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Mesenchymal stem cells expressing GD2 and CD271 correlate with breast cancer-initiating cells in bone marrow

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Purpose: The bone marrow microenvironment is considered a critical component in the dissemination and fate of cancer cells in the metastatic process. We explored the possible correlation between bone marrow mesenchymal stem cells (BM-MSC) and disseminated breast cancer-initiating cells (BCIC) in primary breast cancer patients.

Results: The percentages of BCIC (Aldefluor$^+$CD326$^+$CD44$^+$CD24$^-$) correlated with the percentages of BM-MSC, either CD45$^-$GD2$^+$CD200$^+$CD271$^+$ (Kedall's $\tau = 0.684$, $p = 0.004$) or CD45$^-$GD2$^+$CD271$^+$ in the bone marrow (Kedall's $\tau = 0.464$, $p = 0.042$).

Experimental Design: Bone marrow mononuclear cells (BM-MNC) were collected at the time of primary surgery in 12 breast cancer patients. BM-MNC was immunophenotyped and BCIC was defined as epithelial cells (CD326$^+$CD45$^-$) with a "stem-like" phenotype (CD44$^+$CD24$^{low/-}$, ALDH activity). BM-MSC was defined as CD34$^-$CD45$^-$ cells that co-expressed GD2, CD271 and/or CD200 within CD326-depleted BM-MNC.

Conclusions: There was a positive correlation between mesenchymal stem cells expressing GD2 and CD271 and breast cancer-initiating cells in BM of patients with primary breast cancer.

Introduction

Breast cancer-initiating cells (BCIC) can metastasize and form new tumors.[1,2] BCIC (CD44$^+$CD24$^{low/-}$) have been detected within the disseminated tumor cells (CD326$^+$) in bone marrow (BM) of early stage breast cancer patients.[1,3] Moreover, breast cancer with high aldehyde dehydrogenase (ALDH) activity, Aldefluor$^+$, may contain the tumorigenic cell fraction and ALDH-positive cells that have been shown to be responsible for metastasis.[2,4]

Human bone marrow mesenchymal stem cells (BM-MSC) represent a phenotypically heterogeneous population of cells with the potential for multidirectional differentiation to bone, fat, cartilage and other mesenchymal tissues.[5] In recent years, much attention has been focused on the plasticity of BM-MSC and other BM-derived progenitor cells, and their contribution to tumor-associated stroma formation and tumor progression.[6,7] Moreover, these cells could play a specific role in the BM colonization of disseminated breast cancer cells.[8] Finally, other BM precursors, the BM-derived hematopoietic stem cells that co-express the vascular endothelial growth factor receptor-1 (CD34$^+$VEGFR1$^+$) could play a role in establishing a pre-metastatic niche in distant organs in mouse models, though unconfirmed in humans.[9]

Recently, GD2, CD271 and CD200 were proposed as specific BM-MSC markers[10-12] and the role of BM microenvironment and its specific components in supporting BCIC have been investigated.[13] In the current study, we test the hypothesis that there is a correlation between either the percentages of BM-MSC or BM CD34$^+$VEGFR1$^+$ cell population and of BCIC in BM of primary breast cancer patients.

Results

We evaluated BM samples from 12 primary breast cancer patients, median age 51 years, range 27–75 years. Tumor histology was of infiltrating ductal carcinoma in 11 cases and of mixed lobular and undifferentiated breast carcinoma in one case; estrogen receptor was positive in ten cases and negative in two cases; HER2 was amplified in one patient. Tumor size was pT1 in seven cases and pT2-pT4 in five cases; nodal status was positive in five cases and negative in seven cases. Five patients received neoadjuvant chemotherapy. After a median follow-up of 12 months (range 6–12 months), one patient had a disease relapse with brain metastases (**Table 1**).

To test the hypothesis that a high proportion of BM-MSC is associated with an increased representation of BCIC within the

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Table 1. Patient and disease characteristics

	No.	(%)
Age (years)		
Median (range)	51	(27–75%)
Histology		
Infiltrating ductal carcinoma	11	(92%)
Infiltrating lobular carcinoma	0	(0%)
Other	1	(8%)
Estrogen receptor status		
Positive	10	(83%)
Negative	2	(17%)
HER2 status		
Amplified	1	(8%)
Normal	11	(92%)
Tumor size (pT)		
pT1	7	(58%)
pT2-pT4	5	(42%)
Nodal status		
Positive	5	(42%)
Negative	7	(58%)
Neoadjuvant chemotherapy		
Yes	5	(42%)
No	7	(58%)

bone marrow, we evaluated the phenotype of bone marrow mononuclear cells (BM-MNC) by flow cytometry (**Table 2**). All 12 BM samples contained epithelial cells with the BCIC phenotype and ALDH activity ($Aldefluor^+CD326^+CD44^+CD24^-$). BCIC correlated with BM-MSC that co-expressed GD2 and CD271 (either $CD45^-GD2^+CD200^+CD271^+$, Kedall's $\tau = 0.684$, $p = 0.004$; or $CD45^-GD2^+CD271^+$, Kedall's $\tau = 0.464$, $p = 0.042$) (**Fig. 1**). On the other hand, BM-MSC that expressed either GD2 or CD271 did not correlate with BCIC (data not shown). In addition, $VEGFR1^+CD34^+VEGFR2^-CD31^-$ expressing cells did not correlate with BCIC (data not shown).

Discussion

BCIC have the capacity to seed in the BM at an early stage.[4,8] In our study, all 12 BM samples contained BCIC epithelial cells ($CD326^+CD44^+CD24^-$) and ALDH activity. A 70–80% incidence of BCIC in BM of primary breast cancer patients was previously reported by Balic et al.[3] The presence of BCIC in the BM appeared related to BM-MSC subsets that co-expressed CD271 and GD2. The 1-year median follow-up precludes any conclusion on the metastatic growth potential of this correlation. Larger studies with longer follow-up are needed to correlate the percentage of the BCIC and/or the association to BM-MSC to the tumor status and therapy.

This study was hindered by the small sample size and the relative paucity of cells expressing the BM-MSC phenotype, (on average 18 BM MSC per 10^6 BM-MNC for the $GD2^+CD271^+$ phenotype or 9 BM MSC per 10^6 BM-MSC for the more restrictive $GD2^+CD271^+CD200^+$ phenotype). Nevertheless, the positive correlation between BM-MSC subsets and BCIC with ALDH activity could be interpreted as favoring BCIC survival or as an inhibition of tumor growth. Although the bidirectional crosstalk between BM-MSC and tumor cells is known to occur and critical for tumor survival,[6,14,15] naïve BM-MSC have been shown to inhibit tumor growth.[16-18] In murine models BM-derived haematopoietic progenitor cells ($CD34^+VEGFR1^+$) formed a pre-metastatic niche at the sites of metastasis formation before the infiltration of tumor cells.[9] This might explain the homing of tumor cells to the BM observed in patients.[13] However, no correlation was found between these cells and BCIC in the current study.

Others showed that BM-MSC mixed with otherwise weakly metastatic human breast carcinoma cells caused the latter to increase their metastatic potency.[19] Breast cancer cells stimulate de novo secretion of the chemokine CCL5 from BM-MSC, which then acts in a paracrine fashion on the tumor cells to enhance their motility, invasion and metastatic potential. This metastatic ability, that depends on CCL5 signaling through the chemokine receptor CCR5, is reversible and illustrates that the *niche* or microenvironment determines the fate of stem cells by modifying their biological properties responsible for the ability to invade and metastatic potential.[6] Finally, recently, other authors showed that BM-MSC may accelerate human breast tumor growth by generating cytokine networks that regulate the cancer stem cell population.[20]

Research on cancer dissemination is currently focused on new therapeutic strategies consisting of molecular targeting of distinct oncogenic signaling components activated in the cancer-initiating cells and/or the cells in the microenvironment. To be effective, the putative targeted therapy should prevent disease relapse and enhance patient survival possibly by modulating the critical role of the microenvironment to support survival of cancer initiating cells.

In conclusion, our results suggest that BM-MSC subsets positively correlate with BCIC with ALDH activity in BM of primary breast cancer patients. Additional research is warranted to further investigate the relationship between BM-MSC and disseminated BCIC.

Materials and Methods

Bone marrow specimens were obtained from primary breast cancer patients (Protocol LAB04-0657; Chair: A. Lucci) at The University of Texas MD Anderson Cancer Center. The study was approved by the Institutional Review Board. Following informed consent, 10 mL of bilateral BM aspirates from the upper anterior iliac crests of patients were collected at the time of surgery. BM mononuclear cells (BM-MNC) were obtained by Ficoll density gradient centrifugation and the remaining erythrocytes were lysed with ammonium chloride solution, as previously reported.[19]

BM samples were interrogated for ALDH function using the Aldefluor® assay (Stem Cell Technologies, Vancouver, BC). Briefly, BM-MNC were suspended in Aldefluor buffer containing an ATP-binding cassette transport inhibitor. A BM-MNC aliquot was reacted with the ALDH inhibitor,

Table 2. Relative frequencies of hematopoetic stem cells, mesenchymal stem cells and breast cancer initiating cells

	Cells per million BM MNC					
Subject	**$CD34^+$**	**$ALDH^+$**	**$ALDH^+$ $CD326^+CD45^-$ $CD44^+CD24^{lo}$ (BCIC)**	**$GD2^+$ $CD271^+$ (BM MSC)**	**$GD2^+CD271^+$ $CD200^+$ (BM MSC)**	**$CD34^+$ $VEGFR1^+$**
1	3800	15900	5603	19	0	1794
2	7700	39400	27355	31	31	2975
3	9900	25000	15203	67	23	2683
4	13800	19700	15902	37	32	32
5	9500	9700	4091	0	0	2347
6	11500	4800	2449	7	0	2197
7	4400	47900	16133	0	0	704
8	4700	16300	4280	0	0	17374
9	7300	11100	3371	34	14	1716
10	8500	14300	2914	0	0	723
11	4600	14900	4766	13	4	1339
12	5300	16400	3994	4	0	1240

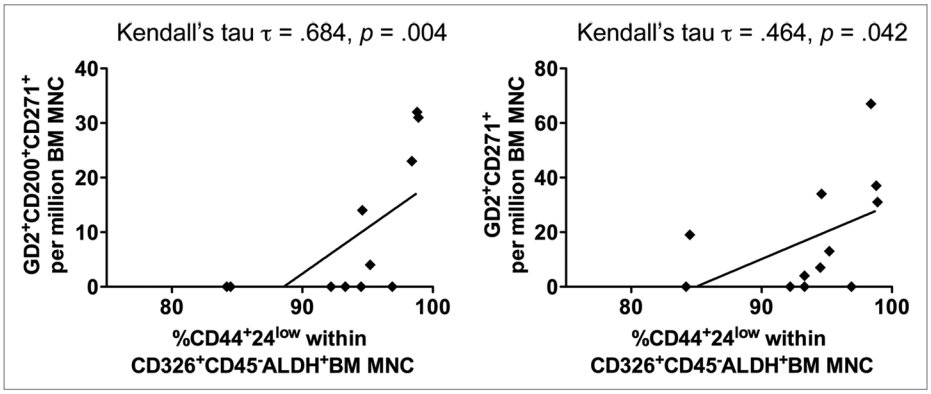

Figure 1. Correlation between the BCIC and BM-MSC. BM samples from primary breast cancer patients were interrogated for ALDH function using the Aldefluor® assay (Stem Cell Technologies, Vancouver, BC). Within the $Aldefluor^+$ BM-MNC subset, BCIC phenotype was defined as $CD326^+CD45^-CD44^+CD24^{low}$ and its percentage is correlated with BM-MSC that co-expressed GD2 and CD271 (either $CD45^-GD2^+CD200^+CD271^+$ or $CD45^-GD2^+CD271^+$).

diethylamino-benzaldehyde (DEAB), as a negative control. Both the test reaction and the negative control were analyzed on a LSR II flow cytometer (BD Biosciences, San Jose, CA). Alexa700 (Invitrogen, Carlsbad, CA) labeled anti-CD44 monoclonal antibody (BD Pharmingen, San Jose, CA) and pre-conjugated antibodies to CD24 (PE-Cy5, BD Pharmingen), CD45 (PE-Cy7, BD Pharmingen) and CD326 (APC, Miltenyi Biotec, Auburn, CA) were used to label cells. Appropriate isotype-matched controls were used. Within the $Aldefluor^+$ BM-MNC subset, BCIC phenotype was defined as $CD326^+CD45^-CD44^+CD24^{low}$.

Another aliquot of BM-MNC was reacted with anti-CD326 antibody coated magnetic beads and then processed in a MACSPro cell separator (MACS, Miltenyi Biotec) to isolate $CD326^+$ cells. Thereafter, the CD326-depleted cells were reacted with the corresponding antibodies and analyzed on the flow cytometer to identify BM-MSC expressing CD200, GD2 or CD271 but not CD45. In addition, the CD326-depleted cells were reacted with the corresponding antibodies and analyzed on the flow cytometer for $VEGFR1^+CD34^+$ $VEGFR2^-CD31^-$ cells.

The Kendall's τ non-parametric correlational test was used to determine the relationship between either BM-MSC or $VEGFR1^+CD34^+$ cells and BCIC.

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Conflicts of Interest

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