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Myeloid Derived Suppressor Cells in Breast Cancer

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Abstract

Myeloid Derived Suppressor Cells (MDSCs) are a population of immature myeloid cells defined by their suppressive actions on immune cells such as T cells, dendritic cells, and natural killer cells. MDSCs typically are positive for the markers CD33 and CD11b but express low levels of HLADR in humans. In mice, MDSCs are typically positive for both CD11b and Gr1. These cells exert their suppressive activity on the immune system via the production of reactive oxygen species, arginase, and cytokines. These factors subsequently inhibit the activity of multiple protein targets such as the T cell receptor, STAT1, and indoleamine-pyrrole 2,3-dioxygenase. The numbers of MDSCs tend to increase with cancer burden while inhibiting MDSCs improves disease outcome in murine models. MDSCs also inhibit immune cancer therapeutics. In light of the poor prognosis of metastatic breast cancer in women and the correlation of increasing levels of MDSCs with increasing disease burden, the purposes of this review are to 1) discuss why MDSCs may be important in breast cancer, 2) describe model systems used to study MDSCs *in vitro* and *in vivo*, 3) discuss mechanisms involved in MDSC induction/function in breast cancer, and 4) present pre-clinical and clinical studies that explore modulation of the MDSC-immune system interaction in breast cancer. MDSCs inhibit the host immune response in breast cancer patients and diminishing MDSC actions may improve therapeutic outcomes.

Keywords

Breast Cancer; Myeloid Derived Suppressor Cells; Therapy; Murine models

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Introduction

Metastatic breast cancer is incurable and is associated with a poor prognosis. Only 23% of patients are alive 5-years after the diagnosis of stage IV breast cancer [1]. Targeted immunotherapy (e.g. cancer vaccines and monoclonal antibodies) to promote anti-tumor immune responses may be one of the more promising means of treating this disease. Preclinical evidence has demonstrated that vaccination could be useful in breast cancer patients via the use of viral vectors, dendritic cells, peptides/carbohydrates or whole cells [2, 3]. However, this approach is severely limited in the clinical setting by cancer-associated immune-suppression mechanisms.

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells which inhibit innate and adaptive immunity. MDSCs do so by multiple mechanisms, including depletion of arginine, production of reactive nitrogen and oxygen species, and secretion of inhibitory cytokines. Since their discovery, it has been recognized that minimizing MDSC-mediated immunosuppression may be important to developing anti-tumor immune responses. MDSCs commonly express the cell surface markers CD33 and CD11b, have reduced expression of HLADR, and have differential expression of monocytic and granulocytic markers (e.g. CD14 and CD15, respectively) in humans [4]. Mouse MDSCs are $CD11b^{+}$ and $Gr\text{-}1^{+}$ with Ly6C/Ly6G epitopes of the Gr1 antigen defining monocytic ($Ly6G^{-}$ $Ly6C^{hi}$) and granulocytic ($Ly6G^{+}Ly6C^{low}$) populations, respectively [5]. MDSCs have been shown to be increased in breast cancer patients, with the highest levels of circulating MDSCs being present in patients with metastatic disease [6].

MDSC function has been studied in breast cancer. These studies demonstrated that MDSC can cause inhibition of T cells, NK cells, and dendritic cells (DCs) whereas they can be stimulatory to immune regulators such as Th2 T cells, T regulatory cells (Treg) and tumor associated macrophages (TAMs). MDSCs also secrete cytokines such as IL-6 and IL-4 allowing for MDSC expansion and subsequent sequestration of essential amino acids such as arginine and cysteine which are required for the survival of T cells. MDSCs are also thought to mediate their inhibitory functions through reactive oxygen species such as nitric oxide [4, 5]. In light of the poor prognosis of metastatic breast cancer in women and the correlation of increasing levels of MDSCs with increasing disease burden, the purposes of this review are to 1) discuss why MDSCs may be important in breast cancer, 2) describe model systems used to study MDSCs *in vitro* and *in vivo*, 3) discuss mechanisms involved in MDSC induction/function in breast cancer, and 4) present pre-clinical and clinical studies that explore modulation of the MDSC-immune system interaction in breast cancer.

Review

I. POTENTIAL IMPORTANCE OF MDSC IN BREAST CANCER

Levels of MDSCs are predictive of patient survival—MDSCs may provide prognostic and possibly predictive information in patients with breast cancer. The use of MDSCs as biomarkers of disease have involved several small clinical trials during which MDSC levels were measured in the peripheral blood of patients with breast cancer and other malignancies. In the earliest experiments involving measurements of different immune subsets in the peripheral blood of patients with head and neck, breast and lung cancer it was noted that levels of mature dendritic cells are decreased while levels of immature myeloid cells lacking markers of mature hematopoietic cells are markedly higher compared with disease-free volunteers. The levels of these immature myeloid cells correlated closely with the tumor burden and duration of the disease. Furthermore, removal of tumors from study patients resulted in partial reversal of immature myeloid cell accumulation in the peripheral blood [7]. Further work established that approximately 30% of these myeloid cells

represented immature dendritic cells and immature cells of macrophage lineage that are capable of suppressing T cell responses, while the remaining populations were probably cells at earlier stages of myeloid differentiation (i.e., MDSC) [8]. Additional work provided support for the hypothesis that levels of MDSC can correlate with clinical stage and metastatic disease burden in breast cancer patients [6, 7]. In a study by Diaz-Montero *et al.*, the percentage of whole blood MDSCs was measured by flow cytometry and found to be increased in patients with later stage breast cancer. The average peripheral blood level of MDSC in patients with early stage I/II breast cancer was 1.96%. In stage III and IV, these levels increased to 2.46% and 3.77%, respectively, and in stage IV patients with 3 or more organ systems involved by the cancer the levels increased to a mean of 4.37%. MDSC levels greater than 25% were seen in some patients with stage IV cancers. Similarly, a pilot study of 25 subjects with metastatic breast cancer showed that patients with higher than average levels of peripheral blood MDSCs following palliative systemic therapy had shorter overall survival [9]. In that study, peripheral blood levels of MDSCs corresponded to levels of circulating tumor cells, another emerging prognostic marker [9]. These studies suggest that MDSC levels correlate with disease burden and are a potential novel and promising prognostic biomarkers for use in breast cancer. However, more research is needed to validate the findings of these early pilot studies.

Levels of MDSCs vary with chemotherapy—Treatment of patients with operable breast cancer involves modalities aimed at reducing primary tumor burden (breast surgery, radiation therapy) and therapies that allow elimination of microscopic distant disease (systemic chemotherapy, endocrine therapy). Based on clinical experience, chemotherapy appears to influence levels of peripheral blood MDSCs in patients with breast cancer. However, many confounding factors such as concurrent use of colony stimulating factors (employed to increase neutrophil numbers) and steroids (to reduce drug reactions) during chemotherapy as well as inclusion of patients with various histologic types and stages of breast cancer make studying chemotherapy effects on peripheral blood MDSC levels difficult. The effects of these confounding factors cannot be easily separated. Diaz-Montero and colleagues evaluated 17 patients with stage II-III breast cancer receiving adjuvant chemotherapy that consisted of dose-dense doxorubicin plus cyclophosphamide (ddAC) followed by dose dense paclitaxel (ddT). These patients also received pegylated granulocyte colony stimulating factor (pegylated G-CSF) to facilitate white blood cell recovery. Increased numbers of MDSCs were associated with increased metastatic tumor bulk and clinical stage. They found that following ddAC chemotherapy, granulocytic MDSCs (defined as $HLADR^{-}$,$Lin^{-/low}$,$CD11b^{+}$,$CD33^{+}$) were significantly elevated compared to the baseline. The mean percentage of MDSC at baseline was 2.2% and mean levels after ddAC chemotherapy were 11.7%, p=0.0002. During sequentially administered dose-dense paclitaxel (ddT), the composite mean MDSC level increased by only 1.45% from the baseline [6].

Given that adjuvant chemotherapeutics are used to treat breast cancer patients, it is important to understand the impact of various chemotherapy regimens on MDSC populations. Chemotherapeutic regimens are meant to eliminate microscopic foci of disease, but they may also provide an immunosuppressive environment where cells such as MDSCs may help residual cancer to persist. In another clinical trial, 42 women with stage II-III, HER2/neu negative breast cancer were treated with standard neo-adjuvant chemotherapy consisting of 4 cycles of doxorubicin and cyclophosphamide (AC) every 3 weeks followed by 4 cycles of weekly docetaxel (DT) with the addition of a glutathione disulfide mimetic (NOV-002). The primary endpoint of this study was pathologic complete response defined as no metastatic tumor in axillary lymph nodes; and either no invasive tumor in the breast (ypT0) or residual invasive tumor less than or equal to 10 mm in maximum dimension (ypT1a or ypT1b). Patients with lower peripheral blood levels of

$Lin^{neg}HLADR^{neg}CD11b^{+}CD33^{+}$ MDSC at baseline and prior to the last cycle of chemotherapy had a significantly higher chance of achieving pathologic complete response to chemotherapy (p=0.02). The baseline levels of MDSC in non-complete responders vs. complete responders were 257.4 vs. 124.3 MDSC/μL, respectively ($p < 0.001$). Similarly, circulating MDSC levels were significantly higher in non-responders compared to complete responders during the final 4 cycles of docetaxel (363.7 vs. 171.5 MDSC/μL; $p = 0.006$). Peripheral blood levels of MDSCs levels remained low and did not change in complete responders, while the levels increased in patients who did not achieve complete pathologic response. In this study, 23% of patients received GCSF [10]. These studies suggest that reduced levels of circulating MDSCs may be correlated with the efficacy of adjuvant chemotherapy.

II. MODEL SYSTEMS USED TO STUDY THE FUNCTION AND INHIBITION OF MDSC

Murine models are utilized to study MDSC immune inhibition in breast cancer—Mouse models are important tools to understand the genetics, immune response and immunotherapeutic outcomes in cancer. Mouse models of human breast cancer can be grouped into two broad categories. 1) Spontaneously developing tumor models due to predisposing mutations facilitating tumor development (i.e. genetically engineered mice (GEMs) with specific transgenes, knock-outs or knock-ins, etc.) or due to the administration of carcinogens such as chemicals, viral agents or ionizing radiation. 2) Transplantation of tumors (e.g. xenograft, orthotopic, etc.) into mice with traditional genetic backgrounds (C57Bl/6, Balb/C), or into genetically engineered mice (GEMs) with specific genetic deficiencies or “knockins” [11]. Importantly, some breast cancer models rely on a combination of the above categories such as carcinogen or radiation induction of breast cancer in a genetically deficient mouse [12]. The selection and use of a particular mouse model depends on the specific question, tumor type and pathway of interest.

Spontaneous and induced breast cancer models in genetically-engineered mice (GEMs)—These models are useful in studying the effects of immunity on breast cancer in immunocompetent mice. Murine models typically either have a loss of tumor suppressor genes (e.g. TRP53, BRCA1, PTEN), or gain of functional oncogenes (e.g. Erbb2, Myc, Ccnd1, PyMT, Hras) that facilitate the spontaneous development of tumors. Hormonally regulated promoters such as mouse mammary tumor virus (MMTV), whey acidic protein (Wap) and metallothionein [13] also provide useful modeling in elucidating the genetics and hormonal induction of breast cancer. One mouse model that mimics the biology of $HER2^{+}$ breast cancer is the FVB-HER murine model that was developed by Dr. Leder at Harvard [14]. In a study by Habibi *et al.*, using mouse mammary carcinoma (MMC) cells derived from the FVB-HER model, FVBN202 transgenic mice were injected with MMC cells and HER2 positive tumors were given intra-tumoral injections of IL-7 and IL-15 after radiofrequency ablation. These mice exhibited increased immune responses to tumors, inhibited tumor development and lung metastasis, reduced MDSC compared to baseline, and had decreased expression levels of known determinants of MDSC suppressive activity such as arginase I, and NOS2 [15]. However, even with expression of HER2, the limitations of this model and other spontaneous models are that they may not directly or fully correspond to the expression levels or cellular functionality of proteins, genes, or regulatory elements seen in human mammary tissue. Additionally, the variability in timing and incidence of tumor development is a significant limitation in providing sufficient animal numbers for robust evaluation of treatment, therapeutics and prognostic outcome. Although new technologies (e.g. inducible and conditionally-expressed transgenes (e.g. tetracycline-regulatable, Cre/loxP) technologies) are enhancing the utility of these models, the protracted time of onset remains a challenge. In one study using *LSL-Kras*^{G12D} *Cre*inducible transgenic mice on a mixed 129SvJ-C57BL/6 strain background responsible for lung

carcinoma and indoleamine-pyrrole 2,3-dioxygenase $(IDO)^{-/-}$ mice on the C57BL/6 background the group was able to demonstrate that IDO is important for development of both primary lung carcinoma and breast metastasis to the lung [16].

Models involving transplanted tumors in mice—Perhaps one of the most widely used model systems in breast cancer is that of transplantable tumors. Tumors may be of xenogenic origin (different organism, i.e. human), syngeneic origin (same organism with the same genetic background) or allogeneic (same organism with a different genetic background) and the recipients may be wild-type or genetically engineered mice (e.g. knock-outs, humanized mice, etc.). Transplants can be placed orthotopically (in mammary gland for breast cancer) or subcutaneously. [17–19]. The most widely used orthotopic model in immunocompetent mice is the 4T1 model in Balb/c mice which has provided evidence that MDSCs are involved in the development and progression of breast cancer [20–24]. These findings show that, similar to studies in human patients with breast cancer, MDSCs are increased in mice with mammary cancers and eliminating MDSCs can result in increased immune-mediated anti-tumor responses and decreased tumor-burden [6]. The C57Bl/6 strain is resistant to breast cancer compared to the C3H and Balb/c strains [25], although some spontaneous or inducible models are available on the C57Bl/6 background. Xenograft models typically involve transplantation of human tumors into immunodeficient mice and as such their role for studying immune cells such as MDSCs is severely limited. For these reasons, most studies in MDSC have employed the immunocompetent C3H and Balb/c strains. Transplantation of syngeneic tumor models also occurs in immune competent recipients. Data from the 4T1 mouse studies suggest that limiting MDSCs in breast cancer could improve the efficacy of breast cancer therapies and enhance anti-tumor immunity. An example was published by Mundy-Bosse *et al.* where they implanted 4T1 cells into the flanks of Balb/C mice and demonstrated that MDSC produce nitric oxide and limited the IFN responsiveness of T cells [26]. In the study described by Smith *et al.*, $IDO^{-/-}$ mice were orthopically transplanted with 4T1 breast carcinoma cells and were found to have fewer MDSC [16]. Another example of a GEM was studied by the Gabrilovich group. They created a murine model in which gp91phox was deleted. The gp91phox is a component of the NADPH complex that generates reactive oxygen species. This murine model is useful as a control as MDSCs produced by these mice are unable to produce reactive oxygen species [27]. In summary, murine models have provided abundant evidence for a role of MDSC in the development and progression of breast cancer.

III. MECHANISM INVOLVED IN MDSC INDUCTION/FUNCTION IN BREAST CANCER

MDSCs are derived from bone marrow progenitor cells—Myelopoiesis is the process by which hematopoietic stem cells differentiate into mature myeloid cell populations (i.e. granulocytes, monocytes, macrophages and dendritic cells). MDSCs are considered to represent heterogeneous populations of immature myeloid cells which have failed to fully differentiate [4]. MDSCs generated *in vitro* from human bone marrow (BM-MDSC) using G-CSF and GM-CSF phenotypically and functionally resemble promyelocytes but suppress T cell activity in a contact dependent manner similar to MDSCs [28]. Similarly, mouse and canine myeloid precursors can suppress T cell proliferation responses and are putative MDSCs (Papenfuss, unpublished data). In a study by Solito, *et al.*, activated T cells block the differentiation of MDSC and maintain proliferation of BM-MDSC $CD11b^{low/neg}$ cells. This group further showed that BM-MDSCs also down-regulate the T cell CD3 ζ chain. Interestingly, BM-derived MDSCs are phenotypically and functionally similar to MDSC obtained from breast and colon cancer patients. The suppressive activity of BM-MDSCs resides in those cells containing the $lin^{neg}CD11b^{low/neg}CD16^{neg}$ phenotype. Similar to the phenotype in bone marrow, in human stage IV breast cancer the population of MDSCs were described phenotypically as being Lin^{neg}, $HLA\text{-}DR^{neg/low}$, $CD33^{+}$, $CD11b^{+}$. Circulating

MDSCs greater than 3.17% correlated with poorer survival (5.5 versus 19.32 months) [28]. Due to phenotypic and functional similarity, MDSCs in breast cancer are likely derived from bone marrow precursors.

Induction of MDSC from progenitor cells in breast cancer likely relies on a combination of cytokines, growth factors and tumor-derived soluble factors— MDSC induction is likely caused by a general inflammatory state in cancer and can be related to a number of cytokines [4]. Multiple cytokines have been shown to impact MDSC generation and cancer growth (e.g. IL-7, IL-15, IL-12, G-CSF, IL-6, IL-10, IL-17, IL1β among others) and both inhibitory and stimulatory effects on MDSCs and cancer growth have been reported [4]. Cytokines such as GM-CSF and IL-6 may even be used to generate MDSC *in vitro* [29]. To study MDSCs *in vitro*, an immortalized MDSC cell line was constructed using the 4T1 murine cancer model. Recently, using the MDSC cell line, it was determined that IL-1β from MDSC induced $CD4^+$ T cells to secrete IL-17, limiting the effectiveness of cytotoxic chemotherapy such as 5FU or gemcitabine [30]. MDSCs may also be generated in vitro by culturing PBMC from normal donors with breast cancer cell lines [29]. In this model, Flt3L and TGF-β contribute to the induction of $CD11b^+$ MDSCs by breast cancer cell lines whereas IL-6, IL-1β and GM-CSF contribute to the induction of $CD33^+$ MDSCs [31]. GM-CSF administered to FVBN202 mice implanted with mouse mammary carcinoma cells demonstrated an increase percentage of suppressive MDSCs [32]. Intra-tumoral injection of Balb/c mice containing mouse mammary carcinoma (MMC) cells derived from the FVB-HER murine model with GM-CSF increased MDSC levels in the spleen, whereas injections of IL-7 and IL-15 decreased numbers of MDSCs [15]. Another cytokine suggested to increase MDSC numbers is VEGF. Blocking VEGFR2 selectively in MMTV-PyMT transgenic mice on the FVB background (which develop breast cancer spontaneously) is effective in limiting tumor growth and suppressing MDSC accumulation after 4 weeks of therapy. IL-1β and IL-6 levels decrease with anti-VEGF antibody treatment and levels of mature dendritic cells ($CD83^+CD11c^+$) increase while MDSC numbers decrease [33].

Kerkar *et al.* demonstrated that the cytokine IL-12 increases the capabilities of professional APCs in the tumor stroma and allows $CD8^+$ T cells to detect antigen cross-presentation [34]. In a 4T1 model, IL-12 has an inhibitory effect on MDSCs by stimulating them to develop into mature myeloid cells. MDSCs obtained from tumors and spleens of tumor bearing mice treated with IL-12 exhibited up-regulation of surface markers of macrophages (F4/80 and MHCII) and dendritic cells (CD80 and CD86) suggesting differentiation into more mature, less immunosuppressive forms. The spleens of tumor-bearing mice also had up-regulation of many dendritic cell and macrophage maturation markers such as CD80, CD86, F4/80 and MHCII. Expression of nitric oxide synthase was also decreased. Treatment with an IL-12 producing adenovirus vector was found to decrease the percentage of MDSC in the tumor microenvironment and increase the percentage of activated CD8+ T cells [35]. Thus, IL-12 treatment, in general, results in infiltration of cytotoxic CD8+ T cells and reduction of metastasis. These data suggest that both the induction of MDSCs and the suppressive activity of MDSCs depend on the cytokines secreted by tumor bearing hosts.

Activation of signaling pathways leads to recruitment of MDSC—Two factors of interest to an analysis of MDSC biology are miR-494 and S100A8/A9 as they both may affect MDSC chemotaxis. MicroRNAs (miRs) are small non-coding RNA molecules that function as regulators of transcription and other biochemical processes. miR-494 is highly expressed in MDSCs derived from tumors and treatment with TGF-β1 causes the increased expression of this miR in MDSCs. When miR-494 levels are decreased using an inhibitor transfected in a lentiviral vector, primary tumor growth and metastatic growth of tumors in the 4T1 murine model are decreased. miR-494 targets PTEN (phosphatase and tensin

homolog) which is a negative regulator of the PI3K/AKT pathway. Thus high levels of miR-494 could lead to increased activation of AKT in the MDSC. Increased levels of miR494 also increase CXCR4-mediated MDSC chemotaxis [36]. Another MDSC chemotactic factor is S100A9. S100 proteins were named for their ability to be soluble in 100% ammonium sulfate and are calcium binding proteins [37]. S100A9 KO mice are capable of rejecting EL4 lymphomas [38] presumably due to the lack of S100A9 ability to enhance MDSC actions. S100A9 serves both as a MDSC chemotactic factor and it inhibits dendritic cell maturation. In the 4T1 murine model levels of the chemotactic protein S100A9 were elevated and were associated with increased numbers of MDSCs [39]. Tumors and MDSCs in general produce chemotactic proteins such as S100A8/A9 that mediate MDSC recruitment to tumor sites via various inflammatory pathways [37]. There are numerous other factors that recruit MDSCs to tumor sites [4]. More work is needed to elucidate these mechanisms in breast cancer.

IV. MDSCs ARE A POTENTIAL TARGET FOR THERAPEUTIC DEVELOPMENT

Adoptive immunotherapy—Adoptive immunotherapy may be combined with agents that inhibit MDSCs such as gemcitabine or MDSC depleting antibodies [40]. T cells specific for the HER2 antigen may be transferred into a tumor bearing host. In a breast murine system, the anti-tumor effects of adoptively transferred CD8+ cytotoxic T cells was aided by depletion of MDSCs with an anti-Gr1 antibody. The combination of adoptive immunotherapy with $HER2^{+}$ specific T cells and depletion of MDSCs ($CD33^{+}/CD11b^{+}/CD14^{neg/+}HLADR^{neg}$) resulted in regression of primary tumors in mice. Furthermore, these studies demonstrated that in the presence of Th1 cytokines such as IL-2 and IFN-γ there are reduced numbers of MDSCs and reduced tumor growth [41]. The literature presented in this review suggests that adoptive transfer with IL-12 may also be useful in altering MDSC maturation in the setting of breast cancer. Adoptive immunotherapy combined with agents that deplete MDSCs is a potential therapeutic strategy in breast cancer.

Oncoloytic viruses and vaccination therapy—Immune therapy may inhibit MDSCs in breast cancer. An oncolytic herpes simplex virus 1 vector has been developed which has a tumor suppressor murine 15-prostaglandin dehydrogenase (15-PGDH) expression cassette. 15-PGDH is a protein that converts prostaglandin E2, a potent pro-tumor cytokine derived from arachidonic acid, to inactive 15-keto-metabolites. Increased expression of 15-PGDH in a murine 4T1 breast cancer model mediated a reduction in prostaglandin E2 (PGE2) and resulted in reduced numbers of MDSCs, decreased IL-4 and GM-CSF production, and reduction in overall size of primary tumor and metastasis [42]. Decreases in IL-4 and GM-CSF are important as these cytokines are known to promote MDSC survival. Thus oncolytic viruses may be modified so that they have effects on immune suppressor cells.

Antibody therapy—Given emerging data on MDSC inhibition of natural killer cell mediated cytotoxicity, blocking MDSC may also be considered in the development of treatments that involve the use of monoclonal antibodies such as trastuzumab [43–45]. Numbers of MDSC also correlate with breast tumor size in mouse models and decreased numbers of T cells [22]. In some murine models, reduction in the number and/or function of MDSCs with agents such as zoledronic acid led to slower tumor growth and improved anti-tumor immune responses in a HER2 breast murine model[46]. Therefore, combination approaches using anti-HER2 antibodies and MDSC inhibition may lead to better responses. A recent study showed that the use of anti-CCL5 antibodies with irradiation decreased tumor growth and attenuated lung metastasis in a 4T1 murine model. CCL5 is important in generating functional MDSCs in 4T1 murine breast cancers. Interestingly, the MDSCs generated in the setting of low CCL5 levels have lower Ly6C expression. Furthermore, CCL5 is important for the immunosuppressive activity of human MDSCs as demonstrated

by increased T cell proliferation in the presence of an anti-CCL5 antibody [47]. These data suggest that therapy with MDSC-modulating agents and anti-CCL5 antibody may have efficacy in triple negative breast cancer by inhibiting the functions of MDSCs.

V. MDSC INHIBITORS IN THE CLINIC

The mechanisms that can be exploited to reverse the immunosuppressive effects of MDSCs can be divided into 4 basic categories that include: 1) forcing differentiation of MDSCs into mature myeloid cells (such as monocytes, granulocytes or dendritic cells), 2) inhibiting MDSC expansion from the precursor stage, 3) preventing MDSC accumulation in peripheral organs and 4) blocking of MDSC function or inhibitory soluble factors [48]. In general, forced differentiation can be achieved in murine models via utilization of all-trans retinoic acid & vitamin D. Compounds that can prevent MDSC expansion from a precursor stage include: STAT3 inhibitors, tyrosine kinase inhibitors, amino-bisphosphonates, and MMP9 inhibitors. Cytotoxic agents that can directly decrease MDSC accumulation include: gemcitabine, 5-FU, CXCR2, CXCR2 antagonists, cisplatin, paclitaxel, Hsp90 inhibitors and IL-13PE. Direct inhibitors of soluble factors include: ROS scavengers, and ARG & NOS inhibitors (e.g. nitroaspirin, PDE5 inhibitors, COX inhibitors) [48]. All of these methods can be combined with immune based therapy in breast cancer.

To date, a limited number of clinical trials have examined whether various MDSC inhibitors are functional in humans and whether such inhibition can lead to clinical benefit in patients. One study is testing the effect of an N-bisophosphonate, zoledronic acid, on the levels of MDSCs in women with hormone receptor positive metastatic breast cancer. In pre-clinical studies, zoledronic acid treatment had led to decreased numbers of MDSCs and slower tumor growth in Balb-T neu transgenic mice [46]. The reduction in MDSC expansion was likely secondary to decreasing the concentration of MMP9.

Modulators of reactive oxygen species may be important for modulating MDSC in breast cancer—Cytotoxic T cells are important for the immune response to cancer and MDSCs are known to inhibit $CD8^+$ T cell antigen recognition via production of ROS [49]. One mechanism of immune escape of cancer is MDSC-mediated nitration of the T cell receptor preventing its binding to MHC complexes [27, 50]. Other mechanisms by which MDSC-derived nitric oxide may facilitate immune escape is by reducing IFN signaling through nitration of STAT1 as was demonstrated in a 4T1 murine model. In this model, increasing numbers of MDSCs caused increased nitration of STAT1, decreased interferon signaling, and increased tumor growth [26]. In other cancers, chemokines such as CCL2 have also been found to be nitrated by MDSCs [51]. As such, inhibitors of free radical formation may be useful in the treatment of breast cancer. NOV-002 is a glutathione disulfide mimetic that induces S-glutathionylation and thereby inhibits free radical formation. A single arm phase 2 breast cancer clinical trial examined whether doxorubicin and cyclophosphamide followed by docetaxel may be combined with NOV- 002. This study demonstrated that patients with lower levels of MDSCs had an increased chance of a complete pathological response [10].

Decreasing stress may decrease numbers of MDSC and promote survival in breast cancer patients—Increased psychological stress has also been associated with a decreased immune response and increased numbers of MDSCs in breast cancer patients. In a study by Mundy-Bosse *et al.*, breast cancer patients filled out a Life Event Scale that measured 5 potentially stressful events in the past year (death of a friend or family member, financial difficulty, divorce or separation from a family member or friend, major conflict with children or grandchildren, robberies or accidents) and provided subjective measure of stress level by the use of the Likert scale. The study revealed that a self-reported low stress

levels as measured with the 4-point Likert scale was associated with an elevation of $CD33^{+}HLA\text{-}DR^{neg}CD15^{+}CD11b^{+}$ MDSCs. However, when stress was measured objectively using the Life Event Scale, MDSC numbers were directly correlated with higher stress levels. Therefore, it is likely that stress may play a role in stimulation of MDSC growth and could contribute to adverse outcomes [52]. Additional studies will evaluate the effect of stress on induction of suppressive immune subsets. The mechanism of this effect likely involves the ability of stress hormones to modulate MDSC function or expansion.

VI. CONCLUSION

MDSCs in breast cancer promote tumor growth, metastasis and suppression of the immune system. Murine models have been established to study breast cancer based on the specific clinical questions. Studies of MDSC induction and function in these murine models have led to new therapeutic approaches. Pre-clinical studies have demonstrated that adoptive immunotherapy and oncolytic/vaccination therapy may be combined with therapies that decrease MDSCs to treat breast cancer. Current clinical studies are examining whether depletion of MDSCs in combination with immune based mechanisms in breast cancer patients will result in overall survival advantages. Given the emerging importance of MDSCs in breast cancer, modulation of MDSCs is an attractive avenue of further research for those breast cancers not curable by conventional therapies.

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