

Expression of Surface Molecules on Multiple Myeloma Cells and their Potential Role in Pathogenesis, Prognosis, and Treatment

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Abstract

Multiple myeloma cells express a number of surface molecules capable of activating downstream anti-apoptotic signaling pathways which could favor their growth and survival. Such cell surface molecules have been considered to be prognostic factors for multiple myeloma (MM), as detailed in the following review, and, we speculate, may become possible therapeutic targets in the future.

Introduction

Multiple myeloma (MM) is a hematological malignancy characterized by the clonal proliferation of malignant plasma cells in bone marrow, bone lesions, renal failure and immunodeficiency [1]. MM is responsible for ~1% of all cancers and ~2% of cancer deaths [2,3]. Both biologically based and immune based therapies offer significant opportunities for improved patient outcome [4,5]. However, despite recent advances in treatment, multiple myeloma remains an incurable disease. Accordingly it is recognized that improved knowledge of novel, as-yet unidentified, molecules or pathways that improve our understanding of the development of malignant clones of plasma cells will foster development of new therapies.

For many years a combination of serum levels of β 2-microglobulin (β 2-m), the number of plasma cells in S-phase, cytogenetics, age and patient performance status (PS) have proven to represent the best predictor of survival in MM patients [132,144]. However, newer data has emerged to characterize the phenotype of malignant plasma cells [6,7] and, as discussed below, these novel antigens may represent new candidate prognostic factors for disease outcome.

Markers Associated with Immune Escape and Malignancy

CD200

CD200, initially referred to as OX-2, is a highly conserved, 48 kDa type 1a transmembrane glycoprotein related to the B7 family of costimulatory receptors [8, 9]. The gene encoding CD200 is located on 3q12-q13 in man. Expression of CD200 is transcriptionally dependent on C/EBP- β [10] which regulates IFN- γ , IL-6-, IL-1- and TNF- α -induced responses [11,12,13]. IFN- γ and TNF- α have been shown to induce CD200 expression in a pathway mediated by NF-kappaB, STAT1 and IRF-1 [14].

CD200 is structurally related to the T cell costimulatory molecules CD80 and CD86 and is a member of the immunoglobulin supergene family [15]. The molecule consists of a single (V+C) extracellular IgSF domain, a transmembrane region, and a short cytoplasmic tail lacking signaling motifs [9,16,37]. It is widely expressed by most cells with the exception of skeletal muscle cells [17,18,26,146]. Because CD200 itself lacks signaling domains, interest has focused on the receptor, CD200R, as the primary signaling molecule in this dyad. CD200R is similar in structure to CD200 from which it probably evolved by gene duplication. Although also encoded by gene(s) on chromosome 3 [19,147] its cellular expression pattern is more limited than that for CD200 [17, 20]. CD200R in experimental animals exists in multiple isoforms [21] but

in human the best characterized is CD200R1 which is expressed only on monocyte/myeloid lineage and certain T cells [17,20,22,23,24,27].

Experimental studies have shown that CD200 interacts with its receptors through the IgSF domains [25], resulting in delivery of an immunosuppressive signal which can affect multiple processes including suppressing tumor-specific T cell immunity [22] and inflammatory-type responses in macrophages [29,30]; "switching" of cytokine production profiles from Th1 to Th2 [28]; and induction of regulatory T cells, including Tr1 [21] and CD4⁺25⁺ Treg cells [29].

Given these well-described effects, it is no surprise that overexpression of CD200 has been associated with a poorer outcome in many human hematological [30,31,32] and non-hematological cancers [33,34,35,36]. Despite high-dose chemotherapy and ASCT, MM patients with cells overexpressing CD200 have shorter event free-survival (EFS) when compared with patients whose cells do not (over) express CD200 [7]. In separate studies it has been reported that CD200 positive tumor cells can attenuate the ability of PBMCs to eradicate tumor cells [18].

CD27

CD27, originally described as S152, is a 120-kDa type I transmembrane glycoprotein belonging to the nerve factor/TNF receptor gene family [38]. CD27 is expressed in normal plasma cells, peripheral T cells, and a subset of mature B cells [39]. On binding to its ligand CD70, CD27 induces T dependent B cell immunity [41,43], with differentiation of peripheral blood memory B cells into plasma cells [42], along with proliferation of T cells and efficient T cell priming [61,62,63] with subsequent evidence for interferon c (IFNc)-mediated B-cell depletion [45]. B-cell IgE synthesis activated by IL-10 and IL-4 is inhibited by CD27:CD70 engagement [44]. CD27 has been reported to regulate the differentiation of $\gamma\delta$ T cells, controlling the IFN γ /IL-17 cytokine profiles of peripheral $\gamma\delta$ T cells [40].

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Accepted January 25, 2011; **Published** January 28, 2011

Citation: Kotwica K, Cioch MB, Dmoszynska A (2011) Expression of Surface Molecules on Multiple Myeloma Cells and their Potential Role in Pathogenesis, Prognosis, and Treatment. J Mol Biomark Diagn 2:103. doi:10.4172/2155-9929.1000103

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CD27 is expressed by normal plasma cells (CD19⁺CD38⁺) but its expression is reduced or absent in malignant plasma cells, a process which might have causal implications in progression of disease in MM [46,72]. Thus low expression of CD27 is associated with clinically aggressive cases of multiple myeloma [73,74], and the lack of CD27 expression in plasmacytomas may be a marker for progression to myeloma [75]. Loss of expression of CD27 and overexpression of CD221 (IGF-1R) are reported to be characteristic for aggressive MM [6,76], while higher expression of CD27 is related to complete remission [74].

CD70

CD70, also referred to as CD27L, is a 50 kDa, homodimeric, type II-TNF related transmembrane glycoprotein [47,48,49] which mediates the interaction between B- and T-lymphocytes and enhances the proliferation of activated T lymphocytes [51].

CD70 is present transiently on dendritic cells (DC), and activated B and T cells, but not on resting lymphocytes [52,53,54]. It is constitutively expressed on non hematopoietic cells in the intestine with antigen-presenting capacity (55) and on epithelial cells in the thymic medulla [50] but is absent in most normal tissues. CD70 is, however, overexpressed by a number of transformed cells, both of hematopoietic [57,58] and epithelial origin [56].

CD70 is aberrantly expressed in ~42% of malignant plasma cells derived from BM-aspirates of MM [59] and in 64% of MM cell lines [56]. This overexpression might be associated with the increased proliferation of malignant plasma cells and their shorter overall survival. Interactions between CD27 and CD70 are known to promote the differentiation of peripheral blood memory B cells into plasma cells in association with several cytokines (IL-4, IL-10), CD40:CD40L signalling, and the up-regulation of PRDI-BF1/Blimp-1 [60]. Since CD70 is expressed on activated B and T cells it is possible that loss of CD27 during B cell differentiation in germinal centres (GC) contributes to immune escape and development of malignant clone of plasma cells [46,145].

Interactions between CD27 and CD70 induce apoptosis, following the action of the proapoptotic protein Siva, an intracellular ligand of CD27 which is highly expressed in lymphoid cells [64,65] although absent in multiple myeloma cells [46]. The Siva protein exists as two splice variants, Siva 1 and Siva 2 [66]. Association of Siva and CD27 activates an initiator caspase-8 which in turn activates effector caspases, directly or through a Bid-mitochondrial pathway [66,67]. In the case of Siva 2, caspase activation involves an as yet unknown adaptor protein. Siva-induced apoptosis is accompanied by the translocations of Siva proteins to the nuclear compartment and inhibition of Bcl-XL protein [66].

Siva-induced apoptosis results in proteolytic activation of Bid, allowing its translocation to the outer mitochondrial membrane where it interacts with the pro-apoptotic proteins, Bax and Bak resulting in their oligomerization. This in turn leads to release of cytochrome c to the cytoplasm, activation of the Apaf-1/caspase-9 apoptosome, and activation of caspase-9. Activation of caspase 9 activates caspase-3 which determines the final stages of apoptosis [68,69]. Proteolytic activation of Bid mediated by Siva protein can itself be abrogated by Bcl-2 and Bcl-XL. Forced expression of CD27 in MM cell lines shows a downregulation of Bad protein, which is itself an antagonist of bcl-2 [66].

Interestingly, in addition to this Siva-protein pathway (see Figure1), CD27 together with CD30 and CD40 binds TRAF2, causing activation

of the JNK/SAPK(c-Jun N-terminal kinase/stress-activated protein kinase) pathway which can support Fas-induced apoptosis in MM cells [70,71].

CD40L: CD40 interactions

CD40L is a 32-33 kDa II type transmembrane protein belonging to the TNF-superfamily. It is expressed on the cell surface as one of three splice variants. Two shorter versions of the protein, 18 and 31 kDa respectively retain the ability to form trimers and bind to CD40 and thus can still apparently mediate biological signaling [82,83,84]. Early data suggested that expression of CD40L was associated with activated mature T cells, Th0, Th1 and Th2 cells, CD4⁺ T cells, a subpopulation of CD8⁺ cells, and CD4⁺CD8⁺γδTCR⁺ T cells [77,85]. It is now known also to be present on mast cells, basophils, eosinophils, B cells, NK cells,

PHENOTYPE OF MULTIPLE MYELOMA CELL	
Bone marrow derived malignant PCs	HMCLs
CD138+++	CD138+
CD38+++	CD38+/- (K620,NAN5,U266,XG6)
CD28+	CD28+
CD56+++	CD56+/- (ANBL-6,BCN,JIM3,U266)
CD27+/-	CD27-
CD19-	CD19+/- (JJN3,MDN,U266,NAN2)
CD40++	CD40+
CD70+	CD70+
CD200+/-	CD200+
CD45-	CD45+/- (JJN3,MDN,U266,NAN2,NAN4-6,NCIH929)

Abbreviations: PCs- plasma cells; HMCLs- human myeloma cell lines; +++ - upregulation of the respective CDs present on the myeloma cells In the brackets we cite HMCLs lacking expression of the corresponding surface antigen.

Table 1: Common surface antigens expressed on multiple myeloma cells.

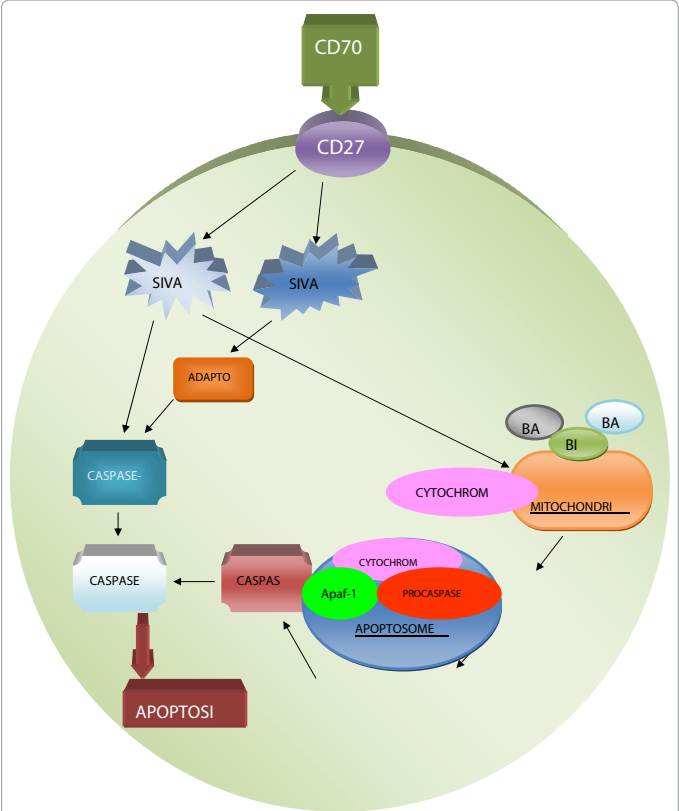


Figure 1: Schematic to show how CD27 might induce apoptosis in bone marrow derived myeloma cells. Given that CD27-CD70 would induce apoptosis in myeloma cells mediated by proapoptotic protein Siva it is speculated that induction of expression of CD27 on bone marrow derived myeloma cells would result in "switching on" the death signal.

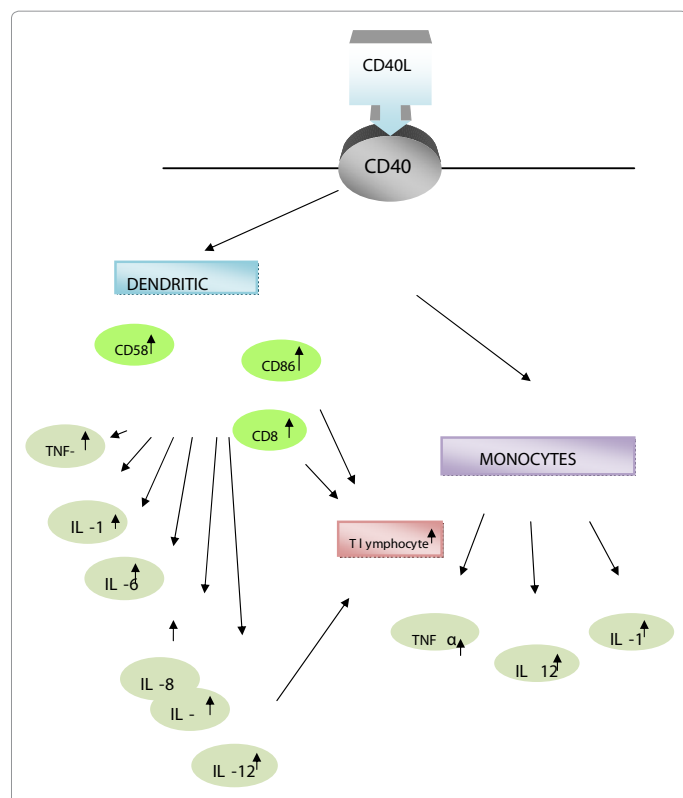


Figure 2: CD40L:CD40 and APCs. Activation of CD40 on APCs results in enhanced survival of DCs and monocytes and secretion of IL-1 α , IL-6, IL-8, IL-10, IL-12 and TNF- α from the corresponding APCs. It also induces maturation of dendritic cells (DCs). CD40L-CD40 alters the phenotype of DCs by upregulating expression of costimulatory molecules including CD54/ICAM-1, CD58/LFA-3, CD80/B7-1. Increased IL-12 secretion and CD80/CD86 expression have an important impact on T cell activation.

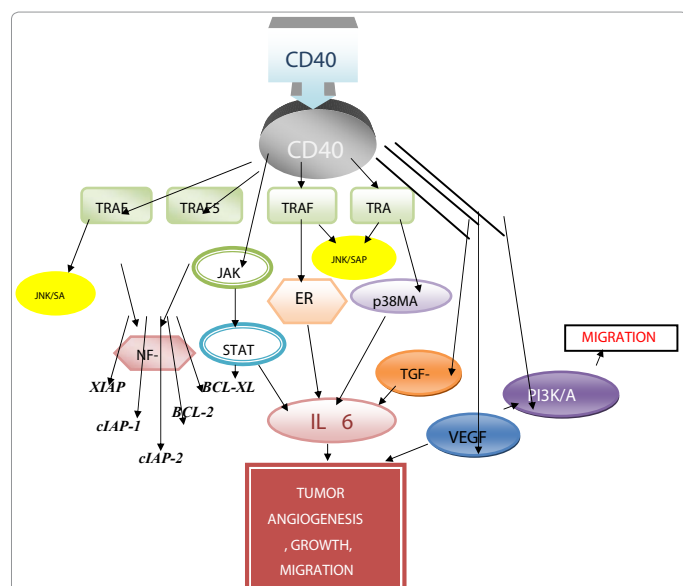


Figure 3: CD40 activated pathways in bone marrow derived myeloma cells. Activation of CD40 on myeloma cells may exert antiapoptotic molecules (mostly belonging to IAP family) and antiapoptotic pathways and lead to enhanced secretion of proinflammatory (IL-6) and proangiogenic (TGF β 1, VEGF) cytokines which are responsible for increased tumor growth, survival, migration and angiogenesis.

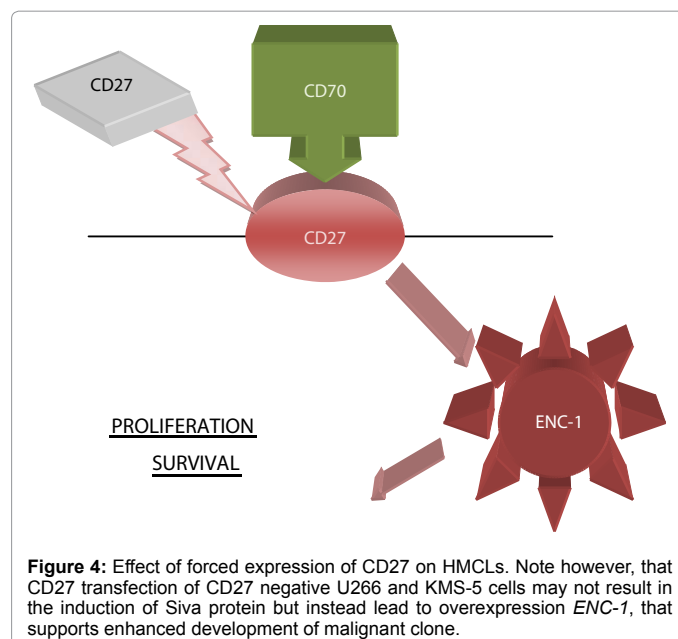


Figure 4: Effect of forced expression of CD27 on HMCLs. Note however, that CD27 transfection of CD27 negative U266 and KMS-5 cells may not result in the induction of Siva protein but instead lead to overexpression *ENC-1*, that supports enhanced development of malignant clone.

monocytes/macrophages and DCs as well as in intracellular stores of thrombocytes, endothelial cells and some megakaryoblastic cell lines [79,80,81,86,87,88]. It has been reported to play a crucial role in the pathogenesis of both chronic inflammatory disease and cancer [89] including MM [90] See Figure1.

CD40, the binding target of CD40L, is a 48 kDa type I-TNF related transmembrane glycoprotein member, which was first identified and characterized on B lymphocytes. CD40 expressed in B cell germinal centres blocks the differentiation of these cells into plasma cells and instead directs development towards memory B cell maturation [91]. CD40 is widely expressed on both haematological and non-haematological cells. Interactions between CD40L and CD40 are known to play a crucial role in regulation of the immune system and thus may play a part in the development of autoimmune diseases [77]. See Figure2.

CD40 is expressed in heterogeneous fashion on >90% of MM cells and > 70% BMSCs (Bone Marrow Stromal Cells) and its expression/activation is correlated with progression of disease [93,94,127]. As shown in Figure 3. below, CD40 induces downstream activation of JAK and STAT proteins following interaction with TRAF2 and TRAF5, TRAF3 and TRAF6 to mediate activation of NF κ B, JNK/SAPK, p38MAPK and ERK signaling [77,111]. Activation of NF- κ B regulates the expression of anti-apoptotic proteins including cIAP-2, XIAP, Survivin, Bcl-2 and Bcl-xL, which play an essential role in the survival of cancer cells [77,111,125]. The JNK/SAPK pathway induced by activation of both TRAF2 and TRAF3 or TRAF6 [77,111] protects from Fas-induced apoptosis [71,112] which is inhibited by IL-6 [113] and mediates proteasome inhibitor PS-341 activity in MM cells [114]. Activation of ERK (extracellular signal-regulated mitogen-activated protein kinase), mediated by TRAF6, results in Sp1 promoter-induced IL-6 transcriptional activation to promote cell growth [124]. Inhibition of TRAF6 function interrupts both CD40 (TNFR family) and IL-1 growth signals, pathways critical to myeloma proliferation [126]. p38 mitogen-activated protein kinase (MAPK) is a member of the MAPK family that induces paracrine MM cell growth, in association with upregulation of IL-6 and VEGF in bone marrow stromal cells [115]. It also leads to the upregulation of Hsp27 (heat-shock protein

27) via MAPKAPK2 (MAP kinase-activated protein kinase 2) and/or p38MAPK- regulated/activated protein kinase (PRAK) [116,117,118, 119,120] that is associated with resistance to dexamethasone (Dex) [121] and bortezomib (proteasome inhibitor PS-341) [98]. Inhibition of p38 activity has been reported both to block CD40-induced gene expression and proliferation [122] and sensitize (or overcome the resistance of) multiple myeloma cells to PS-341 [123]. CD40 also targets PI3K/Akt signaling resulting in migration of MM cells and progressive disease. Urokinase-type plasminogen activator, (uPA), a critical protease mediating tumor invasion and metastasis, which is upregulated in many tumors is a known downstream target of CD40-induced PI3K/AKT/ NF- κ B signaling in MM cells [127].

As shown above, activation of STAT3 mediated by JAK family kinases activity in certain HMCL (U266) leads to overexpression of the anti-apoptotic Bcl family member, Bcl-xL [149]. STAT3 is the major mediator of IL-6 signalling [100,101] and participates in cellular transformation and oncogenesis [102,103]. Activation of STAT3 also plays a key role in IL-6-dependent survival of human myeloma cell lines [104]. Indeed, introduction of a JAK inhibitor causes inhibition of STAT3 activity and sensitizes these cells to Fas-mediated apoptosis [99]. CD40 activation modulates adhesion of multiple myeloma cells to bone marrow stromal cells (BMSC) favoring their survival, growth and drug resistance. The mechanism(s) involved include enhanced adhesion of cells to extracellular matrix proteins and BMSCs, and induction of IL-6, transforming growth factor (TGF)- β 1, vascular endothelial growth factor (VEGF) and insulin-like growth factor-I secretion in BMSCs [105,90,106,107]. IL-6 is an autocrine or paracrine growth factor for MM [92,108,109,110] and inhibition of the secretion of IL-6 in MM cell lines blocks CD40-induced proliferation of those cells [133]. VEGF is key mediator in tumor angiogenesis, growth and migration of MM cells, and its CD40-induced production is p53-dependent and associated with mutation of p53 [107]. Upregulation of TGF- β 1 in multiple myeloma cells induced by CD40 is highly correlated with proliferation and secretion of IL-6 [106].

Cancer-Testis Antigens and their Importance in Treatment of Multiple Myeloma

Cancer testis antigen (CTAg), originally described in patients with malignant melanoma [160], represent a diverse group of genes including more than 40 families [150], expressed in various malignant tumors, testicular (male) germ cells and occasionally placental tissue, but not in normal adult tissues [151,152]. In multiple myeloma cells more than 82 genes encoding CTAg are expressed but only some of them are highly immunogenic and have become especially attractive targets for immunotherapeutic approaches in cancer patients, including multiple myeloma [154,155,156,161]. Previous data have reported on several CTAg expressed in MM, both in samples derived from patients and human myeloma cell lines, belong to the MAGE, GAGE, and SSX families. Expression of these CTags is encoded by genes predominantly located on chromosome X [157,158,162]. Expression of some of these CTAg is present across the entire spectrum of malignant gammopathies, from medullary plasmacytomas through MM to extramedullary plasmacytomas [163].

Commonly expressed cancer testis antigens are capable to induce both antibody-mediated and T-cell-mediated immunity in multiple myeloma (MM) patients [164]. MAGEC1/CT7 (melanoma-associated antigen C1/CT7) and MAGE3/6 (melanoma-associated antigen C3/C6) are two cancer testis antigens commonly expressed in multiple myeloma [159]. *MAGEC1/CT7* seems to be the most frequent gene

overexpressed in multiple myeloma cells derived from patient material [153]. High expression of *MAGEC1/CT7* and *MAGEA3/6* at the mRNA level is associated with advanced stage of MM and at the protein level overexpression of MAGEC1/CT7 and MAGEA3/6 is correlated with an elevated plasma cell proliferation index [159]. Expression of *MAGEC1/CT7* is significantly correlated with the course of multiple myeloma and is strongly associated with an early relapse and reduced survival in patients with MM after alloSCT [158]. Overexpression of *MAGEA3/A6* is positively associated with high level of β 2M (β 2-microglobulin) and shorter EFS (event free survival) [153]. The high immunogenicity of MAGEC1 /CT7 and MAGEA3 in MM results in spontaneous cellular immunity against both of these cancer testis antigens [156, 165], and indeed the immunogenicity of CTags could at least partly be responsible for the good outcome seen in MM after alloBMT (allogeneic bone marrow transplant). Administration of healthy donor PBMCs (peripheral blood mononuclear cells), previously immunized against MAGEA3, to MAGEA3 positive MM patient after alloBMT may support a recipient cellular response without significant affecting the populations of Treg and Th2 lymphocytes [156].

CTAg NY-ESO is a 22 kDa protein expressed in 60% of myeloma patients having a metaphase cytogenetics abnormality (CA) and, like MAGE-3A, is seen more frequently at relapse than at diagnosis. Expression of *NY-ESO-1* at the mRNA level correlates well with protein level in MM. [166,167]. NY-ESO-1 has been reported to be one of the most immunogenic CTags capable of stimulating both humoral antitumor and spontaneous vaccine-induced cellular responses in humans [163,166,168,169,170].

The existence of cellular and humoral immunity to NY-ESO-1 in multiple myeloma and the ability of NY-ESO-1-specific T cells (after expansion) to kill primary NY-ESO-1+ myeloma cells suggests CTags may be a useful immunotherapeutic target [166]. The NY-ESO-1 antibody response is dependent on HTLs, which are also thought to be crucial in priming, expanding and sustaining CD8+ NY-ESO-1 specific T-cell responses. The HTL population also induces expression of MHC class I and II which makes cancer cells superior targets for immunotherapy [180,181,182,183].

Generation of T cells cytotoxic to NY-ESO-1 positive malignant plasma cells may be additionally augmented by transduction of NY-ESO, through its PTD (Protein Transduction Domain) into monocyte-derived dendritic cells [171]. Though it is still unknown if NY-ESO-1 is crucial for the survival of MM PC (multiple myeloma plasma cells) it has been already established that an NY-ESO-1 vaccine may benefit MM patients since it is capable of inducing T cell immune responses and sustaining regression of the disease [184,185,186].

At present a few trials with NY-ESO-1 and MAGE3 vaccines as a supportive treatment in multiple myeloma have been running <http://www.clinicaltrials.gov> but the full reports are not yet in the public domain.

Yet another highly immunogenic CTAg proposed to be a target for immunotherapy in MM is Sp17 (Sperm protein 17) [172]. Expressed at both the mRNA and protein level in multiple myeloma cells, SPA17 is absent in normal bone marrow [173]. Similarly to MAGEA3, immunization with Sp1 can generate both Sp17-specific CD8 positive CTLs from healthy donors able to increase GVM (graft versus myeloma) without GVHD (graft versus host disease) following allogeneic stem cell transplant, as well as tumor-specific CTLs from patients with MM [172,174].

AKAP-4 is a novel CTAg expressed in multiple myeloma plasma

cells, again at both the mRNA and protein level. Existence of an antibody to AKAP-4 in MM patient sera suggests that development of a polyvalent therapeutic vaccine could potentially augment conventional therapy in MM [175,176].

SLLP1 is a unique nonbacteriolytic, c-lysozyme-like CTA_g isolated from human spermatozoa. Expression of the antigen is localized to the acrosome of human spermatozoa and is encoded by the gene *SPACA3* located at *17q11.2* [177]. It may be present in MM cells. Interestingly the presence of antibodies against SLLP1 in the sera of these patients reflects its immunogenicity and development of humoral responses suggests it too may be an attractive target for immunotherapy in MM [178].

Cancer testis antigens belonging to the SSX family comprise four members present on myeloma cells (SSX1,2,4,5). Coexpression of SSX1,2,4,5 in MM is correlated with shorter overall survival, high levels of lactate dehydrogenase, decreased hemoglobin and increased serum creatinine levels [179].

KM-HN-1 is the second most overexpressed CTA_g in multiple myeloma cells derived from patients (seen in ~56% of patients). It is predicted that *KM-NH-1* may be involved in B cell differentiation because it is expressed also by B cells or plasma cells. However the role of this molecule remains unknown [153].

Potential Novel Treatments in MM

Multiple myeloma is a hematological malignancy that despite conventional high-dose chemotherapy with stem cell transplantation is still an incurable disease [128]. Development of novel treatments offer great promise [129,130], as is evident from the discussion (above) regarding CTA_gs in MM, but understanding the molecular pathways which characterize novel gene expression by MM, and contribute to MM growth remains essential to establish a rationale for new therapies [131,132,133,134,135].

MM cells can abrogate the function of critical cells of the immune system by several means. Amongst the negative immune regulators expressed by MM cells is CD200, whose overexpression correlates with poor prognosis of MM patients [7,22]. It might be anticipated that blockade of functional CD200 expression would augment an antigen-specific T cell response and suppress induction of Treg cells, thus sensitizing MM cells to immune-based therapies. Anti-CD200 therapy has already been suggested in the treatment of CLL [131,148]. Evaluation of the activity of two antibodies, anti-CD200-G1 and anti-CD200-G2G4, in a xenograft NOD/SCID human (hu)-mouse model suggested that these reagents showed good antitumor activity for CD200⁺ tumor cells, though apparently their mechanism(s) of action were not the same [18]. To date the value of functional blockade of CD200Rs in tumor therapy has not been described.

As described above, CD27-CD70 interactions can play a dual role in the induction of apoptosis in MM (and other cells). A pro-apoptotic role for the interactions between CD27 and CD70 was confirmed in the presence of simultaneous expression of the pro-apoptotic protein Siva, which mediates caspase-dependent apoptosis. In contrast, an anti-apoptotic activity results from CD27-CD70 interactions in the absence of Siva proteins. Forced expression of CD27 in MM cell lines also contributes to overexpression of ectodermal neural cortex-1 (*Enc-1*), a molecule implicated in lineage differentiation in a variety of cells and which is known to be overexpressed in many other cancers. It is thought that overexpression of Enc1 protein leads to enhanced differentiation of malignant clones while simultaneously inhibiting growth of normal cells [46,134,136] Figure 4.

Based on these observations, introduction of an agonist of CD27 (and/or forced expression of CD27) might be predicted to enhance the immune response and provoke differentiation of malignant clones of MM plasma cells [142]. The crucial molecule for the induction of apoptosis mediated by CD27-CD70 interactions seems to be Siva protein. However, although the loss of expression Siva, both of gene and protein, seems to be a key in the development of MM, forced CD27 expression in MM cell lines did not induce expression of Siva in malignant plasma cells derived from patients [46]. Presumably an as yet unknown mechanism, independent of CD27-CD70 interactions, activates Siva in MM malignant plasma cells, and delineation of this mechanism remains a key to therapies planning to augment apoptosis through this approach. Nevertheless, despite decreased expression of CD27 in MM patients there remains the potential promise of using anti-CD70-antibody therapy to eliminate CD70⁺ tumor cells via antibody mediated cellular cytotoxicity (ADCC), complement fixation, and/or augmented phagocytosis [56,59]. Preclinical data, *in vivo*, using SGN-70, a humanized IgG1 mAb, revealed a significant reduction in the percentage of myeloma cells in the bone marrow and the amount of circulating tumor-derived monoclonal protein in mice, along with prolonged overall survival, support the value of this approach [59].

Interactions between CD40L and CD40 in multiple myeloma cell lines play a dual role in their proliferation, growth and survival [78,92]. CD40 can exert anti-apoptotic events both by stimulating adhesion of MM cells to BMSCs and the extracellular matrix as well as by the secretion of IL-6, VEGF, and IGF-1 by BMSCs [106]. Stimulation of multiple myeloma and bone marrow cocultures by CD40 indeed favors growth and survival of MM cells [90,106,107,137]. In contrast to these effects, activation of CD40 can evoke pro-apoptotic signaling causing growth inhibition [78,113]. CD40 activation on MM patient cell samples correlates with progressive disease [127], and thus one might predict that CD40L-CD40 interactions could be manipulated both to transduce a pro-apoptotic signal to MM cells facilitating their death, and at the same time augment development of therapeutic immunity [138,139]. In support of such an approach, administration of SGN-40, a humanized IgG1 immunoglobulin and a partial agonist of CD40, induced apoptosis and antibody-dependent cellular cytotoxicity against a panel of malignant B cell lines *in vitro* and resulted in tumor regression in MM [133]. The mechanism of SGN-40 apparently relies on either inhibition of IL-6 and VEGF secretion by MM cells and BMSCs, and/or the suppression of anti-apoptotic signaling through Akt and ERK pathways. While SGN-40 alone was able to block AKT signaling in MM cells, simultaneous stimulation of these cells with sCD40L to provide a competitive target binding CD40L eliminated this effect [133]. Thus modulation of CD40L activity might represent a promising therapeutic target to sensitize for apoptosis in cancer cells and indeed studies using a transgenic CD40L revealed a pro-apoptotic activity in MM cell lines associated with enhanced antitumor immune response to those MM cells [141]. It should be noted that the SGN-40 antibody was unable to inhibit all anti-apoptotic signals induced by interactions between CD40L and CD40. Thus it had no documented influence on activation of IGF-1 which is responsible for MM cell growth [133]. This observation has led to the notion that SGN-40 treatment could be used in collaboration with a new, orally bioavailable, small molecule inhibitor of the insulin-like growth factor-1 receptor (IGF-1R), NVP-AEW541, which provokes cell cycle arrest and apoptosis in MM cells. This approach has also been shown to potentiate the action of drugs such as bortezomib, lenalidomide, dexamethasone or melphalan [135,140].

Conclusion

Recent advances in characterizing cell surface antigens expressed

by MM cells has proven valuable not simply for their diagnostic value, but also for what they have taught us concerning the pathways operating with MM cells which contribute to the regulation of their growth and survival. The introduction of monoclonal antibodies for the treatment of human malignancies in general, and MM in particular, is in its infancy, though some success has already been achieved. At present, the discovery of new surface target molecules remains a primary challenge in treatment, including that of MM. Nevertheless, as we have attempted to highlight in this brief review, we believe that improved knowledge of the phenotype of MM cells will revolutionize the way in which we will treat this disease in the future.

Acknowledgements

The authors would like to thank Dr. Reg Gorczynski, University Health Network, Toronto, for his helpful comments during the preparation of this manuscript.

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