Clinical and Experimental Medicine (2019) 19:385–391
https://doi.org/10.1007/s10238-019-00559-6

ORIGINAL ARTICLE

Circulating soluble levels of MIF in women with breast cancer in the molecular subtypes: relationship with Th17 cytokine profile

Guadalupe Avalos-Navarro[1] · José Francisco Muñoz-Valle[2] · Adrian Daneri-Navarro[1] · Antonio Quintero-Ramos[1] · Ramon Antonio Franco-Topete[3,4] · Andres de Jesus Morán-Mendoza[5] · Antonio Oceguera-Villanueva[6] · Luis Alberto Bautista-Herrera[2] · Antonio Topete-Camacho[1] · Alicia Del Toro-Arreola[1]

Received: 30 January 2019 / Accepted: 8 May 2019 / Published online: 18 May 2019

Abstract

Breast cancer (BC) is a health problem worldwide; there is evidence that inflammatory cytokines are increased in BC. Macrophage migration inhibitory factor (MIF) has multiple effects on immune cells, inflammation and cancer. Besides, in previous studies, contradictory and uncertain results have been presented on the implication of Th17 cytokine profile in BC. The aim of this study was to evaluate the plasma levels of MIF and the Th17 cytokine profile in BC and their association with their molecular subtypes and clinical stage. A total of 150 women with BC of Ella Binational Breast Cancer Study and 60 healthy women (HW) were evaluated in cross-sectional study. The molecular subtypes were identified by immunohistochemistry. The plasma levels of MIF were quantified by ELISA and Th17 cytokine profile by multiplex system. MIF and IL-17 were significantly increased in BC versus HW (11.1 vs. 5.2 ng/mL and 14.8 pg/mL vs. 2.5 pg/mL $p < 0.001$, respectively). Our analysis showed that both MIF and IL-17A were associated with increased risk of breast cancer (OR 3.85 CI 95% 1.98–7.50 and OR 4.51 95% 1.83–11.15, respectively), higher in aggressive subtypes Luminal B, HER2 and TN. Likewise, we observed positive correlation between MIF and IL-17A ($p < 0.001$). In addition, IL-17E was lower in BC versus HW ($p < 0.001$). Likewise, we observed a positive correlation between MIF and IL-17A ($p < 0.001$). In conclusion, both MIF and IL-17A were associated with high risk for breast cancer and aggressive molecular subtypes.

Keywords MIF · Th17 · Cytokine profile · Molecular subtypes · Breast cancer

✉ Alicia Del Toro-Arreola
lilydeltoro@gmail.com

[1] Laboratorio de Inmunología, Departamento de Fisiología, CUCS, Universidad de Guadalajara, Sierra Mojada # 950, Colonia Independencia, CP 44340 Guadalajara, Jalisco, Mexico

[2] Instituto de Investigación en Ciencias Biomédicas (IICB), Departamento de Biología Molecular y Genómica, Universidad de Guadalajara, Sierra Mojada 950, Col. Independencia, C.P. 44340 Guadalajara, Jalisco, Mexico

[3] Laboratorio de Patología, Departamento de Patología y Microbiología, CUCS, Universidad de Guadalajara, Sierra Mojada 950, Col. Independencia, C.P. 44340 Guadalajara, Jalisco, Mexico

[4] OPD Hospital Civil de Guadalajara, "Nuevo Hospital Civil, Juan I. Menchaca", Guadalajara, Jalisco, Mexico

[5] Hospital de Especialidades, Centro Medico Nacional de Occidente, IMSS, Guadalajara, Jalisco, Mexico

[6] Instituto Jalisciense de Cancerología, Secretaría de Salud, Guadalajara, Jalisco, Mexico

Introduction

According to the World Health Organization, the breast cancer (BC) is the leading cause of death in women [1, 2]. The biomarkers defined by immunohistochemistry of estrogen receptor (ER), progesterone receptor (PR), receptor 2 of human epidermal growth factor (HER2) and Ki67 have been very useful to define the subrogated molecular phenotypes [3, 4]. The molecular subtypes are classified as Luminal A, Luminal B, HER2 and Triple negative (TN) [5, 6]. BC is a heterogeneous disease with different tumor-infiltrating lymphocyte patterns, depending on the molecular subtype and other tumor microenvironment factors with importance for prognosis and predictive to treatment [7, 8]. Of the molecular subtypes, the TN has a greater lymphocytic cell infiltrate, mainly Foxp3+ cells and high IL-6 levels [9], and tumor-infiltrating lymphocytes (TILs) are currently used as a prognostic and predictive marker in BC mainly in HER2 and TN subtypes [10]. In addition, analysis by

immunohistochemistry reveals a high infiltration of IL-17 producing cells and low CD8+ cells, suggesting that Th17 cells participate against an effective immune response to eliminate the tumor in BC patients [11].

In other tumors, different cytokines, such as MIF and IL-17A, are involved in tumor pathology [12, 13]. MIF is a multifunctional protein composed of 115 amino acids with a molecular weight of 12.5 KD forming a homotrimer; the trimer conformation is the active form. The cells that express MIF are mainly T and B lymphocytes [14], monocytes, macrophages, dendritic cells and granulocytes [15]. Some reports suggest that MIF contributes to tumor progression since soluble and intracellular levels are elevated in cancer patients in correlation with the stage, angiogenic growth and neovascularization [16]. In addition, MIF can also bind to chemokine receptors CXCR2, CXCR4 and CXCR7 regulating the tumor-infiltrating lymphocytes [17, 18]. There is evidence that MIF favors the polarization of activated T lymphocytes toward the Th17 profile in mouse lymph nodes and the production of IL-17. This depends on the production of IL-Iβ and IL-23, which involves MAPK and STAT3 signaling pathways [19]. Th17 infiltrates have been reported in different cancer types, including melanoma, colon, prostate and BC. Cancer cells, tumor-derived fibroblasts, tumor-associated macrophages and antigen-presenting cells secrete several cytokines (IL-1β, IL-6, IL-23 and TGF-β) for the differentiation of Th17 cells [20]. In BC, PGE-2 generates IL-23, necessary for the maintenance and stabilization of Th17 cells [20].

Some reports suggest that MIF is involved in carcinogenesis and progression of BC, but the role and clinical importance of this cytokine are still unclear in BC patients [21]. In this work, for the first time, we evaluated the association of soluble levels of MIF protein with the Th17 cytokine profile, according to the molecular BC subtypes and clinical stage.

Patients and methods

To evaluate the association of soluble levels of MIF with the Th17 cytokine profile, according to the molecular BC subtypes, we used an analytical cross-sectional design. A total of 150 women diagnosed histopathologically with BC, from the Guadalajara, Jalisco Mexico of Ella Binational Breast Cancer Study (Case-Only Study) were included from three different hospitals in Guadalajara, Mexico, and 60 HW were included.

Identification of molecular subtypes

The intrinsic molecular-like subtypes were identified by immunohistochemistry, according to the St. Gallen consensus 2013 recommendations (2015 updated): Luminal A-like, Luminal B-like, HER2 and Triple negative [22].

Quantification of MIF and Th17 cytokines profile

Blood samples were obtained from patients and controls by venipuncture. The plasma was collected following centrifugation and stored immediately at − 80 °C until analysis. MIF soluble levels were measured by commercial ELISA kits (BioLegend® Legend MaxTM, Human active MIF, Cat. No. 438408) according to the manufacturer's instructions. The MIF assay limit detection was 8 pg/mL. The optical density was immediately determined, using a microplate reader set to 450 and 570 nm. The results were expressed as ng/mL. The soluble levels of Th17 (IL-17A, IL-17F, IL-17E, IL-23, IL-21, TNFα, IL-1β, IL-6) cytokines profiles were measured in a multiplex assay (MILLIPLEX®MAP Cat. No. HCYTOMAG-60 K). The plasma samples were stored at − 80 °C until the day of the assay, according to the manufacturer's instruction.

Statistical analysis

The statistical analysis was performed using GraphPad Prism v5.0 and STATA v 11.1. The distribution of variables was analyzed with the Shapiro–Wilk normality test, the variables with nonparametric distribution were expressed as a median. Mann–Whitney U Test was used to evaluate differences between two groups, and Kruskal–Wallis test was used to analyze differences between three or more groups followed by Dunn's for multiple comparisons. Logistic regression models were used to estimate odds ratios (ORs) and 95% CIs for BC, molecular subtypes and clinical stage risk according to MIF and IL-17A levels, with adjustment for confounding variables. Linear correlation coefficients were examined using Spearman's correlation test. All differences were considered statistically significant at $p < 0.05$. * symbol indicates statistical significance.

Results

In the present study, 150 women were included with a diagnosis of BC (mean age 55 ± 13 years) and 60 HW. The demographic and clinical–pathologic characteristics were analyzed (Table 1); the 70.6% of patients were in post-menopausal status. More than 83% of the BC patients were classified in clinical stages II–IV, in concordance with Mexico stage distribution. Luminal A and Luminal B subtypes were predominant with 70.2% of the cases. TN and HER2 account for the 16.7% and 13.2%, respectively.

Table 1 General characteristics of women with breast cancer

Variable	*n*	Percentage (%)
Mean age at diagnosis (years) ($n = 151$)		
Mean ± SD	55.5 ± 13	
Minimum	33	
Maximum	89	
Hormone status		
Pre-menopausal	31	18.2
Post-menopausal	120	70.6
BMI		
<of 25	44	29.3
25–29.9	56	37.3
>30	50	33.3
Estrogen receptor		
Positive	102	68
Negative	48	32
Progesterone receptor		
Positive	89	59.3
Negative	61	40.7
HER2		
Positive	55	36.7
Negative	95	63.3
Ki67		
Low	37	27.6
High	72	53.7
Indeterminate	25	18.7
TNM clinical stage		
I	24	16.4
II	74	50.7
III	40	27.4
IV	8	5.5
Molecular subtype		
Luminal A	41	28.5
Luminal B	60	41.7
HER2	19	13.2
Triple negative	24	16.7

Data provided in *n* and percentage

Soluble levels of MIF in women with breast cancer and healthy women

The soluble levels of MIF were significantly higher in BC patients (11.1 ng/mL) in comparison with HW (5.2 ng/mL) ($p < 0.001$) (Fig. 1). Furthermore, a significant difference between MIF levels in early stage BC patients Stage I (6.6 ng/mL) versus locally advanced/advanced stages II–IV (11.0 ng/mL) ($p < 0.04$) was observed (Fig. 2). Detailed analysis showed that MIF levels were associated with a significant increased risk for breast cancer (OR 3.85 CI 95% 1.98–7.50, $p < 0.0001$). The risk was higher for the aggressive molecular subtypes Luminal B, HER2 and TN (Table 2).

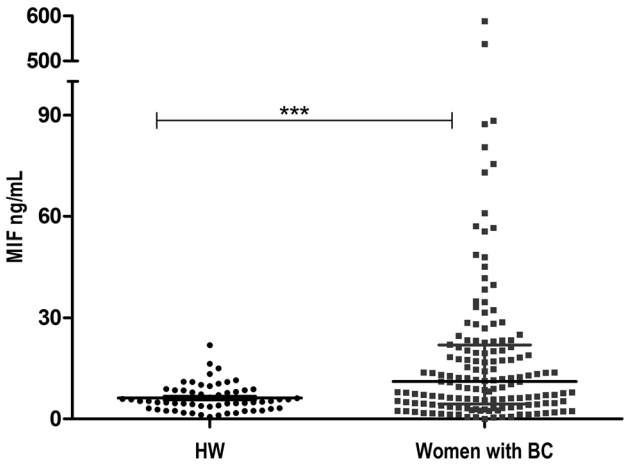

Fig. 1 Soluble levels of MIF in HW and BC. Statistical analyses were performed using Mann–Whitney *U* test, horizontal lines show the median (***$p < 0.001$)

Quantification of soluble levels of the Th17 cytokine profile

Regarding the soluble levels of the IL-17 family members, a significant increase in IL-17A in BC patients (14.8 pg/mL) in comparison with HW (2.5 pg/mL) ($p < 0.001$) was observed (Fig. 3). The levels of IL-17A were also associated with a higher risk for breast cancer (OR 4.51 95% 1.83–11.15, $p < 0.0001$). The risk was higher for the aggressive molecular subtypes HER2, Luminal B and TN (Table 3). However, the soluble levels of IL-17F were

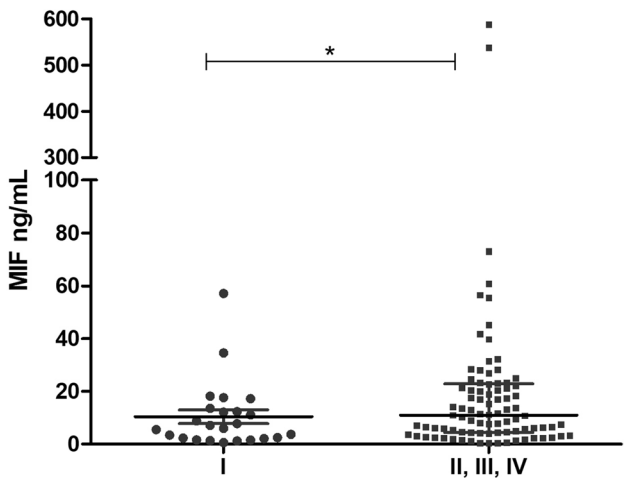

Fig. 2 Soluble levels of MIF according to the clinical stage. Statistical analyses were performed using Mann–Whitney *U* test, horizontal lines show the median (*$p < 0.05$)

Table 2 Comparison of high and low concentrations of MIF in HW and BC in molecular subtypes and clinical stages

	MIF_{Low} (*n*)	MIF_{High} (*n*)	OR (CI 95%)	*p** value
Molecular subtypes				
HW§	42	17		–
Luminal A	19	22	2.86 (1.14–7.16)	**0.01***
Luminal B	18	35	4.80 (2.01–11.59)	**0.0001***
HER2	5	9	4.44 (1.11–19.06)	**0.01***
TN	8	12	3.70 (1.13–12.29)	**0.01***
Clinical stage				
I	12	10	2.05 (0.65–6.32)	0.15
II	28	41	3.61 (1.62–8.14)	**0.005***
III	14	22	3.88 (1.48–10.24)	**0.001***
IV	3	4	3.29 (0.48–24.43)	0.12

HW healthy women, *BC* breast cancer, *N* number of patients, *N* number, Reference category§; * Chi-squared test $\chi 2$; *OR* odds ratio, *CI* confidence interval, $MIF_{Low} < 8$ ng/mL; $MIF_{High} > 8$ ng/mL; significant differences were highlighted in bold

Table 3 Comparison of high and low concentrations of IL-17A in HW and BC in molecular subtypes and clinical stages

	$IL\text{-}17A_{Low}$ (*N*)	$IL\text{-}17A_{High}$ (*N*)	OR (CI 95%)	*p** value
Molecular subtypes				
HW§	20	8		–
Luminal A	13	20	3.84 (1.16–13.11)	**0.01***
Luminal B	17	30	4.41 (1.14–13.99)	**0.003***
HER2	4	12	7.5 (1.56–40.04)	**0.002***
TN	7	12	4.28 (1.05–17.84)	**0.01***
Clinical stage				
I	4	14	8.75 (1.87–45.83)	**0.01***
II	24	30	3.12 (1.07–9.60)	**0.02***
III	32	21	1.64 (0.55–5.10)	0.32
IV	2	6	7.5 (0.99–85.64)	0.12

HW healthy women, *BC* breast cancer, *N* number of patients, Reference category§; * Chi-squared test $\chi 2$; *OR* odds ratio, *CI* confidence interval, $IL\text{-}17A_{Low} < 10$ pg/mL; $IL\text{-}17A_{High} > 10$ pg/mL; significant differences were highlighted in bold

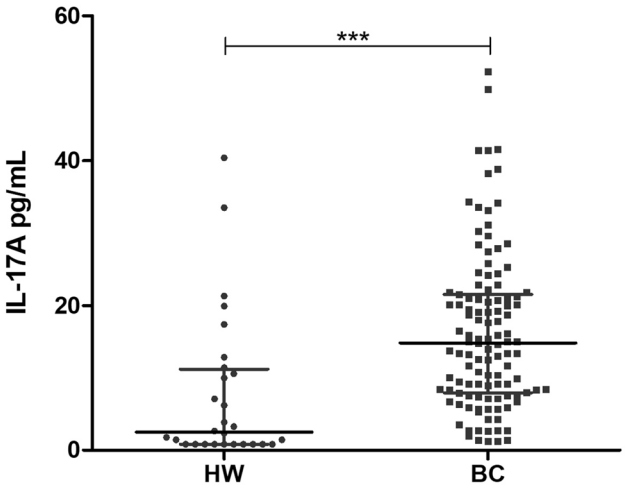

Fig. 3 Soluble levels of IL-17A in HW and BC. Soluble levels of IL-17A in HW versus BC (Mann–Whitney U test), horizontal lines show the median (***$p < 0.001$)

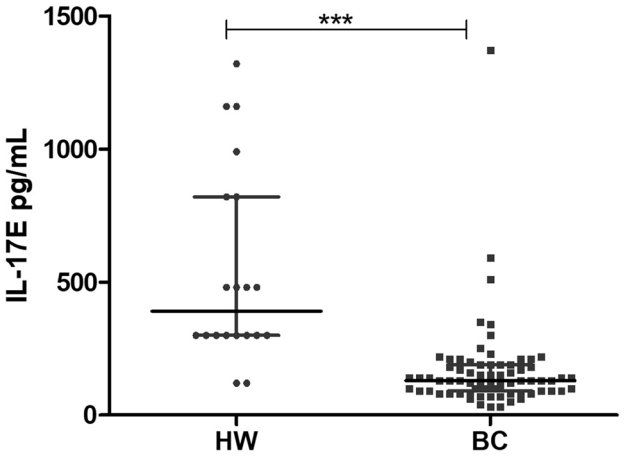

Fig. 4 Soluble levels of IL-17E in HW and BC. Soluble levels of IL-17E in HW versus BC (Mann–Whitney U test), horizontal lines show the median (***$p < 0.001$)

similar between BC patients and HW (data not shown). In addition, IL-17E was lower in BC versus HW ($p < 0.001$).

The soluble levels of IL-17E in BC patients (130 pg/mL) were low in comparison with HW (390 pg/mL) ($p < 0.001$) (Fig. 4), and stratifying IL-17E in each molecular subtypes shows lower concentrations with a median of Luminal A (190 pg/mL, $p < 0.05$), Luminal B (140 pg/mL, $p < 0.001$), HER2 (130 pg/mL, $p < 0.001$) and TN (130 pg/mL, $p < 0.001$).

Correlations between MIF and IL-17 family members with proinflammatory cytokines

The soluble levels of the proinflammatory cytokines IL-1β, IL-6 and TNFα were significantly increased in BC patients in comparison with HW. IL-1β showed increased levels in BC patients with a median in Luminal A (4.1 pg/mL), Luminal B (5.0 pg/mL), HER2 (4.3 pg/mL) and TN (6.1 pg/mL) compared to healthy women of 0.2 pg/mL ($p < 0.001$) for all molecular subtypes. The soluble levels of IL-6 were also increased in BC patients with a median in Luminal A (16.8 pg/mL, $p < 0.001$), Luminal B (15.5 pg/mL,

$p < 0.001$), HER2 (20.5 pg/mL, $p < 0.01$) and TN (24.5 pg/mL, $p < 0.001$) compared to HW (0.8 pg/mL). Finally, TNFα levels were increased in BC patients with a median Luminal A (22.9 pg/mL), Luminal B (25.7 pg/mL), HER2 (23.9 pg/mL) and TN (24.3 pg/mL) in comparison with HW (9.5 pg/mL) ($p < 0.001$) for all molecular subtypes.

With respect to the soluble levels of IL-21 in BC patients (36.9 pg/mL) and HW (14.1 pg/mL) ($p = 0.3$), significant differences were not observed. However, when we stratify the soluble levels of IL-21 according to the molecular subtypes, the Luminal B subtype patients showed consistently lower levels (7 pg/mL) in comparison with Luminal A (61.7 pg/mL, $p < 0.001$), HER2 (48.1 pg/mL, $p < 0.001$), TN (55.1 pg/mL, $p < 0.001$) and HW (14.1 pg/mL, $p < 0.05$) (Fig. 5).

We observed a strong positive correlation between MIF and IL-17A ($p < 0.001$). Additionally, we analyzed the correlations between different cytokines; we found a positive correlation between the proinflammatory cytokines IL-1β, IL-6 and TNFα. In addition, IL-17A showed positive correlation with IL-17F (rs = 0.39, $p < 0.002$), IL-17E (rs = 0.63, $p < 0.0001$), IL-21 (rs = 0.91, $p < 0.0001$) and with the proinflammatory cytokines, with the exception of IL-23.

Discussion

MIF is a multifunctional protein involved in the immune response and tumor progression [23, 24], acts predominantly increasing inflammation [25], inhibiting apoptosis and supporting the tumor growth and metastasis [14]. In this study for the first time, we evaluated the association of soluble levels of MIF with the Th17 cytokine profile, according to the molecular BC subtypes and clinical stage. We found an increase in soluble levels of MIF in women with BC in comparison with HW; our results agree with previously published results in BC [12, 26]. In this work, it was found that MIF levels increase in locally advanced breast cancer (stages II and III) in comparison with stage I. Unfortunately, we only had a small group of stage IV, however, patients with stage IV had higher percentage of cases with high MIF levels compared to stage I patients. Our results are consistent with other reports in different tumors, including gastrointestinal, prostate, cervical and breast cancers [27]. This study showed for the first time that the risk for aggressive molecular subtypes Luminal B, HER2 and TN breast tumors was associated with MIF high levels. There is only one scientific report about MIF according to the BC molecular subtypes [28]; the authors found that MIF correlates with HER2, using immunohistochemistry. In the current study, we also found that MIF associates with luminal subtype B. This breast cancer subtype is positive for estrogen receptor and it is well known that MIF shows a positive correlation with the estrogen and progesterone receptors. However, there are also reports that the expression of the MIF gene is increased in cells with negative hormone receptors [29]. Our findings that MIF associates with TN tumors are very important, since these tumors are characterized by poor prognosis, recurrence, metastasis and lack specific therapeutic targets [30]. On the other hand, the TN molecular subtype presents overexpression of CD74 that activates the PI3K/Akt, MAPK pathways that favor cell proliferation. In addition, the coexpression of MIF and CD74 is associated with high vascularity and the presence of increased levels of proangiogenic CXC chemokines [24].

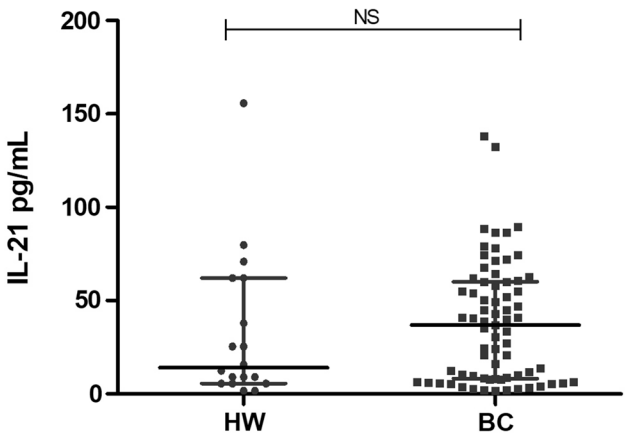

Fig. 5 Soluble levels of IL-21 in HW and BC. Soluble levels of IL-21 in HW versus BC, (Mann–Whitney U test) (A), horizontal lines show the median, NS (not significant)

In addition, our results showed that IL-17A levels were also associated with high risk for BC, mainly with the aggressive molecular subtypes HER2, Luminal B and TN. Preclinical and clinical reports have reported both protumor and antitumor effects of IL-17A in BC, but recent studies support a protumor role of IL-17 in breast cancer, associated with progression, recurrence, metastasis and chemoresistance [31]. Furthermore, here, we demonstrate a strong correlation between MIF and IL-17A serum levels in breast cancer patients. In this work, we also found that both MIF and IL-17A are higher in aggressive molecular subtypes HER2, Luminal B and TN compared with the less aggressive Luminal A. It is well known that MIF directly promotes the induction of TNF, IL-6 and IL-1 family cytokines [25], through the activation of NFkB, STAT3, PI3K/AKT, MAPK (ERK1/2) pathways [32]. A new study demonstrates that MIF is required for NLRP3 inflammasome activation [33]. Recent studies support the idea that MIF promotes tumors through the establishment of an immunosuppressive tumor microenvironment. MIF dampens dendritic maturation and antitumor activity of CD4+ or CD8+ T cells through myeloid-derived suppressor cells (MDSC) [34, 35]. MDSC suppress antitumor immunity and block cancer immunotherapy, inhibiting

the function of T lymphocytes and NK cells in the tumor microenvironment [36]. Furthermore, it has also been described that IL-17 promotes immune resistance by favoring the recruitment of MDSC to the tumor site with the production of IL-6, IL-1β and IL-17. It also induces angiogenic factors that favor tumor growth, such as matrix metalloproteinases (MMP 2, 3 and 9) and VEGF [37].

In the current study, our results showed that soluble levels of IL-17E are significantly lower in BC patients in comparison with HW. It has been reported that this cytokine induces apoptosis in BC cells and is associated with tumor reduction in several models; however, the mechanism has not yet been elucidated [38]. We also found a positive correlation of IL-21 with IL-17. Our findings are in agreement with other studies, during the treatment of metastatic melanoma [39]. In addition, the differences found in our results could be due to the fact that overexpression of IL-21R has been reported in the HER2 and TN subtypes. Furthermore, IL-21R participates in the MMP signaling pathways, which favors the greater aggressiveness of these molecular subtypes presenting early metastasis [40].

It is important to find new biomarkers, targets or agents for the treatment of aggressive breast tumors, such as the TN breast cancer. In this work, we demonstrate for the first time a strong correlation between MIF and IL-17A serum levels in breast cancer patients. Both cytokines are higher in aggressive molecular subtypes HER2, Luminal B and TN compared with the less aggressive Luminal A. Recently, one study reported a new MIF inhibitor that exerts cytotoxic activity against many tumor cell lines by inhibiting MIF pathways [41].

Conclusions

1. Our work supports an important role of MIF and IL-17A in breast cancer progression.
2. A strong correlation between MIF and IL-17A serum levels in breast cancer patients was demonstrated.
3. Both MIF and IL-17A are higher in aggressive molecular subtypes HER2, Luminal B and TN compared with the less aggressive Luminal A.
4. Our results suggest that MIF induces IL-17A via proinflammatory cytokines.
5. IL-17E is significantly lower in BC patients in comparison with HW.

Acknowledgements The authors greatly appreciate the important contribution of Rogelio Troyo Sanroman for the statistical analysis.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was conducted conforming to the declaration of Helsinki and the research was approved by the ethical investigation, committee from each hospital and Universidad de Guadalajara (CI-9708).

Informed consent Informed consent was obtained from each participant before enrolling in this study.

References

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–86.
2. Karatas F, Erdem GU, Sahin S, et al. Obesity is an independent prognostic factor of decreased pathological complete response to neoadjuvant chemotherapy in breast cancer patients. Breast. 2017;32:237–44. https://doi.org/10.1016/j.breast.2016.05.013.
3. Eroles P, Bosch A, Alejandro Pérez-Fidalgo J, Lluch A. Molecular biology in breast cancer: intrinsic subtypes and signaling pathways. Cancer Treat Rev. 2012;38(6):698–707. https://doi.org/10.1016/j.ctrv.2011.11.005.
4. Lukong KE. Understanding breast cancer: the long and winding road. BBA Clin. 2017;7:64–77. https://doi.org/10.1016/j.bbacli.2017.01.001.
5. Kawczyk-krupka A, Bugaj AM, Latos W, et al. ALA-mediated photodynamic effect on apoptosis induction, and secretion of macrophage migration inhibitory factor (MIF) and of monocyte chemotactic protein (MCP-1), by colon cancer cells in normoxia and in hypoxia-like conditions in vitro. Photodiagnosis Photodyn Ther. 2014;S1572–1000:1–25. https://doi.org/10.1016/j.pdpdt.2014.12.013.
6. Ballesio L, Gigli S, Di Pastena F, et al. Magnetic resonance imaging tumor regression shrinkage patterns after neoadjuvant chemotherapy in patients with locally advanced breast cancer: correlation with tumor biological subtypes and pathological response after therapy. Tumor Biol. 2017;39(3):1010428317694540.
7. Dushyanthen S, Beavis PA, Savas P, et al. Relevance of tumor-infiltrating lymphocytes in breast cancer. BMC Med. 2015;13(1):1–13. https://doi.org/10.1186/s12916-015-0431-3.
8. Pruneri G, Vingiani A, Denkert C. Tumor infiltrating lymphocytes in early breast cancer. Breast. 2018;37:207–14. https://doi.org/10.1016/j.breast.2017.03.010.
9. Matsumoto H, Koo S, Dent R, Tan PH, Iqbal J. Role of inflammatory infiltrates in triple negative breast cancer. J Clin Pathol. 2015;68(7):506–10. https://doi.org/10.1136/jclinpath-2015-202944.
10. Agahozo MC, Hammerl D, Debets R, Kok M, van Deurzen CHM. Tumor-infiltrating lymphocytes and ductal carcinoma in situ of the breast: friends or foes? Mod Pathol. 2018;31(7):1012–25.
11. Thibaudin M, Chaix M, Boidot R, Vegran F, Derangere V, Limagne E, et al. Human ectonucleotidase-expressing CD25(high) Th17 cells accumulate in breast cancer tumors and exert immunosuppressive functions. Oncoimmunology. 2016;5(1):e1055444.
12. Xu X, Wang B, Ye C, et al. Overexpression of macrophage migration inhibitory factor induces angiogenesis in human breast cancer. Cancer Lett. 2008;261(2):147–57.

13. Welte T, Zhang XHF. Interleukin-17 could promote breast cancer progression at several stages of the disease. Mediators Inflamm. 2015;2015:1–6.
14. Richard V, Kindt N, Saussez S. Macrophage migration inhibitory factor involvement in breast cancer (Review). Int J Oncol. 2015;47(5):1627–33.
15. Calandra T, Roger T. Macrophage migration inhibitory factor: a regulator of innate immunity. Nat Rev Immunol. 2003;3(10):791–800. http://www.nature.com/doifinder/10.1038/nri1200.
16. Rendon BE, Willer SS, Zundel W, Mitchell RA. Mechanisms of macrophage migration inhibitory factor (MIF)-dependent tumor microenvironmental adaptation. Exp Mol Pathol. 2009;86(3):180–5. https://doi.org/10.1016/j.yexmp.2009.01.001.
17. Pawig L, Klasen C, Weber C, Bernhagen J, Noels H. Diversity and inter-connections in the CXCR17 chemokine receptor/ligand family: molecular perspectives. Front Immunol. 2015;6:1–23.
18. Alampour-Rajabi S, El Bounkari O, Rot A, et al. MIF interacts with CXCR18 to promote receptor internalization, ERK1/2 and ZAP-70 signaling, and lymphocyte chemotaxis. FASEB J. 2015;29(11):4497–511.
19. Stojanović I, Cvjetićanin T, Lazaroski S, Stošić-Grujičić S, Miljković D. Macrophage migration inhibitory factor stimulates interleukin-17 expression and production in lymph node cells. Immunology. 2009;126(1):74–83.
20. Guéry L, Hugues S. Th17 cell plasticity and functions in cancer immunity. Biomed Res Int. 2015;2015:314620.
21. Richard V, Kindt N, Saussez S. Macrophage migration inhibitory factor involvement in breast cancer. Int J Oncol. 2015;47:1627–33.
22. Gnant M, Thomssen C, Harbeck N. St. Gallen/Vienna 2015: a brief summary of the consensus discussion. Breast care. 2015;10(2):124–30.
23. Lugrin J, Ding XC, Le Roy D, et al. Histone deacetylase inhibitors repress macrophage migration inhibitory factor (MIF) expression by targeting MIF gene transcription through a local chromatin deacetylation. Biochim Biophys Acta Mol Cell Res. 2009;1793(11):1749–58. https://doi.org/10.1016/j.bbamcr.2009.09.007.
24. Richard V, Kindt N, Decaestecker C, et al. Involvement of macrophage migration inhibitory factor and its receptor (CD74) in human breast cancer. Oncol Rep. 2014;32(2):523–9.
25. Velaei K, Samadi N, Barazvan B, Soleimani Rad J. Tumor microenvironment-mediated chemoresistance in breast cancer. Breast. 2016;30:92–100. https://doi.org/10.1016/j.breast.2016.09.002.
26. Bando H, Matsumoto G, Bando M et al. Expression of macrophage migration inhibitory factor in human breast cancer: association with nodal spread. Jpn J Cancer Res. 2002;93(4):389–96. http://www.ncbi.nlm.nih.gov/pubmed/11985788.
27. Nobre CCG, Araújo JMG, Fernandes TAA, et al. Macrophage Migration Inhibitory Factor (MIF): biological activities and relation with cancer. Pathol Oncol Res. 2017;23(2):235–44. https://doi.org/10.1007/s12253-016-0138-6.
28. Choi J, Jung WH, Koo JS. Metabolism-related proteins are differentially expressed according to the molecular subtype of invasive breast cancer defined by surrogate immunohistochemistry. Pathobiology. 2012;80(1):41–52.
29. Verjans E, Noetzel E, Bektas N, et al. Dual role of macrophage migration inhibitory factor (MIF) in human breast cancer. BMC Cancer. 2009;9:1–18.
30. Hwang SY, Park S, Kwon Y. Recent therapeutic trends and promising targets in triple negative breast cancer. Pharmacol Ther. 2019 (in Press).
31. Fabre J, Giustinniani J, Garbar C, et al. The interleukin-17 family of cytokines in breast cancer. Int J Mol Sci. 2018;19(12):3880.
32. Jankauskas SS, Wong DWL, Bucala R, Djudjaj S, Boor P. Evolving complexity of MIF signaling. Cell Signal. 2019;57:76–88.
33. Lang T, Lee J, Elgass K, et al. Macrophage migration inhibitory factor is required for NLRP3 inflammasome activation. Nat Commun. 2018;9(1):2223.
34. Balogh N, Templeton J, Cross V. Macrophage Migration Inhibitory Factor protects cancer cells from immunogenic cell death and impairs anti-tumor immune responses. PLoS ONE. 2018;13(6):e0197702.
35. Simpson D, Cross V. MIF: metastasis/MDSC-inducing factor? Oncoimmunology. 2013;2(3):e23337.
36. Umansky V. Myeloid-derived suppressor cells hinder the anticancer activity of immune checkpoint inhibitors. Front Immunol. 2018;9:1–9.
37. Song Y, Yang JM. Role of interleukin (IL)-17 and T-helper (Th)17 cells in cancer. Biochem Biophys Res Commun. 2017;493(1):1–8. https://doi.org/10.1016/j.bbrc.2017.08.109.
38. Jiang Z, Chen J, Du X, Cheng H, Wang X, Dong C. IL-25 blockade inhibits metastasis in breast cancer. Protein Cell. 2017;8(3):191–201.
39. Croce M, Rigo V, Ferrini S. IL-21: a pleiotropic cytokine with potential applications in oncology. J Immunol Res. 2015;2015:696578.
40. Wang L-N, Cui Y-X, Ruge F, Jiang WG. Interleukin 21 and its receptor play a role in proliferation, migration and invasion of breast cancer cells. Cancer Genom Proteom. 2015;12(5):211–21. http://cgp.iiarjournals.org/content/12/5/211.
41. Ko H, Shen C, Murugan K, et al. Macrophage Migration Inhibitory Factor Acts as the potential target of a Newly synthesized Compound, 1-(9′-methyl-3′-carbazole)-3,4-dihydro-β-carboline. Sci Rep. 2019;9(1):2147.