochondrial integrity, cytochrome-c release, and activation of the caspase-9 cascade [28]. However, the mechanisms underlying the mAb-mediated binding to and cross-linking of surface β2M/MHC class I molecules, and transduction of apoptotic signals to cells needs further investigation.

Lipid rafts as a linker mediate anti-β2M mAb-induced tumor apoptosis

Lipid rafts, cholesterol- and glycosphingolipid-enriched dynamic patches in the plasma membrane, function to organize plasma membrane into functional units [86] and act as platforms for conducting different signals into cells [87]. Integral proteins in the cellular membrane, such as caveolins and flotillins, can modify lipid rafts structurally and functionally, and may therefore affect subsequent cellular functions [88, 89]. Our studies showed that lipid rafts play an important role in anti-β2M mAb-induced myeloma cell apoptosis (Figure 1). Anti-β2M mAbs bind to surface β2M/MHC class I molecules and recruit them to lipid rafts, leading to MHC class I binding to caveolin-1 and consequently activating the downstream kinases of JNK [28, 90]. On the other hand, stimulation of myeloma cells by IL-6 or IGF-I leads to relocation of their receptors gp130, or IGF-IRβ, respectively, and its substrate IRS-1, to lipid rafts and an increased affinity of receptor binding to caveolin-1, which regulates the structure and function of lipid rafts. Treatment of anti-β2M mAbs excludes gp130, IGF-IRβ and IRS-1 out of lipid rafts in the presence of IL-6 and/or IGF-I and abrogates IL-6 - or IGF-I-mediated JAK/STAT3, PI3K/Akt, and Ras/Raf/ERK pathway signaling [90]. These findings indicate that modification of lipid rafts may enhance the ability of mAbs to induce tumor cell apoptosis.

3. Therapeutic Efficacy and Potential Toxicity of Anti-β2M mAbs

For future clinical application of the mAbs, a major concern is whether anti-β2M mAbs will be therapeutic in patients, in whom every tissue expresses low densities of β2M/MHC class I molecules and elevated levels of soluble β2M are present in the circulation. In vitro studies showed that anti-β2M mAb treatment kill myeloma cells with the same efficiency even though they are surrounded and outnumbered by β2M/MHC class I-expressing normal peripheral blood mononuclear cells (PBMCs) in the cocultures. In addition, cocultures with BMSCs, such as osteoclasts, has no effects to protect myeloma cells from mAb-induced apoptosis [28]. Furthermore, addition of higher molar concentrations of soluble β2M (50–100 µg/mL), which is 3- to 10-fold higher than those in patients with myeloma, in the culture of myeloma cells does not abrogate the mAb effects on tumor apoptosis [28]. To examine the in vivo anti-myeloma effects of the mAbs, a human-like mouse model, i.e., myeloma-HLA-A2-transgenic NOD/SCID mice, has
been generated [91]. The mice are transgenic for HLA-A2 α-chain but not human β2M. However, with established myeloma, myeloma-derived human β2M forms mature MHC class I molecules with the HLA-A2 α-chain on murine cells, and high levels of circulating human β2M were detected. Moreover, the human MHC class I molecules on murine cells were functional. Although there is a concern that the surrounding, β2M/MHC class I-expressing BMSCs would

Figure 1. Anti-β2M mAbs recruit MHC class I into and exclude IL-6R from lipid rafts. Visualization by confocal microscopy shows the distribution and localization of: (A) MHC class I (MHC-I) and (B) IL-6 receptor (IL-6R) in relationship with lipid rafts on myeloma cells ARP-1 treated with 50 µg/mL of anti-β2M mAbs in presence or absence of IL-6 (10 ng/mL) for 1 hour. Cells treated with mouse Ig control served as controls. After the treatment, cells were fixed with 4% paraformaldehyde and stained with anti-MHC class I mAb W6/32 (A), or with anti-gp130 antibody for IL-6R (B), followed by incubation with Alexa Fluor 488-conjugated secondary antibody (green color). Cells were then stained with anti-CTB antibody (red color) for detection of lipid rafts and DAPI staining (blue color) to visualize nuclei. Representative results of three independent experiments are shown.
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Our recent studies showed that IgM anti-β2M mAbs are more potent than IgG mAbs to induce myeloma cell apoptosis, due to IgM mAb pentameric structure of 10 antigenic binding sites with stronger cross-linking capacity (unpublished data). Disruption of IgM pentamers by beta-mercaptoethanol [92, 93] impaired the ability of IgM anti-β2M mAbs to induce tumor apoptosis. This observation indicates that enhancing the cross-linking ability of anti-β2M IgG mAbs or using IgM anti-β2M mAbs may improve the mAb-induced anti-myeloma activity and their therapeutic potential. In addition, combination therapy has demonstrated more efficacy to eradicate tumor cells than monotherapy in MM. Combination of the mAbs with chemotherapy drugs may improve antmyeloma activity of the mAbs. Furthermore, although anti-β2M mAbs induce strong tumor cell apoptosis, other mechanisms, such as ADCC and CDC, may be utilized in vivo by the mAbs. Therefore, it is necessary to examine whether application of ADCC and/or CDC may improve the antitumor activity of the mAbs in MM in future studies.

Nevertheless, the mechanisms why anti-β2M mAbs selectively kill tumor cells but not normal cells need further investigation. One of the potential mechanisms may be partially due to overexpression of the antigens in tumor cells. Our and other studies have shown that myeloma cells express significantly higher levels of surface β2M than normal cells, even though the expression levels of β2M vary in myeloma cell lines or patient samples [28]. Additionally, it is a possibility that the components of MHC class I or class I-like complexes are different in myeloma and normal cells, which warrants future studies to find out which MHC class I or class I-like molecules the mAbs bind to in tumor cells but not in normal cells. Another potential mechanism may involve mAb-mediated signaling transduction. Our studies have shown that the mAbs do not recruit MHC class I molecules to lipid raft and activate the downstream apoptotic signaling pathways in normal B cells [28]. Therefore, resistance to mAb-induced apoptosis in normal cells may be partially due to the inability of the mAbs to cross-link β2M/MHC class I molecules and activate apoptotic signaling pathways in normal cells. Finally, our studies also showed that the mAbs exclude growth receptors out of lipid rafts and inhibit survival signaling pathways [90]. It is possible that growth receptors may be physically associated with β2M/MHC class I molecules and higher levels of these receptors are expressed in tumor cells than in normal cells. Cross-linking of β2M/MHC class I molecules with the mAbs may exclude more growth receptors out of lipid rafts and induce stronger inhibition of growth factor-mediated anti-apoptotic signaling.

CONCLUSION

Advances in molecular biology and tumor biology over the past years have enhanced our understanding of the mechanisms of MM pathogenesis. Therefore, many potential tumor-associated antigens or targets have been identified, which include those expressed in tumor
cells, involving in tumor-BMSC interaction, and in BM microenvironment. Based on these findings, several mAbs, including anti-β2M mAbs, have been developed and are being examined in preclinical and early clinical studies. Most of them showed remarkable anti-myeloma activity. Although these mAbs have the potential to be therapeutic agents for MM therapy in the future, enhancement of the therapeutic efficacy of the mAbs is still necessary. Moreover, to overcome drug resistance and to improve patient outcome, development of more potent and specific mAbs for myeloma cells and myeloma-associated BM microenvironment are still needed.

ACKNOWLEDGEMENTS

Grant support: National Cancer Institute R01 CA96569, R01 CA103978, and R01 CA138402, the Leukemia and Lymphoma Society Translational Research Grants, Multiple Myeloma Research Foundation, Commonwealth Foundation for Cancer Research, and funds from the University Cancer Foundation and the Center for Targeted Therapy of The University of Texas M. D. Anderson Cancer Center (Q. Yi); and National Cancer Institute K99/R00 CA137158, Lymphoma Research Foundation, and American Society of Hematology (J. Yang).

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