RESEARCH ARTICLE

Editorial Process: Submission:09/13/2023 Acceptance:01/19/2024

Phenotypic Analysis of Circulating Myeloid Derived Suppressor Cells and Their Subpopulations in Egyptian Females with Breast Cancer: A Single-Centre Case-Control Study

Salma M.Saed[1], Shimaa Abbas[1]*, Mervat S. El Ansary[1], Walaa Abdelfattah[1], Karim K Maurice[2], Mohamed Emam Mohamed[3], Dina M. Tawfik Koptan[1]

Abstract

Background: Myeloid-derived suppressor cells (MDSC) are immature myeloid cells with suppressive function that has been thoroughly documented in the setting of cancer. Our purpose was to evaluate levels of MDSC and their subsets in a cohort of Egyptian patients with breast cancer. **Methods:** Evaluation of peripheral blood total MDSC and its subset was done using multicolor flowcytometry in 30 malignant, 10 benign breast tumor patients and 10 healthy control females. **Results:** BC patients had higher total MDSC levels compared to controls (p= 0.01) particularly the Monocytic MDSC (M-MDSC) and abnormal MDSC subsets (p = 0.001 and p <0.001, respectively). A tumor size > 2 cm exhibited significantly higher granulocytic MDSCs (G-MDSCs) compared to tumor size < 2 cm (p= 0.02) whereas abnormal MDSCs were significantly higher in patients with a tumor size < 2 cm (p = 0.037). **Conclusion:** MDSC and its subsets can be used as a prognostic marker of tumor size as well as a potential targets for treatment in breast cancer patients.

Keywords: Breast Cancer- MDSC- Neoadjuvant therapy

Asian Pac J Cancer Prev, **25 (1)**, 257-263

Introduction

Breast cancer (BC) is the most frequently diagnosed malignancy worldwide and is the second leading cause of cancer-related deaths in females [1]. Remarkably, more than half of BC diagnoses and two-thirds of BC related deaths were reported in low- and middle-income countries in 2020 [2]. In Egypt, BC accounts for 38.8% of cancers in females, with the estimated number of BC cases nearly 22,700 in 2020 and is forecasted to rise exponentially over time [3]. Several factors contribute to BC heterogenicity including variations in genomic, epigenomic, transcriptomic, and proteomic characteristics of malignant cells. These factors affect tumor proliferation, apoptosis, metastasis, as well as therapeutic response [4].

Evidence supports that the development and progression of cancer occurs in concert with alterations in the surrounding stroma. The tumor microenvironment (TME) encompasses several stromal cells, fibroblasts, and endothelial cells as well as innate and adaptive immune cells populations. Through cell-cell contact or the production of extracellular matrix complexes and soluble substances that influence the TME, these cells engage in complex interactions with cancer cells [5]. Tumor immunoediting by innate and adaptive immune cells that together form the BC Immune Microenvironment (BCIM) is a vital determinant of tumor progression [6]. Surprisingly, immunosuppressive cells, especially myeloid-derived suppressor cells (MDSC), hinder the anti-cancer immune response generated by immunostimulating cells like macrophages, lymphocytes, natural killer cells, and innate lymphoid cells [7].

MDSC are a heterogeneous population of pathologically activated progenitors and precursors of myeloid cells, which fail to terminally differentiate into mature cells such as dendritic cells and macrophages. MDSC are defined by their morphology, surface phenotype, and functions. Based on their different cell surface antigen expressions, MDSC are phenotypically classified into polymorphonuclear or granulocytic MDSC (G-MDSC), with a morphology that resembles granulocytes, and are CD11b+ CD14− CD15+ CD33+ cells in humans; the monocytic MDSC (M-MDSC), with a typical monocyte morphology, and are CD11b+ CD14+ CD15− CD33+ HLA− DR−/low [8]. The more immature MDSC are characterized as Lin- (including CD3, CD14, CD15, CD19, CD56) HLA-DR-CD33+ the so-called "early stage-MDSC" (e-MDSC) (Sánchez-León et al., 2023). Abnormal early differentiation of myeloid/

*[1]Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Egypt. [2]Department of General Surgery, Faculty of Medicine, Cairo University, Egypt. [3]Department of Anatomic Pathology, Faculty of Medicine, Cairo University, Egypt. *For Correspondence: shi.mo.88@hotmail.com*

monocyte lineage cells generates abnormal MDSC which are CD14+ CD15+ CD33+ HLA− DR−/low [9].

Despite the fact that treatment alternatives have improved BC patients' outcomes, many still develop metastatic disease, which is still challenging to cure [10, 11]. One study provided scientific evidence about the benefits of beetroot extract when given with neoadjuvant regimen in cases of breast cancer which improved the pathological response of cancer cells by increasing the immune response in the tumor environment, especially increasing CD8 T cells and decreasing MDSC [12].

Several studies suggested that MDSC play a detrimental role in BC progression [5]. Research reveals the association between raised levels of MDSC in peripheral blood of BC patients and the disease prognosis, since it's associated with advanced stages, higher tumor burden, and lower progression-free survival and overall survival, and lesser response to chemotherapy, radiotherapy, immunotherapy, and targeted therapies [13]. Interestingly, clinical trials combining immunotherapy with other types of therapies in BC to target MDSC are currently underway [14].

To the best of our knowledge, there is a paucity of information concerning the relationship of MDSC in Egyptian patients with BC [15, 16], hence, we aimed to determine the frequency of different MDSC subsets in a cohort of female patients with BC compared to subjects with benign breast lesions as well as healthy controls. We also aimed to investigate the potential association of MDSC and their subsets with tumor size, distinct tumor histopathology, hormonal markers, and intake of neoadjuvant therapy.

Materials and Methods

Ethical approval

The collection of blood for PBMC isolation and MDSC analysis was approved by the medical ethics committee of Faculty of Medicine, Cairo University (approval number: N-143-2018). All procedures were carried out in accordance with the Helsinki Declaration's ethical principles. The study purpose was clarified to all participants prior to study enrollment. Prior to participating in the study, each subject provided informed consent.

Study population

We enrolled 30 patients that had histologically confirmed BC that was treated in the Department of General Surgery, Cairo University, between March 2019 and November 2019. All patients were staged in accordance with National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology: Breast Cancer Screening and Diagnosis [17]. Fifty percent of patients received preoperative anticancer treatment. Ten patients with benign breast lesions were enrolled. Exclusion criteria for participation were presence of active infection(s), coexistent malignancies or inflammatory or autoimmune disease(s) at the time of sampling. The control group included 10 healthy volunteers without chronic disorders. All the study participants were females.

Blood sampling and isolation of peripheral blood mononuclear cells (PBMNs)

Six mL of blood were collected into K2 ethylene diamine tetra acetic acid Vacutainers™ for the immediate isolation of PBMCs using the Ficoll density gradient centrifugation method (Ficoll-Hypaque; GE Healthcare Life Sciences, Chalfont, UK). After two washes in phosphate buffer saline, cells were assessed for viability by trypan blue exclusion. The number of viable cells present in the cell suspension ranged between 1.68 x 106 and 26 x 106 per ml. For freezing, Roswell Park Memorial Institute medium-1640 (RPMI; ThermoFisher Scientific) supplemented with 40% fetal bovine serum (FBS; Biowest) and 10% dimethyl sulfoxide was used, and cells were stored in liquid nitrogen immediately at −80 °C for analysis later. After collection of all samples which took around one-year, frozen PBMCs were thawed at 37°C in a water bath. Cells were washed twice in 3−5 ml pre-warmed RPMI and resuspended in 1 ml PBS. Cell viability was determined by trypan blue exclusion and all samples showed viability of more than 90%.

Flowcytometric analysis

A multicolor flow cytometric analysis was performed using a series of monoclonal antibodies (Beckman Coulter, Fullerton, CA) including fluorescent isothiocyanate (FITC)-conjugated anti-CD33 (cat. no. IM1135U; 10µl), phycoerythrin (PE)-conjugated anti-CD15 (cat. no. IM1954U; 10µl), allophycocyanin (APC)-conjugated anti-CD14 (cat.no. IM2580U; 5 µl), and PC7-conjugated anti-HLA-DR (cat.no. B49180; 10 µl). Labelled antibodies were added to each sample (100 µl) and incubated for 20 minutes in the dark at room temperature.

Data acquisition and analysis were performed on a FACSCanto 10 flow cytometer (BD Biosciences, San Jose, CA, USA) using BD FACS DIVATM software. The labelled cells were first gated based on their lack of expression of HLA-DR; was composed of HLA-DR− cells. The fraction of cells in this population that expressed the myeloid marker CD33 was then determined. Then within this population, the fraction of cells expressing CD15 and CD14 were evaluated. In the present study, MDSC were defined as DR-/low/CD33+. Moreover, MDSC subsets were determined as G-MDSC: CD15+/CD14-, M-MDSC: CD14+/CD15-, e-MDSC: CD14-/CD15- and abnormal MDSC: CD15+/CD14+ MDSC. Figure 1 presents flowcytometry scatter plot results, based on the gating strategy for one of the study participants.

Statistical studies

Analysis of data was done by IBM computer using statistical program for social science version 21. Description of quantitative variables as mean, standard deviation (SD), median and interquartile range (IQR) for quantitative variables. Description of qualitative variables as number and percent. Mann Whitney test was used instead of independent t-test to compare quantitative variables between two groups in non-parametric data (SD>30%mean). Comparison between quantitative variables between more than two categories done using one-way Anova test in noramally distributed data and

Kruskal wallis test in not normal distributed test. In both conditions pairwise comparison checked. Chi square test used to compare between qualitative variables and exact correction when cell contain count less than 5. P ≤0.05 was considered significant while p <0.01 was considered highly significant.

Results

Patients' characteristics

Our study included thirty female patients who were diagnosed to have BC by tru-cut needle biopsy. Their mean ages were 52 ± 7.8 (range, 35–60). Fourteen (46.7%) patients were classified with stage I cancer, 10 (33.3%) with stage II, 4 (13.3%) with stage III and 2 (6.7%) patients with stage IV. The most prevalent cancer type was invasive duct carcinoma (83.3%), followed by lobular duct carcinoma (10%) and invasive duct carcinoma with co-existing duct carcinoma in situ (6.7%). Among all patients, 10/30 (33.3%) had a positive family history of BC. The family history was considered as positive when the patient had ≥ 1 relative with breast cancer within 3 generations. 3/30 (10%) patients had a previous history of BC. At the time of blood draw, fifteen patients (50%) were untreated, and 15 (50%) patients received neoadjuvant therapy prior to tumor resection in the form of chemotherapy (30%), hormonal therapy (13.3%), combined hormonal and chemotherapy (6.7%). Ten female patients with biopsy-proved breast benign tumors were also recruited. Their mean ages were 34.8 ± 9.8 (range, 28–60). The pattern of benign breast disease, fibroadenoma was the most common lesion constituting 6/10 (60%) cases followed by hamartoma in 2/10 (20%) cases, fibrocystic disease in 1/10 (10%) patient and phylloid in 1/10 (10%) patient. 2/10 (20%) patients with benign tumors had a positive family history of BC. All ten patients had no previous history breast tumors. Control group was 10 age- and gender-matched healthy participants with no chronic disorders, randomly selected from the out-patient clinic with a mean age 48.1 ± 7.65 (range, 35–60) years.

The percentages of circulating MDSC total cells were significantly increased in patients with BC compared to controls (median= 1%, IQR= 0.7–1.3 vs. median= 0.3%,

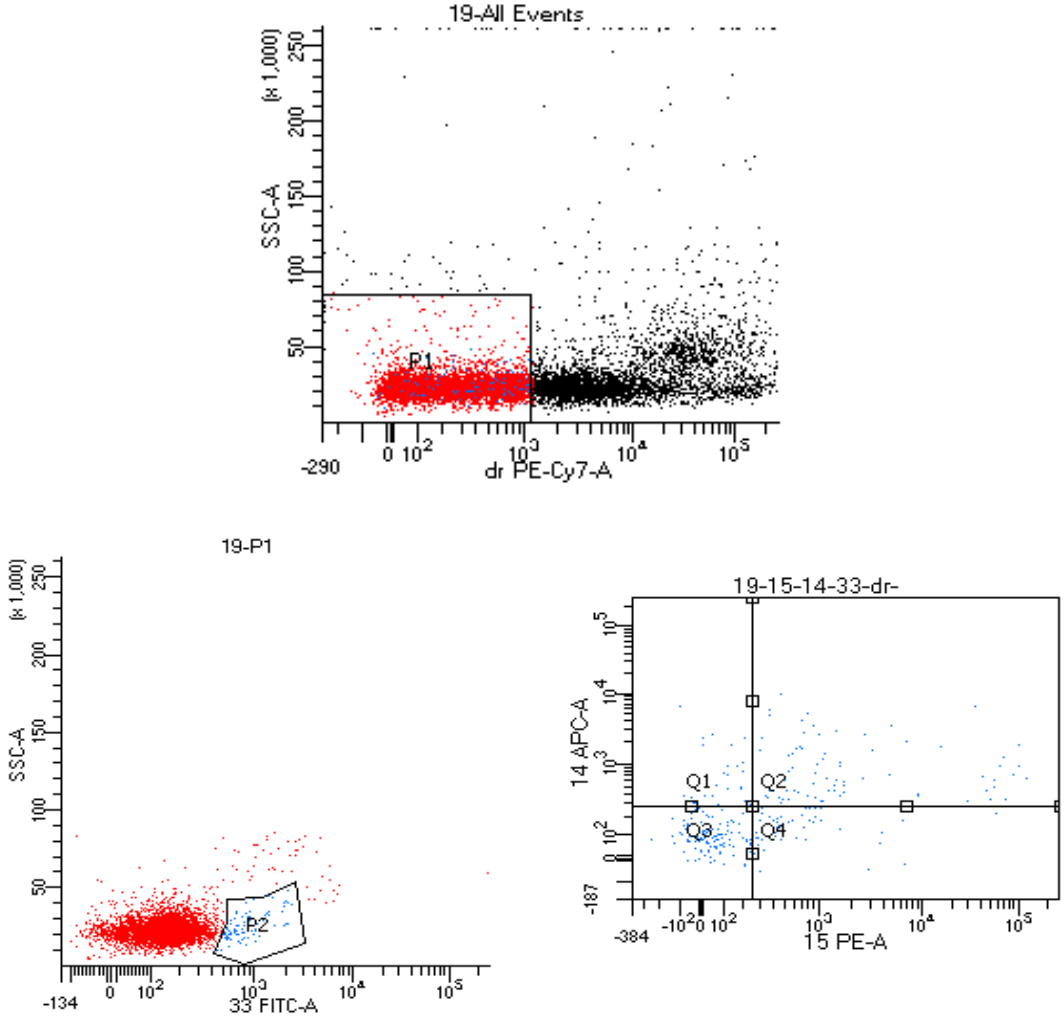

Figure 1. Representative Flow Cytometry Gating for Circulating HLA-DR−/CD33+ myeloid-derived Suppressor Cells in Peripheral Blood Mononuclear Cells of Patients with Breast Cancer. The dot plot shows the expression of cells positive for surface markers of MDSC on gated HLA-DR$^{-/low}$ CD33+ cells including monocytic MDSC (M-MDSC): CD14+/CD15− (Q1), abnormal MDSC (Q2), e-MDSC: CD14−/CD15− (Q3), and granulocytic MDSC (G-MDSC): CD14−/CD15+ (Q4).

Table 1. Comparison between Patient Groups with BC, benign Breast Tumors and Controls Regarding Circulating MDSC Levels.

	Breast cancer (n = 30)[a]	Benign breast tumors (n=10)[a]	Control (n=10)[a]	p value [b]
Total MDSC	1 (0.7–1.3)	0.6 (0.4–0.7)	0.3 (0.2–0.5)	0.01c
M-MDSC	12.7 (8.4–20.3)	4.7 (3.6–9.8)	0.7 (0.3–1.7)	0.001c
G-MDSC	12.7 (9.4–20.2)	10.5 (7–16.7)	7.2 (4.4–10.9)	0.1
e-MDSC	44.5 (31.6–58.1)	77.6 (71.9–86)	90.8 (87.4–94)	<0.001cd
Abnormal MDSC	22.6 (10.5–28.8)	2.1 (1.4–4.2)	0.5 (0.3-0–8)	<0.001cd

[a], Data are presented as median and interquartile range. [b], p-values < 0.05 were considered statistically significant. csignificant p value between breast cancer and control groups. dsignificant p value between breast cancer and benign breast tumors groups. Abbreviations: BC, breast cancer; e-MDSC, early-stage myeloid derived suppressor cells; G-MDSC, granulocytic myeloid derived suppressor cells; M-MDSC, monocytic myeloid derived suppressor cells; n, number.

IQR= 0.2–0.5, respectively; p = 0.01) but not those with benign breast tumors (median= 0.6%, IQR= 0.4–0.7). Likewise, a highly significant increase in M-MDSC in patients with BC was observed compared to controls (median= 12.7%, IQR= 8.4 –20.3 vs. median = 0.7%, IQR= 0.3–1.7, p = 0.001) but not those with benign breast tumors (median= 4.7%, IQR= 3.6–9.8). A highly significant increase was seen in abnormal MDSC in patients with BC compared to those with benign breast tumors and controls (median= 22.6%, IQR= 10.5–28.8, median=2.1%, IQR= 1.4–4.2 and median=0.5%, IQR= 0.3–0.8, respectively; p <0.001). Conversely, a highly significant decrease was seen in e-MDSC in patients with BC compared to those with benign breast tumors and controls (median= 44.5%, IQR= 31.6–58.1, median=77.6%, IQR= 71.9–86 and median= 90.8%, IQR= 87.4–94, respectively; p <0.001). There was no significant difference in the percentages of G-MDSC between patients with BC, subjects with benign breast tumors and healthy donors (median= 12.7%, IQR= 9.4–20.2, median= 10.5%, IQR= 7–16.7 and median = 7.2%, IQR= 4.4–10.9, respectively). Data are shown in Table 1.

In the present study, G- MDSC were increased in patients with positive family history of BC compared to those with negative family history (median= 25.5%, IQR= 11.8–33.5, median=12%, IQR= 8.4–15.1, respectively; p <0.02). Furthermore, we found significantly high rates of G-MDSC in patients with tumor volume greater than 2 cm (median= 28.9%, IQR= 20.2–30.7) compared to tumor volume smaller than 2 cm (median= 11.9%, IQR= 8.7–17.3; p = 0.02). On the contrary, abnormal MDSC were significantly higher in patients with a tumor volume smaller than 2 cm than larger tumors (median= 23.6%, IQR= 15.5–30.4, median=7.2%, IQR= 6.6–10.5, respectively; p = 0.037).

Notably, no differences between patients were observed regarding total MDSC and its subsets in relation to BC histopathology, lymph node involvement, distant metastasis, the presence of estrogen receptors, progesterone receptors or human epidermal growth factor receptor (HER2/neu). Patients who received neoadjuvant therapy compared to those who did not get neoadjuvant therapy prior to surgery did not differ in terms of levels of MDSC and its subsets (The data are summarized in electronic supplementary Tables S1–S8).

Discussion

MDSC have been implicated in sustaining progression of numerous malignancies including BC [18]. We demonstrated that circulating total MDSC were significantly increased in patients with BC, but not in subjects with benign breast lesions, compared with the control group (p = 0.01). Notably, alteration in MDSC levels in BC was reported in several studies, but the results are inconsistent, with some researchers demonstrating elevated MDSC in peripheral blood of patients with BC [19, 20], whereas another report demonstrated that tumor-infiltrating MDSC expansion was not reflected in the peripheral blood of BC patients [21]. The lack of consistency of markers to identify MDSC and the clinical parameters of study groups may explain the discrepancies in the results among different studies.

As a draw back in the current study, the usage of thewed MDSCs may affect quality of the results [22] but as a compensation for that draw back viability testing done directly before using MDSCs in flowcytometry demonstrated that every sample used showed viability more than 90% as mentioned in the methodology section.

No significant differences in MDSC levels were observed among our patients with BC with regards to lymph nodes involvement, lymph vascular emboli and distant metastasis. Intriguingly, MDSC play a fundamental role in orchestrating immunosuppression within the TME via multiple mechanisms that dampen antitumor immunity and promote tumor progression including induction of a highly oxidative microenvironment [23], production of cytokines and immunosuppressive mediators [24], metabolite depletion, and expression of immune checkpoint molecules [25]. Tumor cells recruit MDSC, T-regulatory cells, and M2 macrophages, to shape a pro-tumorigenic microenvironment [26].

Even though immunotherapies improved the outcome of many BC patients, nevertheless, it has been demonstrated that the immune context of the TME plays a key role in the development of resistance mechanisms to immunotherapy in BC [27, 24]. Currently, there are various immunotherapeutic strategies in BC to target MDSC, either decreasing their number or inhibiting their immunosuppressive functions [28]. Interestingly, recent research revealed that MDSC depletion combined with HER2-targeted passive immunotherapy using monoclonal antibodies (mAbs) dramatically reduced tumor growth

and increased tumor rejection in a murine early BC model [29].

We observed a significant increase in circulating M-MDSC in our BC patients, but not in subjects with benign breast tumors, compared to controls ($p = 0.001$). Worth mentioning, earlier studies reported elevated circulating M-MDSC in patients with primary BC, loco-regional recurrence, metastasis to lymph nodes and visceral organs and in more advanced stage of the disease [30, 31]. In contrast to the aforementioned studies, Toor (2017) found no difference between M-MDSC levels in BC patients compared to controls [21]. Remarkably, recent research demonstrated that elevated levels of M-MDSC in peripheral blood favours an immunosuppressive microenvironment that promotes metastasis and BC progression [32].

In line with earlier studies [33, 34], no difference was found between our patients with BC or benign breast lesions and controls regarding G-MDSC. Nevertheless, significantly increased G-MDSC was found in our patients with positive family history of BC compared to patients without affected family members ($p = 0.02$). Moreover, we observed a significant increase in G-MDSC ($p = 0.019$) among our BC patients with tumor size > 2 cm. Worthmentioning, Toor (2017) demonstrated that expansion of tumor-infiltrating G-MDSC in BC was not reflected in peripheral blood. On the contrary, Safarzadeh (2019) [20] reported significantly elevated circulating G-MDSC in BC patients compared with controls. Remarkably, Mehmeti-Ajradini (2020) [35] presented evidence that G-MDSC were generated in patients with metastatic BC, as cells of the neutrophil lineage at a range of maturation stages [35], promoting BC growth and myeloid immune cell exclusion.

In the current work, e-MDSC were significantly decreased in patients with BC compared with subjects with benign tumors and controls ($p <0.001$), nonetheless, no significant difference was observed between patients with benign breast lesions and controls. In contrast to our findings, Yu (2013) demonstrated that e-MDSC were significantly increased in patients with BC compared with controls [33]. The fact that their study included patients with invasive duct carcinoma unlike the current work, could be a plausible explanation, nevertheless, further studies are warranted to confirm this hypothesis. Despite the fact that e-MDSC have potent immunosuppressive capacity and play a vital role in carcinogenesis, nonetheless, the mechanism underlying their development in cancer remain undisclosed [36].

We found that abnormal MDSC were significantly increased in patients with BC compared to patients with benign breast lesions as well as controls ($p <0.01$), yet no significant difference was found between patients with benign tumors and the control group. In line with our findings, Safarzadeh (2019) [20] reported increased abnormal MDSC in patients with BC compared with healthy controls. Furthermore, we observed a significant decrease in abnormal MDSC ($p = 0.037$) among our BC patients with tumor size > 2 cm.

The results of the present work were in agreement with those of other reports, which demonstrated no differences regarding MDSC levels between BC patients with positive estrogen, progesterone or HER2/neu receptor subtypes compared to patients that were receptor–negative [33, 20]. There were no differences among our patients with BC with regards to MDSC levels in relation to BC history, tumor histopathology or Ki-67. There was no difference in MDSC levels between patients who received neoadjuvant therapy compared to those who didn't receive neoadjuvant therapy prior to surgery.

In conclusion, the present study showed that the frequencies of circulating total MDSC, M- MDSC and abnormal MDSC were significantly increased in an Egyptian cohort with BC. The frequency of G-MDSC positively correlated with tumor size and positive family history of BC, while abnormal MDSC negatively correlated with tumor size. The current study elaborated the importance of MDSCs in breast cancer follow up and prognosis. A deeper understanding of the myeloid derived suppressor cell populations' effects on cancer progression and response to therapies may contribute to the development of more effective treatments for BC.

Limitations of the study

1. The current work didn't study the MDSC in the tumor micro-environment which is important hence studies about whether peripheral blood MDSC really reflect their percentage in tumor microenvironment were controversial.
2. The relatively small number of the studied group.

Author Contribution Statement

Mervat El Ansary designed the study and supervised all steps of the study, Shimaa Abbas conducted almost all the experiments and wrote the first draft of the manuscript, Salma M.Saed, Walaa Abdelfattah, Dina M.T. Koptan checked over the laboratory work and the manuscript, Mohamed Emam performed the pathological laboratory aspect of the study and Karim K Maurice helped in study design, recruitment and diagnosis of patients participating in the study.

Acknowledgements

Part of the data is used for approved MD thesis of Shimaa Abbas with some significant improvements.

We thank the main laboratory of faculty of medicine, Cairo university in providing facilities for experiments.

Conflict of Interest

We declare that we have no conflict of interest.

Ethical issue

The study protocol was approved by the medical ethics committee of Faculty of Medicine, Cairo University (approval number: N-143-2018). All procedures were carried out in accordance with the Helsinki Declaration's ethical principles. The study purpose was clarified to all participants prior to study enrollment. Prior to participating in the study, each subject provided informed consent.

References

1. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, et al. Cancer statistics for the year 2020: An overview. Int J Cancer. 2021. https://doi.org/10.1002/ijc.33588.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-49. https://doi.org/10.3322/caac.21660.
3. Azim HA, Elghazawy H, Ghazy RM, Abdelaziz AH, Abdelsalam M, Elzorkany A, et al. Clinicopathologic features of breast cancer in egypt-contemporary profile and future needs: A systematic review and meta-analysis. JCO Glob Oncol. 2023;9:e2200387. https://doi.org/10.1200/go.22.00387.
4. Visvader JE. Cells of origin in cancer. Nature. 2011;469(7330):314-22. https://doi.org/10.1038/nature09781.
5. Salemme V, Centonze G, Cavallo F, Defilippi P, Conti L. The crosstalk between tumor cells and the immune microenvironment in breast cancer: Implications for immunotherapy. Front Oncol. 2021;11:610303. https://doi.org/10.3389/fonc.2021.610303.
6. Lorenzo-Sanz L, Muñoz P. Tumor-infiltrating immunosuppressive cells in cancer-cell plasticity, tumor progression and therapy response. Cancer Microenviron. 2019;12(2-3):119-32. https://doi.org/10.1007/s12307-019-00232-2.
7. Gatti-Mays ME, Balko JM, Gameiro SR, Bear HD, Prabhakaran S, Fukui J, et al. If we build it they will come: Targeting the immune response to breast cancer. NPJ Breast Cancer. 2019;5:37. https://doi.org/10.1038/s41523-019-0133-7.
8. Millrud CR, Bergenfelz C, Leandersson K. On the origin of myeloid-derived suppressor cells. Oncotarget. 2017;8(2):3649-65. https://doi.org/10.18632/oncotarget.12278.
9. Nishimoto N, Murakami M. Appearance of cd14+ cd15+ poplulation during the differentiation from ra-ips cells into monocytes. . Ann Rheum Dis. 2014;73:820.
10. Hu M, Yao J, Cai L, Bachman KE, van den Brûle F, Velculescu V, et al. Distinct epigenetic changes in the stromal cells of breast cancers. Nat Genet. 2005;37(8):899-905. https://doi.org/10.1038/ng1596.
11. Miglietta F, Bottosso M, Griguolo G, Dieci MV, Guarneri V. Major advancements in metastatic breast cancer treatment: When expanding options means prolonging survival. ESMO Open. 2022;7(2):100409. https://doi.org/10.1016/j.esmoop.2022.100409.
12. Susilowati S, Istiadi H, Suhartono S, Prajoko YW, Riwanto I, Susilaningsih N, et al. The role of beetroot extract in overcoming chemoresistance of neoadjuvant adriamycin cyclophosphamide regimen by targeting immune response in tumor microenvironment: A preclinical study in mammary adenocarcinoma rats. Asian Pac J Cancer Prev. 2022;23(3):1061-8. https://doi.org/10.31557/apjcp.2022.23.3.1061.
13. De Cicco P, Ercolano G, Ianaro A. The new era of cancer immunotherapy: Targeting myeloid-derived suppressor cells to overcome immune evasion. Front Immunol. 2020;11:1680. https://doi.org/10.3389/fimmu.2020.01680.
14. Sánchez-León ML, Jiménez-Cortegana C, Silva Romeiro S, Garnacho C, de la Cruz-Merino L, García-Domínguez DJ, et al. Defining the emergence of new immunotherapy approaches in breast cancer: Role of myeloid-derived suppressor cells. Int J Mol Sci. 2023;24(6). https://doi.org/10.3390/ijms24065208.
15. Moaaz M, Lotfy H, Motawea MA, Fadali G. The interplay of interleukin-17a and breast cancer tumor microenvironment as a novel immunotherapeutic approach to increase tumor immunogenicity. Immunobiology. 2021;226(2):152068. https://doi.org/10.1016/j.imbio.2021.152068.
16. Elbasateeny SS, Yassin MA, Mokhtar MM, Ismail AM, Ebian HF, Hussein S, et al. Prognostic implications of malat1 and bach1 expression and their correlation with ctcs and mo-mdscs in triple negative breast cancer and surgical management options. Int J Breast Cancer. 2022;2022:8096764. https://doi.org/10.1155/2022/8096764.
17. Bevers TB, Anderson BO, Bonaccio E, Buys S, Daly MB, Dempsey PJ, et al. Nccn clinical practice guidelines in oncology: Breast cancer screening and diagnosis. J Natl Compr Canc Netw. 2009;7(10):1060-96. https://doi.org/10.6004/jnccn.2009.0070.
18. Ostrand-Rosenberg S, Fenselau C. Myeloid-derived suppressor cells: Immune-suppressive cells that impair antitumor immunity and are sculpted by their environment. J Immunol. 2018;200(2):422-31. https://doi.org/10.4049/jimmunol.1701019.
19. Andersen MH. Comment on "myeloid-derived suppressor cells suppress antitumor immune responses through ido expression and correlate with lymph node metastasis in patients with breast cancer". J Immunol. 2013;190(11):5341. https://doi.org/10.4049/jimmunol.1390023.
20. Safarzadeh E, Hashemzadeh S, Duijf PHG, Mansoori B, Khaze V, Mohammadi A, et al. Circulating myeloid-derived suppressor cells: An independent prognostic factor in patients with breast cancer. J Cell Physiol. 2019;234(4):3515-25. https://doi.org/10.1002/jcp.26896.
21. Toor SM, Syed Khaja AS, El Salhat H, Faour I, Kanbar J, Quadri AA, et al. Myeloid cells in circulation and tumor microenvironment of breast cancer patients. Cancer Immunol Immunother. 2017;66(6):753-64. https://doi.org/10.1007/s00262-017-1977-z.
22. Lee EJ, Jung S, Park KH, Park SI. Flow cytometry-based immunophenotyping of myeloid-derived suppressor cells in human breast cancer patient blood samples. J Immunol Methods. 2022;510:113348. https://doi.org/10.1016/j.jim.2022.113348.
23. Markowitz J, Wang J, Vangundy Z, You J, Yildiz V, Yu L, et al. Nitric oxide mediated inhibition of antigen presentation from dcs to cd4(+) t cells in cancer and measurement of stat1 nitration. Sci Rep. 2017;7(1):15424. https://doi.org/10.1038/s41598-017-14970-0.
24. Groth C, Hu X, Weber R, Fleming V, Altevogt P, Utikal J, et al. Immunosuppression mediated by myeloid-derived suppressor cells (mdscs) during tumour progression. Br J Cancer. 2019;120(1):16-25. https://doi.org/10.1038/s41416-018-0333-1.
25. Ballbach M, Dannert A, Singh A, Siegmund DM, Handgretinger R, Piali L, et al. Expression of checkpoint molecules on myeloid-derived suppressor cells. Immunol Lett. 2017;192:1-6. https://doi.org/10.1016/j.imlet.2017.10.001.
26. Shou D, Wen L, Song Z, Yin J, Sun Q, Gong W. Suppressive role of myeloid-derived suppressor cells (mdscs) in the microenvironment of breast cancer and targeted immunotherapies. Oncotarget. 2016;7(39):64505-11. https://doi.org/10.18632/oncotarget.11352.
27. Conti L, Ruiu R, Barutello G, Macagno M, Bandini S, Cavallo F, et al. Microenvironment, oncoantigens, and antitumor vaccination: Lessons learned from balb-neut mice. Biomed Res Int. 2014;2014:534969. https://doi.

org/10.1155/2014/534969.
28. de la Cruz-Merino L, Gion M, Cruz J, Alonso-Romero JL, Quiroga V, Moreno F, et al. Pembrolizumab in combination with gemcitabine for patients with her2-negative advanced breast cancer: Geicam/2015-04 (pangea-breast) study. BMC Cancer. 2022;22(1):1258. https://doi.org/10.1186/s12885-022-10363-3.
29. Khaki Bakhtiarvand V, Ramezani-Ali Akbari K, Amir Jalali S, Hojjat-Farsangi M, Jeddi-Tehrani M, Shokri F, et al. Myeloid-derived suppressor cells (mdscs) depletion by cabozantinib improves the efficacy of anti-her2 antibody-based immunotherapy in a 4t1-her2 murine breast cancer model. Int Immunopharmacol. 2022;113(Pt B):109470. https://doi.org/10.1016/j.intimp.2022.109470.
30. Bergenfelz C, Larsson AM, von Stedingk K, Gruvberger-Saal S, Aaltonen K, Jansson S, et al. Systemic monocytic-mdscs are generated from monocytes and correlate with disease progression in breast cancer patients. PLoS One. 2015;10(5):e0127028. https://doi.org/10.1371/journal.pone.0127028.
31. Bergenfelz C, Roxå A, Mehmeti M, Leandersson K, Larsson AM. Clinical relevance of systemic monocytic-mdscs in patients with metastatic breast cancer. Cancer Immunol Immunother. 2020;69(3):435-48. https://doi.org/10.1007/s00262-019-02472-z.
32. Palazón-Carrión N, Jiménez-Cortegana C, Sánchez-León ML, Henao-Carrasco F, Nogales-Fernández E, Chiesa M, et al. Circulating immune biomarkers in peripheral blood correlate with clinical outcomes in advanced breast cancer. Sci Rep. 2021;11(1):14426. https://doi.org/10.1038/s41598-021-93838-w.
33. Yu J, Du W, Yan F, Wang Y, Li H, Cao S, et al. Myeloid-derived suppressor cells suppress antitumor immune responses through ido expression and correlate with lymph node metastasis in patients with breast cancer. J Immunol. 2013;190(7):3783-97. https://doi.org/10.4049/jimmunol.1201449.
34. Jin G, Zhang Y, Chang X, Zhang Y, Xu J, Wei M, et al. Increased percentage of mo-mdscs in human peripheral blood may be a potential indicator in the diagnosis of breast cancer. Oncol Res Treat. 2017;40(10):603-8. https://doi.org/10.1159/000478933.
35. Mehmeti-Ajradini M, Bergenfelz C, Larsson AM, Carlsson R, Riesbeck K, Ahl J, et al. Human g-mdscs are neutrophils at distinct maturation stages promoting tumor growth in breast cancer. Life Sci Alliance. 2020;3(11). https://doi.org/10.26508/lsa.202000893.
36. Jiang M, Zhang W, Zhang R, Liu P, Ye Y, Yu W, et al. Cancer exosome-derived mir-9 and mir-181a promote the development of early-stage mdscs via interfering with socs3 and pias3 respectively in breast cancer. Oncogene. 2020;39(24):4681-94. https://doi.org/10.1038/s41388-020-1322-4.