Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

Mesenchymal stromal cells in adipose tissue of breast cancer patients: a cross-sectional study

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Received November 15, 2019; **Accepted** May 3, 2020; **Published** May 15, 2020

Doi: http://dx.doi.org/10.14715/cmb/2020.66.2.11

Abstract: Mesenchymal stromal cells (MSCs) are important components of the tumor microenvironment and are believed to facilitate cancer growth affecting cancer cells. We investigated this issue in breast cancer patients. In this cross-sectional study biopsy or mastectomy specimens from breast cancer patients who underwent surgery were evaluated based on the presence of MSCs. Flow cytometric analysis was used to evaluate the expression of CD73, CD90, CD105, and absence of CD11b or CD14, CD79, CD45, and HLA-DR expression as the criteria of MSC presence. Patients were divided into MSC (+) or (-). SPSS software was used to evaluate the association of age, tumor type, tumor size, disease grade, stage, and metastasis with the presence of MSC cells in adipose tissue of specimens. 82 patients with a mean age of 44.43±9.2 years were included in the study. MSC (+) happened in 13 (15.83) patient's samples. A regression model was conducted to evaluate the association of patient characteristics and the MSC positivity. The patient's age was associated with MSC positivity (B=-0.194, p=0.006); and the disease stage was associated with MSC positivity (B=-0.734, p=0.019). Significantly lower age of patients was seen in MSC (+) patients vs. MSC (-) patients (p=0.002). While no difference in the case of tumor type, tumor size, disease grade, stage, and metastasis was seen between two groups (P>0.05). Our result indicated that Mesenchymal stromal cells are seen more in the tumor microenvironment of younger breast cancer patients. Also, patients having mesenchymal stromal cells may have milder disease and lower disease stage.

Key words: Mesenchymal stromal cells; Breast cancer; Flow cytometry.

Introduction

Breast cancer is one of the most common cancers among women in the world, and global statistics show that the prevalence of this disease is increasing (1). Although several risk factors for breast cancer have been identified, the exact cause of cancer remains unclear (2). Stem cells are present in many different somatic tissues and play an important role in their physiology (3). In terms of origin, stem cells are divided into two main categories: embryonic cells and adult stem cells. One of the most important adult stem cells that have attracted the attention of most researchers today is the mesenchymal stem cells (4). Mesenchymal stromal cells (MSCs) are fresh and cells stem, which could be seen in numerous tissues, including the bone marrow, adipose tissue, umbilical cord, and placenta. Mesenchymal stem cells are involved in repairing tissues of mesenchymal origin, such as bone, cartilage, muscle, tendon, and fat, and are also supporting cells for hematopoietic cells in the bone marrow. The most readily available source of these cells is the bone marrow, although they can be isolated from other sources such as infants, umbilical cord blood, and amniotic fluid (5). Interestingly, they show the ability to multiply in the laboratory as sticky fibroblast-like cells attached to the bottom of plastic containers. No specific markers have been obtained for these cells, but these cells lack the hematopoietic markers of CD34, CD45, CD14, as well as the endothelial markers of CD34, CD31, VWF (Von Willebrand factor), and a large number of adhesive factors like CD90 and CD29, CD44 and Indicators of SH-2, Sh-3, SH-4 stromal cells and cytokine receptors such as interleukin 1 receptor (IL1-R) and TNF receptor are expressed in these cells (6). TNFα will transmit signals of direct interactions between MSC and certain breast cancer cells that support an unusual MSC/breast cancer cell fusion mechanism that results in the development of hybrid breast cancer cells (7). While another study indicated that Human MSCs can inhibit the growth of human breast cancer cells (8). Minimal criteria for defining multipotent mesenchymal stromal cells are known to be positive for CD90, CD73, and CD105 markers and are negative for CD45, CD34, CD31, CD14, CD19, and HLA-DR (9). As in previous studies, researchers had tended to evaluate in-vitro interaction of these cells and breast cancer, we used minimal criteria of MSCs to investigate their relevance with clinical aspects of breast cancer.

Materials and Methods

A biopsy or mastectomy specimen from breast cancer patients who underwent surgery between 2017 and 2018 was selected. Initially, histopathological slides of these patients were evaluated, tumor type and its stage on the basis of the Bloom-Richardson system. The TNM method was used to determine Tumor metastasis. Metastases were identified and recorded by a biochemical method, X-ray, ultrasound or elevation in blood markers approved by a specialist physician. Appropriate slides and paraffin blocks of each patient were selected for immunohistochemical examination.

Adipose tissue-derived mesenchymal stem cells were derived from the patient's adipose tissue of the breast, following written informed consent. After physical destruction and enzymatic degradation of the adipose tissue, the cells were harvested in a minimum essential medium (MEM-5-007) (Sigma-Aldrich, St. Louis, MO); Complemented with 1 percent penicillin/ streptomycin solution (P / S; 100 IU / mL penicillin, 100 IU / mL streptomycin; Lonza, Verviers, Belgium), 2 mM L- (Lonza) and 15 percent bovine fetal serum (FBS; Lonza) (MSC medium); and sown in T175 culture flasks (Greiner Bio-, Kremsmunster, Germany) at 37 ° C, 5 percent CO2, and 95 percent humidity. Cultures were renewed twice a week. When cultures crossed 90 percent confluence, MSC was extracted from culture flasks using 0.05 percent trypsin-EDTA (Life Technology, Bleiswijk, Netherlands). CD73, CD90, CD105, CD45, CD34, CD14, CD79, and HLA-DR markers were assessed in the extracted cells by the flow cytometer (BD Biosciences) and evaluated with the program Kaluza Analysis 1.3 (Beckman-Coulter, Brea, CA) (10).

Results

The mean age of evaluated patients was 44.43±9.2 years. The results of the present study showed that out of 82 breast cancer patients we evaluated, not otherwise specified (NOS) breast cancer was the most prevalent carcinoma type (82.9%), following lobular carcinoma (9.8%), tubular carcinoma (2.4%), Modular carcinoma (2.4%), and Papillary carcinoma (1.2%) and cribriform carcinoma (1.2%) (Table 1).

Samples assessment for presence of MSC were shown in Figure 1, as the incidence of criteria of MSC. CD73, CD90, CD105, CD45, CD34, CD14, CD79, and HLA-DR were seen in 57.31%, 47.56%, 59.75%, 51.21%, 43.90%, 46.34 %, 41.46%, and 37.80% of samples, respectively. MSC (+) happened in 13 (15.83) patient's sample.

A regression model was conducted to evaluate the association of patient characteristics and the MSC positivity. As shown in Table 2, the patient's age was associated with MSC positivity (B=-0.194, p=0.006); and the disease stage was associated with MSC positivity (B=-0.734, p=0.019).

As shown in Figure 2, further evaluation comparing the age of MSC positive and negative patients revealed a significantly lower age of patients who were MSC (+)

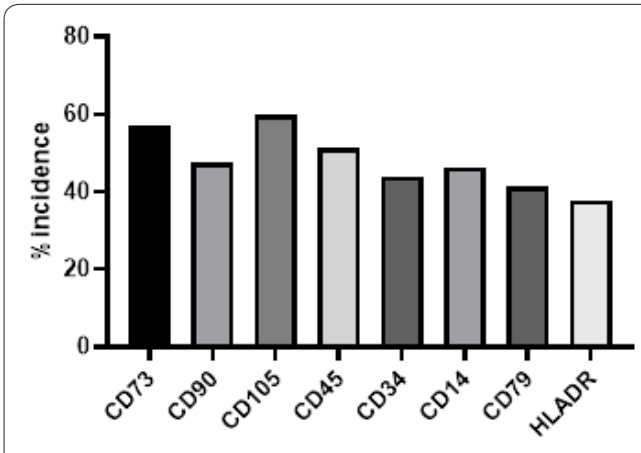

Figure 1. Incidence of criteria of MSC.

Table 1. Study participant's characteristics.

		Frequency	Percent
Type of carcinoma	NOS	68	82.9
	Lobular Carcinoma	8	9.8
	Papillary carcinoma	1	1.2
	Tubular carcinoma	2	2.4
	Cribriform carcinoma	1	1.2
	Modular carcinoma	2	2.4
Tumor size	2> cm	36	43.9
	2-5 cm	25	30.5
	<5 cm	21	25.6
Grade	I	21	25.6
	II	28	34.1
	III	33	40.2
stage	Ia	14	17.1
	IIa	15	18.3
	IIb	14	17.1
	IIIa	12	14.6
	IIIc	12	14.6
	IIIc	15	18.3
Metastasis	Yes	43	52.4
	No	39	47.6

Table 2. Regression of study variables association with MSC positivity.

Variable	B	SE	Sig.
Age	-0.194	0.071	0.006
Type	0.107	0.456	0.814
Tumor size	-0.107	0.476	0.822
grade	-0.045	0.480	0.925
Stage	-0.734	0.313	0.019
Metastasis	1.151	0.923	0.212

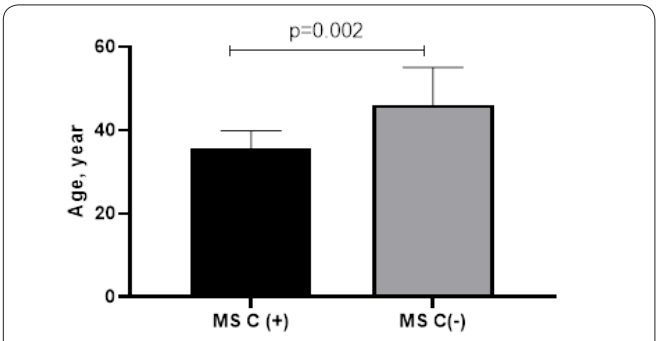

Figure 2. Comparison of age between MSC positive and negative patients.

in their samples (p=0.002). Other comparisons of MSC positive and negative patients by the chi-square test, didn't reveal any significant difference in the case of tumor type, tumor size, disease grade, stage, and Metastasis (P>0.05).

Discussion

Our result indicated that Mesenchymal stromal cells are seen more in the tumor microenvironment of younger breast cancer patients. Also, patients having mesenchymal stromal cells may have milder disease and lower disease stage.

Multipotent Cell stem cells are non-hematopoietic and have a high proliferation capacity. These cells have the ability to differentiate into various cell types. This feature is completely dependent on the conditions and factors in the environment. These cells have the ability to become mesodermal cell lines such as bone cells, chondrocytes and fats; ectodermal cell lines such as neurocytes and endodermal cell lines such as hepatocytes. As shown in our study, it should be noted that the potential for proliferation and differentiation depends on the source of their separation, as the genetic stability of mesenchymal cells derived from the source of adult stem cells is affected by age and environmental stress (11,12).

One of the most important features of these cells, like other stem cells, is the tendency of the tumor. Evidence suggests that adipose tissue-derived mesenchymal cells stimulate tumor growth and metastasis in cancer (13). Obesity, which is associated with increased adipose tissue and increases mesenchymal cells in adipose tissue, is associated with a variety of cancers, such as breast and colon cancer (14), which is not consistent with our study. On the other hand, other studies have found that MSC presence in the tumor environment could have positive effects on disease improvement and have a better progress. Sun et al. stated that MSCs can inhibit the growth of human breast cancer cells. Confusion about these controversies could be due to different definitions of MSCs in experimental methods of detecting cells (15). The causes of cancer can be genetic (16-20).

MSCs extracted from breast malignant tumors have not been yet investigated at the clinical level and most studies are restricted to in-vitro approaches. So their role in breast cancer is still poorly described. Our finding showed that Mesenchymal stromal cells are detected mostly in the tumor microenvironment of younger breast cancer patients. Patients having mesenchymal stromal cells can often have milder diseases and a lower stage of the disease.

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