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Gpnmb/osteoactivin, an attractive target in cancer immunotherapy

Minireview

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Cancer is a complex disease with interactions between normal and neoplastic cells. Since current therapies for cancer largely rely on drugs or radiation that kill dividing cells or block cell division, these treatments may have severe side effects on normal proliferating cells in patients with cancer. Recently, immunotherapeutic approaches for cancer therapy, by which monoclonal antibodies (Mabs) target tumor specific antigens, have shown great potential. Glycoprotein non-metastatic melanoma protein B (Gpnmb)/Osteoactivin (OA) is a transmembrane glycoprotein highly expressed in various types of cancer. Gpnmb/OA promotes the migration, invasion and metastasis of tumor cells. CR 011-vcMMAE is a Mab-drug conjugate being developed for the treatment of Gpnmb/OA-expressing cancers. Gpnmb/OA represents an attractive target in cancer immunotherapy and CR011-vcMMAE holds promise as a reagent in targeted therapy for Gpnmb/OA-expressing malignancies.

Key words: Glycoprotein non-metastatic melanoma protein B; Osteoactivin; cancer immunotherapy

In targeted cancer therapy, studies have been based on the assumptions that tumor cells carry a set of altered cell surface antigens that are being specific and could be used to boost the immune system for an appreciable immune response and (or) could be used for tumor specific targeted therapy. Ideally, an antigen to be used as a target for therapy would mean that the same should be present and over-expressed exclusively on human cancers but not in normal cells, should induce broad immune response and T-cell recoginition independent of any specific major histocompatibility complex (MHC) restricting component. However, in reality, very few recorded tumor associated antigens or tumor specific antigens that are unique to tumor cells only are known.

There are several reasons for growing interest in the development of Gpnmb/OA-targeted therapies for cancer. First, it is highly expressed at the surface of cancer cells [1], but is predominantly expressed intracellularly in normal cells, such as macrophages or melanocytes [2, 3].This expression pattern makes Gpnmb/OA particularly attractive for antibody-based therapies because, as a target, it would be more readily accessible in cancer cells than in normal cells, thereby reducing potential complications owing to bystander effects. It is also highly expressed in several aggressive cancers, including melanoma (a cancer with high affinity to metastasize to bone) [1], glioma [4, 5] and breast cancer [6]. Finally, ectopic expression of Gpnmb/OA, or its murine orthologue OA, in glioma, hepatocellular carcinoma and breast cancer cells is sufficient to enhance invasive phenotypes in vitro and metastasis capabilities in vivo [4, 6, 7].

Gpnmb, also known as OA [8], dendritic cell-heparin intergrin ligand (DC-HIL) [9] or hematopoetic growth factor inducible neurokinin (HGFIN)-1 type [10], is a type I transmembrane protein [3]. Gpnmb shares the highest amino acid sequence homology with: QNR-71 (responsible for melanin production in quail neuroretina cells) (48%); a melanosome protein Pmel-17/ap100 (25%) and lysosome-associated membrane protein-1 (LAMP-1) (13%) [9]. Gpnmb/OA is expressed on various tumor cells including hepatocellular carcinoma, glioma, melanoma and breast cancer cells and may offer a poteintial target for cancer immunotherapy.

Gpnmb/OA Structure and Homology. Gpnmb, also known as OA, is a type I transmembrane glycoprotein that consists of at least three different domains: an N-terminal domain with a signal peptide, a polycystic kidney disease domain (PKD) domain, and a transmembrane domain (TRD). The protein also contains an Arg–Gly–Asp (RGD) cell attachment domain, for integrin-mediated cell attachment and dspreading. The rat OA protein shares 88% homology with mouse OA, also known as DC-HIL; 77% homology with human HGFIN and human nonmetastatic gene B (nmb); and 65% and 60% homology, with quail neuroretina protein (QNR71) and human Pmel-17/gP100 melanocyte specific protein, respectively. All of these proteins contain all of the domains described for Gpnmb/OA. OA also contains a sorting signal sequence in close proximity to the C-terminal domain. This signal sequence contains di-leucine amino acids with a consensus sequence of EXXPLL, and is located 7 amino acids away from the C-terminal end of the protein. There is another signal sequence located 29 amino acids upstream of the sorting sequence, and this sequence has been suggested to play a role in protein sorting through the rough endoplasmic reticulum and the Golgi complex . This signal sequence contains one tyrosine with a consensus sequence of YXPI. Bioinformatic analysis of the amino acid sequence of the OA protein showed that the first 22 amino acids constituted a signal peptide [8]. Further analysis showed that the OA protein had a transmembrane hydrophobic amino acid sequence from 499 to 521. Analysis of the secondary structure of the OA protein sequence demonstrated that OA had an alpha helical structure from 500 to 521. Analysis of the native/immature MW of OA showed that OA protein had a predicted MW of 63.8 kDa [8] and had a potential 11 N-linked [11] and 19 O-linked glycosylation sites. Collectively, these data suggest that OA protein is highly glycosylated and has two isoforms, secreted and transmembrane.

Gpnmb/OA expression in normal tissue and cancer. Gpnmb/OA was expressed in a wide array of normal tissue and cell types including: osteoblasts [11, 12] and osteoclasts [13, 14] in the bone, macrophages [2] and dendritic cells [15] in the hematopoietic system, and melanocytes, keratinocytes, Langerhans cells in the skin [16, 17]. In addition to its diverse roles in normal cells, aberrant Gpnmb/OA expression had been linked to various pathological disorders such as glaucoma [18], kidney disease [19-21] and osteoarthritis [22]. Gpnmb/OA was also expressed at higher levels in several malignant human tissues, such as hepatocellular carcinoma [7], glioma [4, 5], melanoma [1, 23] and breast cancer cells [6, 24], relative to corresponding normal tissue.

Gpnmb/OA was first cloned and described in 1995 [25]. It was initially designated nmb, a name that is now understood to be a misnomer. It was given this name after a partial Gpnmb/OA cDNA was transfected into a highly invasive melanoma cell line and suppressed spontaneous metastasis in one of three transfected clones. However, further research revealed that Gpnmb/OA was expressed in the majority of metastatic melanomas and that the frequencey of Gpnmb/OA expression in cutaneous melanomas was 80% [1] and in uveal melanomas was 85.7% [23]. The role of Gpnmb/OA in tumor progression was next re-visited in 2003 when it was reported to promote the invasion of glioma cells [4]. They found that tumor cells expression Gpnmb/OA acquired a highly invasive phenotype when implanted intracranially in immunocompromised mice and in vitro, Gpnmb/OA had no impact on tumor cellular growth or death but increased invasiveness and expression of MMP-9 and MMP-3. Gpnmb/OA overexpression also increased the invasiveness and metastasis of rat hepatoma cells in vitro and in vivo [7]. Kuan et al [5] revealed that 35 of 50 glioblastoma multiforme (GBMs) patients (70%) were positive for Gpnmb/OA mRNA and 52 of 79 GBMs patients (66%) were detected Gpnmb/OA in a membranous and cytoplasmic pattern. Rose et al [6] had identified Gpnmb/OA as a gene that was highly and selectively expressed in aggressive bone-metastatic sub-populations of 4T1 breast cancer cells, and demonstrated that, when Gpnmb/OA was overexpressed in an independent, weakly bone metastatic 66cl4 breast cancer cells, it promoted breast cancer metastasis to bone in vivo. Then, they employed IHC-based analysis of tissue microarrays to investigate the relevance of Gpnmb/OA expression in human breast cancer, and found that Gpnmb/OA was expressed in the tumor epithelium of approximately 10% of human breast cancers and the stromal compartment of nearly 70% of breast tumors. Moreover, epithelial, but not stromal, Gpnmb/OA expression was a prognostic indicator of cancer recurrence across all breast cancer subtypes, and specifically within "triple negative" breast cancers [24].

Function of Gpnmb in cancer. Several recent studies investigated biological roles of Gpnmb/OA. These studies demonstrated that Gpnmb/OA played several important biological roles in T cells activation, fibroblast differentiation, osteoblast differentiation, and osteoclast development. The molecular functions of Gpnmb/OA in tumor growth, invasion and metastasis were just beginning to be elucidated [2, 3, 9, 26] and it was perhaps involved in cancer pathophysiology through following ways: (1) Through MMP pathway. Material analysis of glioma cells expressing Gpnmb/OA with specific MMP-2, MMP-9 and MMP-3 inhibitors ablated the increase in invasion associated with Gpnmb/OA expression relative to vector controls [4]. Ogawa et al [27] found that overexpression of Gpnmb/OA in NIH-3T3 fibroblasts induced expression of MMP-3 and in Gpnmb/OA-transgenic mice, denervation further enhanced expression of MMP-3 and MMP-9 in fibroblasts infiltrated into gastrocnemius muscle, compared with wild-type mice. Gpnmb/OA-mediated induction of pro-invasive matrix metalloproteases, such as MMP-3 and MMP-9, may represented one mechanism by which it promoted metastasis. (2) By inhibiting the activation of tumor-reactive T cells. Tomilari et al found that Gpnmb/OA knockdown markedly reduced the growth of B16F10 melanoma cells in vivo following their s.c. injection into synogeneic immunocompetent mice. Gpnmb/OA was capable of suppressing the activation of T-cell activation, by binding to syndecan-4 and on the surface of activated T cells

and inducing its auto-phosphorylation, thereby allowing melanoma to evade immunologic recognition and destruction [28-32]. (3) By decreasing apoptosis and increasing vascular density. Some researchers injected 66cl4 mouse mammary carcinoma cells into the mammary fat pads of Balb/c mice. They removed the primary tumors and by quantifying the number of apoptotic cells in non-necrotic regions of mammary tumors and found that, on average, fewer cells in Gpnmb/OA-expressing tumors (1.1%) were undergoing apoptosis when compared to control mammary tumors (2.6%). They assessed the vascular density of these tumors by quantifying the degree of CD31 positivity, a routinely used endothelial cell marker. These analyse revealed that the vascular density in Gpnmb/OA expressing mammary tumors (3.5%) was significantly higher when compared to control tumors (0.9%). They ectopically expressed Gpnmb/OA in BT549 cells, a basal breast cancer model and revealed that matrigel plugs containing Gpnmb/OA-expressing BT 549 cells displayed greater endothelial recruitment (11.8%) when compared to matrigel plugs composed of empty vector control cells (8.5%). Primary human breast cancers characterized by high vascular density also displayed elevated levels of Gpnmb/OA when compared to those with low vascular density. Gpnmb/OA ectodomain was shed from the surface of breast cancer cells and the shed ECD of Gpnmb/OA could promote endothelial migration in vitro[33]. These data suggested a role for Gpnmb/OA in promoting endothelial recruitment during mammary tumorigenesis. Gpnmb/OA-expressing tumors displayed elevated endothelial recruitment and reduced apoptosis when compared to vector control-derived tumors [33]. (4) Gpnmb/OA function in melanocytes appeared to aid in anchoring to keratinocytes [3] with similar function noted in dendritic cells anchoring to endothelial cells [9]. A human SV40-transformed endothelial line(SVEC) adhered to immobilized DC-HIL in a RGD-dependent manner. Using PAM212 keratinocytes (which do not express Gpnmb) as a surrogate, we radio-labelled these cells and incubated them in 96-ELISA wells immobilized with Gpnmb-Fc. PAM212 cells adhered to immobilized Gpnmb in a dose-dependent manner (but not to control IgG). Addition a RGDs tetramer peptide inhibited adhesion by up to 40% of the maximum adhesion activity. The demonstration, Gpnmb adhered to PAM212 keratinocytes in a RGD-dependent fashion, suggested a role for intergrin-mediated binding between melanocytes and keratinocytes that may be involved in the development to melanocytes, melanin synthesis and generation of melanoma. Leukocytes and antigen-presenting cells, such as macrophages and dendritic cells, expressed Gpnmb/OA, which could promote adhesion to endothelial cells in an RGD-dependent manner[2, 9, 15, 32, 34].

Gpnmb/OA as a therapeutic target. CR011–vcMMAE, which is now referred to as glembatumumab vedotin or CDX-011, combines the tumor-targeting specificity of a Mab (CR011) and the cytotoxic activity of a potent antimitotic compound (MMAE). It is a Gpnmb/OA-targeted therapeutic that belongs to a class of drugs known as antibody-drug conjugates (ADCs) [1]. These drugs consist of antibodies that bind to cell surface molecules, which are linked to highly potent cytotoxins. In the case of CDX-011, the cytotoxin is auristatin E-a tubulin destabilizer. ADCs bind to the extracellular domain of their target protein on the surface of cancer cells, which is then rapidly internalized. ADCs are pro-drugs that require the release of the cytotoxin for activation. Upon internalization, the drug is released and induces cell-cycle arrest and apoptosis of the cancer cell [35]. At concentrations as low as 2.5 mg/kg, CDX-011 was capable of inducing complete regression in 100% of Gpnmb/OA expressing SK-Mel-2- and SK-Mel-5-xenografted melanoma tumors [36]. Treatment with imatinib and inhibitors of the Erk pathway enhance cell-surface expression of Gpnmb/OA in cancer cells, which, in turn, increases sensitivity to CDX-011. This suggests that CDX-011 efficacy could be further enhanced by combining it with additional targeted therapies [37].

The results of completed phase I studies and the preliminary results of ongoing phase II trails in patients with advanced metastatic melanoma of cutaneous origin indicated that CDX-011 was safe and active [35]. In clinical trials, CDX-011 was currently being investigated in two multicenter Phase II trials; one for patients with unresectable melanoma (NCT00412828), and the other for patients with locally advanced or metastatic breast cancer (NCT00704158). To date, with 34 melanoma and 18 breast cancer patients treated with CDX-011, tumor shrinkage was reported in 58% of melanoma patients and 50% of breast cancer patients [35]. CDX-011 does appear to target Gpnmb/OA expressed in normal tissues – most notably in the skin and can induce rash formation. Interestingly, patients who experienced rash within their first cycle of treatment also had significantly longer progression-free survival (PFS) than CDX-011- treated patients who did not develop rash, suggesting that rash may be indicative of a patients' ability to tolerate and respond to the drug. Moreover, in a subset of 13 melanoma patients, there was a striking tendency for patients with the highest levels of tumoral Gpnmb/OA expression to experience the longest PFS times. These preliminary data suggest that tumoral Gpnmb/OA expression and incidence of rash will serve as important predictors for response to CDX-011 therapy in the future. These insights will be useful for sparing patients who are unlikely to benefit and selecting a group of patients who are most likely to respond for CDX-011 therapy.

F6V-PE38 demonstrated sighificant protein-synthesis-inhibition activity on Gpnmb/OA-expressing glioma and malignant melanoma cells, a 60-fold improvement over G49 activity, but no cytotoxicity on Gpnmb/OA-negative cells. F6V-PE38 exhibited significant antitumor activity against subcutaneous malignant glioma xenografts in two nude-mouse models and a melanoma neoplastic meningitis model in athymic rats. These Gpnmb/OA-specific scFv antibodies and imunotoxins hold pormise as reagents in targeted therapy for HGGs and other Gpnmb/OA-expressiong malignancies [38].

An important consideration for any novel targeted therapy is what effects they may have on target-expressing, nonma-

lignant tissue. The first link between Gpnmb/OA expression and bone physiology was made when OA was identified as highly expressed in osteopetrotic bones relative to normal bones in rats. This study demonstrated that, in bones, OA was predominantly expressed by mature, matrix producing osteoblasts [8]. Subsequent studies had demonstrated that inhibition of OA, using neutralizing antibodies or siRNA, in developing osteoblasts inhibited their differentiation and ability to produce bone matrix [11, 39]. These results suggest that Gpnmb/OA-targeted therapies for bone metastasis may, by inhibiting osteoblast function, have the detrimental effects resulting in enhanced osteolysis. In addition to these studies, it was recently reported that OA was abundantly expressed in differentiated osteoclasts, and played an important role in mediating cell fusion to produce multinucleated osteoclasts. Neutralizing antibodies against OA resulted in fewer osteoclasts that were smaller, possessed fewer nuclei and were less able to resorb bone [14]. This observation was consistent with reports that Gpnmb/OA was induced by the transcriptional activator micropthalimia-associated transcription factor [40], which played a critical role in regulating osteoclastogenesis. Clearly, more studies will be needed to better understand the complex role of Gpnmb/OA in normal bone physiology. In the meantime, special care should be taken to monitor for indicators of increased bone resorption in on-going clinical trials using Gpnmb/OA-targeted therapies for cancer.

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