Osteoclast-like stromal giant cells in breast cancer likely belong to the spectrum of immunosuppressive tumor-associated macrophages

Elham Sajjadi1,2, Gabriella Gaudioso3, Andrea Terrasi4, Francesca Boggio3, Konstantinos Venetis1,2, Maria Ivanova4, Letizia Bertolasi3, Gianluca Lopez3, Letterio Runza3, Alice Premoli3, Daniele Lorenzini3, Elena Guerini-Rocco1,2, Stefano Ferrero3,5, Valentina Vaira3,6† and Nicola Fusco1,2*†

1Division of Pathology, IEO, European Institute of Oncology IRCCS, Milan, Italy, 2Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy, 3Division of Pathology, Fondazione IRCCS Ca’ Granda—Ospedale Maggiore Policlinico, Milan, Italy, 4Division of Molecular Biology, Biomedical Center, Faculty of Medicine, LMU Munich, Planegg-Martinsried, Munich, Germany, 5Department of Biomedical, Surgical, and Dental Sciences, University of Milan, Milan, Italy, 6Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy

Background: Breast cancer with osteoclast-like stromal giant cells (OSGC) is an exceedingly rare morphological pattern of invasive breast carcinoma. The tumor immune microenvironment (TIME) of these tumors is populated by OSGC, which resemble osteoclasts and show a histiocytic-like immunophenotype. Their role in breast cancer is unknown. The osteoclast maturation in the bone is regulated by the expression of cytokines that are also present in the TIME of tumors and in breast cancer tumor-associated macrophages (TAMs). TAMs-mediated anti-tumor immune pathways are regulated by miRNAs akin to osteoclast homeostasis. Here, we sought to characterize the different cellular compartments of breast cancers with OSGC and investigate the similarities of OSGC with tumor and TIME in terms of morphology, protein, and miRNA expression, specifically emphasizing on monocytic signatures.

Methods and Results: Six breast cancers with OSGC were included. Tumor-infiltrating lymphocytes (TILs) and TAMs were separately quantified. The different cellular populations (i.e., normal epithelium, cancer cells, and OSGC) were isolated from tissue sections by laser-assisted microdissection. After RNA purification, 752 miRNAs were analyzed using a TaqMan Advanced miRNA Low-Density Array for all samples. Differentially expressed miRNAs were identified by computing the fold change (log2Ratio) using the Kolmogorov-Smirnov test and p values were corrected for multiple comparisons using the false discovery rate (FDR) approach. As a similarity analysis among samples, we used the Pearson test. The association between pairs of variables was investigated using Fisher exact test. Classical and non-classical monocytic miRNA signatures were finally applied. All OSGC displayed CD68 expression.
Introduction

Breast cancer with osteoclast-like stromal giant cells (OSGC) is a rare tumor showing a variable number of OSGC at the periphery of the neoplastic nests and/or within the tubular lumens, in the context of a hypervascular microenvironment composed of lymphocytes, histiocytes, and monocytes (Zhou et al., 2014; Marchiò et al., 2015; Peña-Jaimes et al., 2018; WHO Classification of Tumours Editorial Board and WHO Classification of Breast Tumours, 2019). As their name suggests, OSGC resemble osteoclasts (Ohashi et al., 2018a; Caetano Oliveira and Schmitt, 2018; Bonsang et al., 2019; Hoda et al., 2020). They are morphologically characterized by an abundant intensely-eosinophilic cytoplasm containing well-developed organelles and numerous oval non-atypical nuclei with prominent nucleoli (Ellis et al., 2020; Behzatoglu, 2021). By immunohistochemical analysis, OSGC show the expression of histiocytic surface markers (e.g., CD68) and are negative for cytokeratins, S100, actin, and markers of cell proliferation such as Ki67 (Ohashi et al., 2018a; Inoue et al., 2021; Kao et al., 2021; Venetis et al., 2021). Researchers have been looking for further parallels between OSGC and osteoclasts, whose formation is regulated by the expression of cytokines, including tumor necrosis factor-a (TNFα), interleukin-1a (IL1a), macrophage-colony stimulating factor (M-CSF), and receptor activator of NF-κB ligand (RANKL) (Kolb et al., 2019; Invernizzi et al., 2020b; Liang et al., 2021). These molecules have been documented both in the tumor immune microenvironment (TIME) of other neoplasms with an OSGC component (Bennàssar et al., 2011; Hatano et al., 2014; Fusco et al., 2017a; Liang et al., 2021) and in breast cancer tumor-associated macrophages (TAMs) (Lala et al., 2018; Guo et al., 2020). In breast cancer, the presence of tumor-infiltrating lymphocytes (TILs) and TAMs within the TIME are related to the secretion of specific cytokines that are involved in immunosuppression, angiogenesis, tumor progression, and metastasis (Mao et al., 2013; Pagni et al., 2019; Horimoto et al., 2020; Criscitiello et al., 2021; Fusco et al., 2022). It has become increasingly evident that miRNAs play a crucial role in regulating TAMs-mediated anti-tumor immune pathways as well as in osteoclast differentiation, function, and survival (Inoue et al., 2021; O’Brien et al., 2018; McGuire et al., 2015; Fumagalli et al., 2017; Weivoda et al., 2021). However, their role in breast cancer with OSGC is essentially unexplored and based on a handful of morphology-based case reports available in the literature.

We hypothesized that OSGC share not only phenotypic but also molecular features with macrophages/monocytes populating breast cancer TIME. In this study, we sought to characterize the different cellular compartments of breast cancers with OSGC and investigate the similarities of OSGC with tumor and TIME in terms of morphology, protein, and miRNA expression, specifically emphasizing on monocytic signatures.
Materials and methods

Patients and tissue specimens

This study is in line with the local ethical guidelines and was approved by the Institutional Review Board (IRB) of Fondazione IRCCS Ca’ Granda - Ospedale Maggiore Policlinico under the protocol number # 620-2018bis. Only therapy-naïve patients and their corresponding surgical specimens were included in this study. Taken together, 6 cases of breast cancers with OSGC were retrieved from the pathology archives of the aforementioned Institution. Hematoxylin and eosin-stained serial sections of each case were centrally reviewed, re-classified, and re-graded by two pathologists (F.B. and N.F.), according to the latest WHO recommendations (WHO Classification of Tumours Editorial Board and WHO Classification of Breast Tumours, 2019) and the Nottingham histologic grading system (Rakha et al., 2008), respectively. Pathologic re-staging was performed following the 8th edition of the American Joint Committee on Cancer (AJCC) Cancer Staging Manual (Amin et al., 2017).

Immunohistochemical analysis

Representative 4-μm-thick sections of all cases were subjected to immunohistochemical analysis (IHC) with antibodies against estrogen receptor (ER), progesterone receptor (PgR), Ki67, HER2, CD68, Tartrate-resistant acid phosphatase (TRAP), and receptor activator of nuclear factor κ B (RANK), as previously described (Fusco et al., 2018; Lopez et al., 2020). Briefly, the protocol uses the Dako automated staining platform (Dako Omnis; Dako, Carpinteria, CA, United States) and anti-human prediluted antibodies. For each antibody, positive and negative controls were included in each slide run. ER, PgR, and HER2 status were tested and reported according to the latest breast biomarker reporting guidelines published by the College of American Pathologists (CAP) (Allison et al., 2020; Wei et al., 2021). The proliferation index was assessed by Ki67 IHC as the global (average) score across the section. According to the updated recommendations from the International Ki67 in Breast Cancer Working Group, a cut-off value of ≥30% was used to define the high proliferation group (Nielsen et al., 2020). Cut-off point for CD68, TRAP, and RANK was calculated as the number of immunoreactive cells (membrane and/or cytoplasm staining of any intensity) divided into lower and higher groups according to the median of a larger cohort (Kuroda et al., 2021). Based on this assumption, a cut-off value of ≥26 identified high expressors. The methods and scoring systems employed are detailed in Table 1.

Tumor-infiltrating lymphocytes and tumor-associated macrophages assessment

The presence and relative proportion of stromal TILs were determined according to the recommendation of the International TILs Working Group (Dieci et al., 2018).
particular, TILs within the borders of the tumor areas and/or nests were defined as stromal TILs, while the lymphocytes in direct cell-to-cell contact with the tumor cells with no intervening stroma were defined as intratumoral TILs and disregarded from the analysis (Sajjadi et al., 2020; Esposito et al., 2021). The density of stromal TILs was recorded as a continuous percentage and categorized for analyses as negative if 0%, low in between 1 and 10%, intermediate in between 11 and 50%, and high if > 51% (Fusco et al., 2020a). The evaluation and quantification of TAMs were carried out semiquantitatively based on the presence and relative proportion of CD68+ mononuclear cells within the TIME per high power field, as previously described (Kuroda et al., 2021).

Microdissection and RNA extraction

Representative FFPE tissue blocks of 6 patients were selected based on the amount of OSGC; 4-μm-thick sections were then cut and stained with hematoxylin. Subsequently, the histologically distinct components of each case and matched normal breast tissues were separately microdissected using a combination of laser-capture and manual microdissection, as previously described (Fusco et al., 2017b). Specifically, a laser-capture microscope (LMD 6000 System; Leica Biosystems, Wetzlar, Germany) was used for the isolation and microdissection of OSGC. Afterward, the neoplastic epithelial component was isolated from the TIME and manually microdissected using a sterile needle under a stereomicroscope (Zeiss, TIEsse Lab) to ensure > 80% of tumor cell content. Finally, matched normal breast tissue containing non-neoplastic terminal duct-lobular units were manually microdissected. The OSGC (n = 6), tumor (n = 6), and normal tissue samples (n = 6) of each case were collected into separate tubes (n = 18) and subjected to total RNA purification using the Master Pure RNA purification kit (Epicenter Biotechnologies, Illumina) as described (Faversani et al., 2021). Next, the RNA content and quality were measured using a spectrophotometer (Thermo Scientific NanoDrop™ 1,000 Spectrophotometer). Samples with poor quantities of miRNA were re-cut, re-microdissected, and re-extracted. All preparation and handling procedures were conducted under RNase-free conditions.

miRNA profiling

100 ng of total RNA per sample was reverse transcribed using the TaqMan Advanced miRNA cDNA synthesis kit. Then, miRNA profiles were obtained using the TaqMan™ Advanced miRNA Human A and B Cards. A total of 752 miRNAs were assessed (Supplementary Table S1). As a threshold for expression, we set a Cycle threshold (Ct) less than 35. All miRNAs with a Ct value > 35 were considered not expressed. If a miRNA was undetectable (Ct > 35) in the majority plus one sample of our series, it was excluded from further analysis. According to this criterion, 130 miRNAs were available for the study (Supplementary Table S2).

Statistical and bioinformatics analysis

For miRNA analysis, miRNA raw data were normalized using the most stable miRNAs. As normalizer, miRNAs with a mean < 32 Ct and standard deviation < 2.5 were selected. Then, miRNA relative quantities were median-normalized, log2-transformed, and imported in R environment for statistical analysis. Corrplot packages was used for correlation analysis (https://github.com/taiyun/corrplot). Supervised clustering analysis was performed using the ComplexHeatmap package available within Bioconductor (https://bioconductor.org/packages/release/bioc/html/ComplexHeatmap.html), as previously described (Gu et al., 2016; Faversani et al., 2021). Differentially expressed miRNAs according to clinical or outcome variables were identified by computing the fold change (log2Ratio) between the 2 classes and applying the Wilcoxon test. The accuracy of the data was assessed using p value the false discovery rate (FDR) approach. The associations between pairs of variables were investigated using Fisher exact test (MedCalc software). Differences among samples were analyzed using a non-parametric two-sided Student’s t-test, or Wilcoxon signed-rank test. Statistical analyses were performed using GraphPad Prism version 4 for Windows. A p < 0.05 was considered statistically significant.

Results

Clinicopathological features and tumor immune microenvironment composition

All patients (n = 6, age range, 35–69; mean ± SD, 56.8 ± 8.8) were diagnosed with intermediate/high grade, highly proliferative (Ki67 ≥ 30) breast cancer. Lymphovascular invasion was observed in 2 (33.3%) cases. All OSGC within each case displayed high CD68 and TRAP expression, and low/null RANK positivity (Supplementary Figure S1), supporting their monocytic origin. In addition to OSGC, in all tumors, both the presence of stromal TILs (range, 45–85%) and the presence of high intratumoral and stromal TAMs (range, 35–75%) were observed. The clinicopathologic characteristics and subtypes of the patients included in this study are listed in Table 2 and shown in Figure 1.
Recurrent miRNA signatures between OSGC and breast cancer cells

From each of the 6 cases, the neoplastic cells, OSGC, and breast normal cells containing non-neoplastic terminal ductal lobular units, were separately microdissected, for a total number of 18 samples (i.e., 3 samples for each case). Samples failing to reach the optimal RNA quality were excluded from subsequent analyses. Altogether, a total of 6 OSGC, 4 normal, and 4 tumors microdissected samples were analyzed (n = 14).
Applying the Pearson similarity analysis, we could document that the majority (4 out 6; 67%) of OSGC clustered together separately from cancer samples and non-neoplastic breast epithelium. By unsupervised separate analysis we asked whether the miRNA found within OSGC samples are more similar to cancer or to the non-neoplastic breast samples. Considering all available miRNAs (n = 130) we found that OSGC had a more similar miRNA expression pattern to cancer samples, as shown by the dot color and size (Figure 2A). Next, we searched for miRNAs that were differentially expressed between cancer and non-neoplastic breast samples and we found four dysregulated miRNAs (i.e., miR-181a-5p and miR-181b-5p were upregulated, while miR-143a-3p and miR-195a-5p were significantly downregulated in the neoplastic cells compared to the non-neoplastic ones (L2R>2; p = 0.02)) (Figure 2B). Subsequently by considering these four miRNAs, we tested whether OSGC is more similar to the cancer cells or normal breast epithelial cells. According to Pearson analysis, the majority
Expression of monocytic miRNA is considered.

Compartment signiﬁcantly diverges from the latter when the expression of monocytic miRNA expression patterns.

Expression of monocyte-related miRNAs in OSGCs

To test the hypothesis of the monocytic origin of OSGC cells, we retrieved a publicly available miRNA signature characteristics of classical (CD14+/CD16−) and non-classical (CD14+/CD16++) monocyte subsets and we applied those signatures to our samples (Duroux-Richard et al., 2019). Generally, monocytes of either subtypes clustered separately from normal/neoplastic breast samples and OSGCs (Figure 3A). Then, the previously identified monocytic-speciﬁc miRNA signature composed of 18 miRNAs (Duroux-Richard et al., 2019) was investigated in our series (Figure 3B). The monocyte-associated miR-29a-3p and miR-21-3p were different in OSGCs compared with non-neoplastic or breast cancer tissue. Speciﬁcally, the relative expression of miR-29a-3p was lower in OSGC than in tumor and normal tissue samples, while the relative expression for miR-21-3p was lower in OSGC compared with cancer samples. Nevertheless, the remaining monocytes-associated miRNAs were not differentially modulated in breast OSGC cells. Furthermore, the signatures of monocyte-related miRNAs were completely different between the two monocytic populations and the OSGC compartment. Even though OSGC share some phenotypic similarities (i.e., CD68) with TAMs and monocytes, this cellular compartment signiﬁcantly diverges from the latter when the expression of monocytic miRNA is considered.

Discussion

Here, we analyzed the TIME composition along the miRNAome of the different cellular elements within breast cancers with OSGC. Our analyses show that these tumors are enriched in both TILs and TAMs, indicating an activation of the anti-tumor immune response. Furthermore, we provide previously unavailable evidence that OSGC are more similar to the breast neoplastic cells than to the non-neoplastic epithelial counterpart in terms of miRNA expression. This observation suggests that shared epigenetic events might occur between breast cancer tumorigenesis and OSGC phylogenesis. Finally, we demonstrated that despite OSGC show some phenotypic similarities with monocytes such as the expression of CD68 and TRAP, there is no similarity in terms of monocytic miRNA expression patterns.

The observation of OSGC within breast cancer TIME is a rare event with an unclear clinical relevance. To date, only 200 cases have been described in the literature; for this reason, their biology is substantially undetermined (Zhou et al., 2014). The World Health Organization (WHO) classiﬁes the carcinoma with OSGC among rare variants of invasive breast carcinomas NST (WHO Classiﬁcation of Tumours Editorial Board and WHO Classiﬁcation of Breast Tumours, 2019). The carcinomatous part of these lesions is most frequently described as a well-to-moderately differentiﬁed (Zhou et al., 2014; Ohashi et al., 2018a). In our study group, however, the majority (n = 5, 83.3%) of cases were mostly poorly differentiﬁed invasive breast carcinomas NST, although metaplastic features were seen in one case. Our observation conﬁrms the great morphological heterogeneity of these neoplasms, where a wide range of breast cancer special types [e.g., cribriform, mucinous (micro) papillary, lobular, and metaplastic] with variable histological grades can be accompanied by OSGC (Marchiò et al., 2015; Ohashi et al., 2018a; Bonsang et al., 2019; Hoda et al., 2020). Previous studies on solid tumors associated with OSGC have unraveled that these cells have a histiocytic origin based on their phenotype and molecular features (Bauditz et al., 2006; Dahm, 2017). To the best of our knowledge, this is an extremely limited information regarding the speciﬁc composition of the TIME in breast cancer with OSGC, thus, the information provided in our study could constitute the basis for additional research focused on the correlation of this morphological features with the clinical course, tumor aggressiveness, and application of therapeutic strategies (Pruneri et al., 2016; Sajjadi et al., 2020; Criscitiello et al., 2021). By analyzing the composition of TIME, we observed the steady presence of TILs and high TAMs within the TIME of all cases, providing circumstantial evidence to suggest that OSGC might be a contributor to the host anti-tumor immunity. In addition, we demonstrated for the first time in the literature that the OSGC miRNA expression landscape shares similar characteristics with that of breast cancer cells. Hence, in our microdissected samples, we found that OSGC could be grouped with cancer cells based on four markers, namely hsa-miR-181a-5p, hsa-miR-181b-5p, hsa-miR-143-3p, and hsa-miR-195-5p. These miRNAs have been previously described as regulators of genes involved in breast cancer tumorigenesis and immune inﬁltration (Allantaz et al., 2012; Alsaweed et al., 2015; Johannessen et al., 2017; Liu et al., 2017; Rizzo et al., 2017; Li et al., 2021). Among these miRNAs, interestingly, miR-143 regulates the machinery of the breast epigenome (Ng et al., 2014; Humphries et al., 2019). In breast cancer, miR-143 directly targets and regulates DNMT3A. DNMT3A overexpression could alter the methylation status of PTEN and TNFRSF10C which contribute to tumorigenesis (Ng et al., 2014; Humphries et al., 2019). This highlights the tumor-suppressive role of miR-143 in epigenetic aberration of breast cancer.
To assess whether OSGCs are biologically related to TAMs, we performed targeted miRNA expression profiling of monocyte signatures in the different cellular components of our samples. Altogether, we found two markers that were differentially expressed within the OSGC, namely miR-29a-3p and miR-21-3p. Interestingly, miR29a-3p has been recently reported to be involved in the regulation of TAMs-derived exosomal long non-coding (Inc) RNAs that can promote proliferation, invasion, and restrain cell apoptosis in several conditions associated with abnormal osteoclast-mediated osteolysis, such as osteosarcoma, osteoporosis, bone loss, and breast cancer metastatic to the bone (Li et al., 2019; Wu et al., 2019; de Sire et al., 2020; Cascini and Chiodoni, 2021; Hrdlicka et al., 2021; Norregaard et al., 2021; Venetis et al., 2021; Zhang et al., 2021). In addition, several studies have documented that miR-21 is the most abundant miRNA in macrophages and TAMs (Wang et al., 2015a; Canfrán-Duque et al., 2017). In contrast to macrophage function in normal tissue, TAMs are classified into two major distinctive phenotypes: the pro-inflammatory (tumorigenic) M1 macrophages and immunosuppressive (tumor-promoting) M2 macrophages (Rivera and Bergers, 2013). In particular, miR-21 is considered as a homeostatic regulator of macrophage differentiation, as its deficiency prompts M1 polarization in TAMs (Xi et al., 2018). For this reason, tumor cells may stimulate miR-21 expression in TAMs to prevent tumorigenic M1 polarization (Sahraei et al., 2019). Recently, it has been observed that miR-21 increases the M2 macrophage-mediated chemoresistance and downregulates major histocompatibility complex (MHC) class I surface antigens, while upregulating programmed death-ligand 1 (PD-L1) expression in TAMs, which is known to inhibit phagocytic anti-tumor activity (Caescu et al., 2015; An and Yang, 2020; Subbarayan et al., 2021). This negative regulator of inflammation and phosphatase and tensin homolog (PTEN)/phosphoinositide 3-kinase (PI3K) axis has also been studied for its role in balancing apoptosis and oncogenic transformation in normal epithelial cells and as a prognostic biomarker in breast cancer (Buscaglia and Li, 2011; Wang et al., 2015b; Ma et al., 2015; Fusco et al., 2020b; Amirfallah et al., 2021; Fusco et al., 2021; Sajjadi et al., 2021). Interestingly, we found several similarities between OSGC and M2-TAMs, particularly in their morphology and immunophenotype, and a miRNA monocytic signature.

Our study has several limitations. First, given the rarity of breast cancers with OSGC, we could only analyze a relatively small number of cases, thus this study should be regarded as hypothesis-generating. It should be noted, however, that this study, to the best of our knowledge, represents the first integrated miRNA analysis of breast cancer and associated OSGC. Second, given the relatively limited quantity of OSGC and that all cases were microdissected from sections obtained from formalin-fixed paraffin-embedded (FFPE) blocks, we could only perform targeted miRNA expression profiling; hence, potential somatic genetic alterations and dysregulations affecting miRNAs not included in the panel studied could play a role in the development of OSGC and modulation of TILs and TAMs within breast cancer TIME. Finally, due to the retrospective nature of this cohort, risk and survival analyses have not been performed. Large prospective multicentric studies are needed to investigate the specific outcome of breast cancer with OSGC and the risk of development of systemic (including bone) metastases conferred by the presence of OSGC. In this respect, it would be of great interest to perform multi-level high-throughput analyses, coupled with bioinformatics investigations, to include selected miRNA and predicted target genes accompanied by functional analysis and GO terms clustering, in order to assess further differences and similarities among immune and cancer cells, in different tumor types.

Despite these limitations, this study, together with previous observations, challenges the notion that OSGC is a mere histologic curiosity within the wide spectrum of breast cancer. Larger multicentric clinical studies and cancer registries, coupled with comprehensive molecular analyses will be required to 1) determine whether OSGC may constitute a prognostic/predictive biomarker, 2) identify potential founder genetic/epigenetic events in these tumors, and 3) to define whether breast cancers with OSGC would be associated with a hyperactivated anti-tumor immune response.

In conclusion, our findings on the presence of stromal TILs with a high proportion of TAMs suggest an activated TIME in breast cancer with OSGC. We found that OSGC miRNAs profile were more similar to cancer cells than to non-neoplastic epithelial counterparts implying the potential role of epigenetic events on both neoplastic and OSGC component. Finally, all OSGC within each case displayed CD68 expression and partially a monocyes’ miRNA signature, suggesting that OSGC might be resident elements of TIME and likely belong to the spectrum of immunosuppressive, tumor-promoting, M2 TAMs.

**Data availability statement**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, upon reasonable request.

**Ethics statement**

The studies involving human participants were reviewed and approved by Institutional Review Board (IRB) of Fondazione IRCCS Ca’ Granda–Ospedale Maggiore Policlinico. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.
Author contributions

Conceptualization, NF. Methodology, GG, FB, GL, ES, AP, NF. Formal analysis, AT. Data curation, VV, ES, GG. Writing-original draft preparation, ES. Writing-review and editing, ES, VV, KV, MI, LB, DL, EG-R, and NF. Resources, SF, NF. Supervision, NF, VV. Project administration, NF and VV. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the Italian Ministry of Health with Ricerca Corrente funds.

Acknowledgments

The authors acknowledge support from the University of Milan through the APC initiative and would like to thank Marco Brevi for his help in the manuscript revision.

References

Allantaz, F., Cheng, D. T., Bergauer, T., Ravindran, P., Rossier, M. F., Ebeling, M., et al. (2012). Expression profiling of human immune cell subsets identifies miRNA-mRNA regulatory relationships correlated with cell type specific expression. PLoS One 7 (1), e32979. doi:10.1371/journal.pone.002979

Allison, K. H., Hammond, M. E. H., Dowsett, M., McKernin, S. E., Carey, L. A., Fitzgibbons, P. L., et al. (2020). Estrogen and progesterone receptor testing in breast cancer: American society of clinical oncology/college of American pathologists guideline update. Arch. Pathol. Lab. Med. 144 (5), 545–563. doi:10.5858/arpa.2019-0904-SA

Alsaweed, M., Hartmann, P. E., Geddes, D. T., and Kakulas, F. (2015). MicroRNA-21 in breastmilk and the lactating breast: potential immunoprotectors and developmental regulators for the infant and the mother. Int. J. Environ. Res. Public Health 12 (11), 13981–14020. doi:10.3390/ijerph121113981

Amin, M. B., Edge, S. B., Greene, F. L., Byrd, D. R., Brookland, R. K., Washington, M. K., et al. (2017). AJCC cancer staging manual. Eighth Edition ed. New York: Springer International Publishing.

Amirfallah, A., Knutzdottir, H., Arason, A., Þímarsdottir, B., Johannsson, O. T., Aagnarsson, B. A., et al. (2021). Hsa-miR-21-3p associates with breast cancer patient survival and targets genes in tumor suppressive pathways. PLoS One 16 (11), e0260327. doi:10.1371/journal.pone.0260327

An, Y., and Yang, Q. (2020). MiR-21 modulates the polarization of macrophages and increases the effects of M2 macrophages on promoting the chemoresistance of ovarian cancer. Life Sci. 242, 117162. doi:10.1016/j.lfs.2019.117162

Bauditz, J., Rudolph, B., and Wermke, W. (2006). Osteoclast-like giant cell tumors of the pancreas and liver. World J. Gastroenterol. 12 (48), 7876–7883. doi:10.3748/wjg.v12.i48.7878

Behzatoglu, K. (2021). Osteoclasts in tumor biology: metastasis and epithelial-mesenchymal-ymediation transition. Pathol. Oncol. Res. 27, 609472. doi:10.3389/pore.2021.609472

Bennaisar, A., Mas, A., Guilabert, A., Juliá, M., Mascaro-Galy, J. M., and Herrero, C. (2011). Multicentric reticulohistiocytosis with elevated cytokine serum levels. J. Dermatol. Res. 38 (9), 905–910. doi:10.1111/j.1468-8138.2010.01446.x

Bonsang, B., Charles, F., Conan-Charlet, V., Guilbert, S., Marcocelles, P., and Talagas, M. (2019). Mammary carcinoma with osteoclast-like giant cell: Fine needle aspiration and cytological diagnosis of a rare and misleading subtype of invasive ductal carcinoma. Cytopathology. 30 (3), 337–339. doi:10.1111/cyt.12669

Buscaglia, L. E. B., and Li, Y. (2011). Apoptosis and the target genes of microRNA-21. Chin. J. Cancer 30 (6), 371–380. doi:10.5732/cjc.30.0371

Cascu, C. I., Guo, X., Teșa, L., Bhagat, T. D., Verma, A., Zheng, D., et al. (2015). Colony stimulating factor-1 receptor signaling networks inhibit mouse macrophage inflammatory responses by induction of microRNA-21. Blood 125 (8), e1–13. doi:10.1182/blood-2014-06-608000

Caetano Oliveira, R., and Schnitt, F. C. (2018). Stromal cellular fragments in breast fine needle aspirates: Think outside of the box. Acta Cytol. 62, 450.

Canfrán-Duque, A., Rolllan, N., Zhang, X., Fernández-Fuertes, M., Ramirez-Hidalgo, C., Araldi, E., et al. (2017). Macrophage deficiency of miR-21 promotes apoptosis, plaque necrosis, and vascular inflammation during atherogenesis. EMBO Mol. Med. 9 (9), 1244–1262. doi:10.15252/emmm.201607492

Cascini, C., and Chiiodoni, C. (2021). The immune landscape of osteosarcoma: implications for prognosis and treatment response. Cells 10 (7), 1668. doi:10.3390/cells10071668

Crisciuttiello, C., Guerini-Rocco, E., Viale, G., Fumagalli, C., Sajjadi, E., Venetis, K., et al. (2021). Immunotherapy in breast cancer patients: A focus on the use of the currently available biomarkers in Oncology. Anticancer. Agents Med. Chem. 22, 787–800. doi:10.2174/1871520621666210706144112

Dahm, H. H. (2017). Non-small cell carcinoma of the lung with osteoclast-like giant cells. Int. J. Surg. Pathol. 25 (3), 258–261. doi:10.1016/j.ijsupa.2016.07.014

de Sire, A., Baricich, A., Renò, F., Casari, C., Fusco, N., and Invernizzi, M. (2020). Mysstatin as a potential biomarker to monitor sarcopenia in hip fracture patients undergoing a multidisciplinary rehabilitation and nutritional treatment: a preliminary study. Aging Clin. Exp. Res. 32 (5), 959–962. doi:10.1007/s40520-019-01436-8

Dei, M. V., Radosovic-Robin, N., Fineberg, S., van den Eynden, G., Ternes, N., Penault-Llorca, F., et al. (2018). Update on tumor-infiltrating lymphocytes (TILs) in breast cancer, including recommendations to assess TILs in residual disease after neoadjuvant therapy and in carcinoma in situ: a report of the international immuno-oncology biomarker working group on breast cancer. Semin. Cancer Biol. 52, 16–25. doi:10.1016/j.semcancer.2017.10.003

Durox-Richard, A., Robin, M., Pellex, C., and Apparailly, F. (2019). MicroRNA-338. Fine tuners of monocyte heterogeneity. Front. Immunol. 10, 2145. doi:10.3389/fimmu.2019.02145
Invermizzi, M., de Sire, A., Renò, F., Cisar, C., Runza, L., Barich, A., et al. (2020). Spinal cord injury as a model of bone-muscle interactions: therapeutic implications from in vitro and in vivo studies. Front. Endocrinol. 11, 204. doi:10.3389/fendo.2020.00204

Johannessen, C., Moi, L., Kiselev, Y., Pedersen, M. I., Dalem, S. M., Bratтен, T., et al. (2017). Expression and function of the miR-143/145 cluster in vitro and in vivo breast cancer. PLoS One 12 (10), e0186658. doi:10.1371/journal.pone.0186658

Kuo, Y. F., Tu, M. C., Chai, H. J., Lin, Y. L., and Chen, Y. C. (2021). Suppressive effects of an apoptotic mimic prepared from jumbo-flying squid-skin phospholipids on the osteoosteosclerosis in receptor activator of nuclear factor kappa B ligand/macrophage colony-stimulating factor-induced RAW 264.7 cells. J. Clin. Med. 10 (4), 1. doi:10.3390/jcm1004001

Kobli, A. D., Shupp, A. B., Mukhopadhyay, D., Marini, F. C., and Bussard, K. M. (2019). Osteoblasts are “educated” by crosstalk with metastatic breast cancer cells in the bone tumor microenvironment. Breast Cancer Res. Treat. 21 (1), 31. doi:10.1007/s10549-019-1711-0

Kurzeda, H., Jamyian, T., Yamaguchi, R., Kakumoto, A., Abe, A., Harada, O., et al. (2021). Tumor microenvironment in triple-negative breast cancer: the correlation of tumor-associated macrophages and tumor-infiltrating lymphocytes. Clin. Transl. Oncol. 23, 2513–2525. doi:10.1007/s12094-021-02562-3

Laia, P. K., Nandi, P., and Majumder, M. (2018). Roles of progestins in tumour-associated lymphangiogenesis with special reference to breast cancer. Cancer Metastasis Rev. 37 (2), 369–384. doi:10.1007/s10555-018-9734-0

Li, J., Han, T., Wang, X., Wang, Y., and Yang, Q. (2021). Identification of novel survival-related IncRNA-miRNA-mRNA competing endogenous RNA network associated with immune infiltration in colorectal cancer. Am. J. Transl. Res. 13 (6), 5815–5834.

Lian, W. S., Ko, J. Y., Chen, S. Y., He, H. J., Hsueh, C. K., Kuo, C. W., et al. (2019). MicroRNA-29a represses osteosclerosis formation and protects against osteoporosis by regulating PGC-1α-mediated RANKL and CXCL12. Cell Death Dis. 10 (10), 705. doi:10.1038/s41419-019-1942-1

Liang, M., Ma, Q., Ding, N., Luo, F., Bai, Y., Kang, F., et al. (2019). IL-11 is essential in promoting osteolyis in breast cancer bone metastasis via RANKL-independent activation of osteoclasts. Cell Death Dis. 10 (5), 353. doi:10.1038/s41419-019-1594-1

Liang, M., Yin, X., Zhang, S., Ai, H., Luo, F., Xu, J., et al. (2021). Osteoclast-derived small extracellular vesicles induce osteogenic differentiation via inhibiting ARHGP1. Mol. Ther. Nucleic Acids 23, 1191–1203. doi:10.1016/j.omtn.2021.01.031

Liu, K., Xie, F., Gao, A., Zhang, R., Zhang, L., Xiao, Z., et al. (2017). SOX2 regulates multiple malignant processes of breast cancer development through the SOX2/SOX18-1/5p, miR-30e-5p/TUSC3 axis. Mol. Cancer 16 (1), 62. doi:10.1186/s12943-017-0632-9

Liu, T., Jiang, L., Li, J., Sun, J., Li, H., Gao, J., et al. (2021). A huge malignant phyllodes tumor of the breast with osteoclast-like giant cells: a case report in “Gland surg. 2010. 31: gland surgery”. All rights reserved, 1508

Liu, Y., Zhang, R. X., Yuan, W., Chen, H. Q., Tian, D. D., Li, H., et al. (2018). Knockdown of bone morphogenetic proteins type 1a receptor (BMP1RA) in a breast cancer cells protects bone from breast cancer-induced osteolysis by suppressing RANKL expression. Cell. Physiol. Biochem. 43 (5), 1759–1771. doi:10.1159/000487784

Lopez, G., Noale, M., Corti, C., Gaudio, G., Sajjadi, E., Venetis, K., et al. (2020). PTEN expression as a complementary biomarker for mismatch repair testing in breast cancer. Int. J. Mol. Sci. 21 (4), E1461. doi:10.3390/ijms21041461

Ma, X., Conklin, D. J., Li, F., Dai, Z., Hua, X., Li, Y., et al. (2015). The exocytic microRNA miR-21 promotes regulated necrosis in mice. Nat. Commun. 6, 7351. doi:10.1038/ncomms8151

Mao, Y., Keller, E. T., Garfield, D. H., Shen, K., and Wang, J. (2013). Stromal cells in tumor microenvironment and breast cancer. Cancer Metastasis Rev. 32 (1-2), 303–315. doi:10.1007/s10555-012-9413-5

Marchiò, C., Pietribiasi, F., Castiglione, R., Fusco, N., and Sapino, A. (2015). Giants in a microcosm: multinucleated giant cells populating an invasive micropapillary carcinoma of the breast. Int. J. Surg. Pathol. 23 (8), 654–655. doi:10.1177/1076327X15065016

McGuire, A., Brown, J. A., and Kerin, M. J. (2015). Metastatic breast cancer: the potential of miRNA for diagnosis and treatment monitoring. Cancer Metastasis Rev. 34 (1-2), 145–155. doi:10.1007/s10555-015-9551-7

Mori, D., Hiraki, M., Yamaji, K., Miyoshi, A., Koga, K., Yamamoto, T., et al. (2021). Non-invasive undifferentiated carcinoma with osteoclast-like giant cells of the common bile duct. Pathol. Int. 71 (1), 161–163. doi:10.1111/pin.13035

Ng, E. K. O., Li, R., Shin, V. Y., Sui, J. M., Ma, E. S. K., and Kwong, A. (2014). MicroRNA-143 is downregulated in breast cancer and regulates DNA
methyltransferases 3A in breast cancer cells. Tumour Biol. 35 (3), 2591–2598.
doi:10.1007/s13277-013-1341-7

Nielsen, T. O., Leung, S. C. Y., Rimm, D. L., Dodson, A., Acs, B., Badve, S., et al. (2020). Assessment of K67 in breast cancer: updated recommendations from the international K67 in breast cancer working group. J. Natl. Cancer Inst. 113 (7), 808–819. doi:10.1093/jnci/dja201

Nørregaard, K. S., Jürgensen, H. J., Gårdsvoll, H., Engelholm, I. H., Behrendt, N., and See, K. (2021). Osteosarcoma and metastasis associated bone degradation-A tale of osteosarcoma and malignant cell cooperation. J. Int. Mol. Sci. 22 (13), 6865. doi:10.3390/jm22136865

O’Brien, J., Hayder, H., Zayed, Y., and Peng, C. (2018). Overview of MicroRNA biogenesis, mechanisms of actions, and circulation. Front. Endocrinol. 9, 402. doi:10.3389/fendo.2018.00402

Offri, A., Noudsi, F., and O’Toole, S. (2020). Invasive breast carcinoma with osteoclast-like giant cells (olgc): A rare entity causing diagnostic confusion. Breast J. 26 (9), 1831–1832. doi:10.1111/bjc.13890

Ohashi, R., Hayama, A., Matsubara, M., Watarai, Y., Sakatani, T., Naito, Z., et al. (2018). Breast carcinoma with osteoclast-like giant cells: a cytological-pathological correlation with a literature review. Ann. Diagn. Pathol. 33, 1–5. doi:10.1016/j.anndiagpath.2017.11.003

Ohashi, R., Yanagihara, K., Namimatsu, S., Sakatani, T., Takei, H., Naito, Z., et al. (2018). Osteoclast-like giant cells in invasive breast cancer predominantly possess M2-macrophage phenotype. Pathol. Res. Pract. 214 (2), 253–258. doi:10.1016/j.prp.2017.11.002

Pagni, F., Guerini-Rocco, E., Schultheis, A. M., Grazia, G., Rijavec, E., Ghidini, M., et al. (2019). Targeting immune-related biological processes in solid tumors: we do need biomarkers. Int. J. Mol. Sci. 20 (21), E5452. doi:10.3390/ijms20215452

Peña-James, L., González-Garcia, J., Reguerro-Callejas, M. E., Pinilla-Pagnon, I., Pérez-Mies, B., Albarran-Artaizona, V., et al. (2019). Pleomorphic lobular carcinoma of the breast with osteoclast-like giant cells: a case report and review of the literature. Diagn. Pathol. 13 (1), 62. doi:10.1186/s13000-018-0744-6

Pruner, G., Vingiani, A., Bagnardi, V., Rotmensz, N., De Rose, A., Palazzo, A., et al. (2016). Clinical validity of tumor-infiltrating lymphocytes analysis in patients with triple-negative breast cancer. Ann. Oncol. 27 (2), 249–256. doi:10.1093/annonc/mdw571

Rakhia, E. A., El-Sayed, M. E., Lee, A. H., Elston, C. W., Grainge, M. J., Hidi, Z., et al. (2008). Prognostic significance of Nottingham histologic grade in invasive breast carcinoma. J. Clin. Oncol. 26 (19), 3153–3158. doi:10.1200/JCO.2007.15.5986

Rivera, L. B., and Bergers, G. (2013). Location, location, location: macrophage positioning within tumors determines pro- or antitumor activity. Cancer Cell 24 (6), 687–689. doi:10.1016/j.ccr.2013.11.014

Rizzo, S., Cangemi, A., Galvano, A., Fanale, D., Buscemi, S., Giacio, M., et al. (2017). Analysis of miRNA expression profile induced by short term starvation in breast cancer cells treated with doxorubicin. Oncotarget 8 (42), 71924–71932. doi:10.18632/oncotarget.18028

Sahrai, M., Chalbe, B., Liiu, Y., Sun, J., Kaplan, A., Price, N. L., et al. (2019). Supersizing miR-21 activity in tumor-associated macromolecules promotes an antitumor immune response. J. Clin. Invest. 129 (12), 5518–5536. doi:10.1172/JCI137125

Sajjadi, E., Venetis, K., Piciotto, R., Gambini, D., Blundo, C., Runza, L., et al. (2021). Combined analysis of PTEN, HER2, and hormone receptors status: remodeling breast cancer risk profiling. BMC cancer 21 (1), 1152. doi:10.1186/s12885-021-08889-z

Sajjadi, E., Venetis, K., Scatena, C., and Fusco, N. (2020). Biomarkers for precision immunotherapy in the metastatic setting: hope or reality? Ercanercamedicinecare14, 1156. doi:10.33320/ercancer.2020.1150

Shishido-Hara, Y., Kurata, A., Fujwara, M., Itoh, H., Imoto, S., and Kamma, H. (2010). Two cases of breast carcinoma with osteoclastic giant cells: Are the osteoclastic giant cells pro-tumoural differentiation of macrophages? Diagn. Pathol. 5 (1), 55. doi:10.1186/1746-1596-5-55

Subbarayan, K., Massa, C., Lazaridou, M. F., Ulagappan, K., and Seliger, B. (2021). Identification of a novel miR-21-3p/TGF-β signaling-driven immune escape via the MHC class I/biglycan axis in tumor cells. Clin. Transl. Med. 11 (3), e306. doi:10.1002/ctm.2.306

Venetis, K., Piciotto, R., Sajjadi, E., Inverrizzii, M., Morganti, S., Criscitiello, C., et al. (2021). Breast cancer with bone metastasis: Molecular insights and clinical management. Cells 10 (6), 1377. doi:10.3390/cells10061377

Wang, C. Z., Yuan, P., and Li, Y. (2015). MR-126 regulates breast cancer cell invasion by targeting ADAM9. Int. J. Clin. Exp. Pathol. 8 (6), 6547–6553.

Wang, Z., Brandt, S., Medeiros, A., Wang, S., Wu, H., Dent, A., et al. (2015). MicroRNA 21 is a homostatic regulator of macrophage polarization and prevents prostaglandin E2-mediated M2 generation. PLOS ONE 10 (2), e0115855. doi:10.1371/journal.pone.0115855

Wei, C. H., Garcia, L., Murata-Collins, J., Schmolze, D., and Apple, S. (2021). Quantitative impact of the 2018 american society of clinical oncology (ASCO)/College of American pathologists (CAP) practice guideline update on human epidermal growth factor receptor 2 testing in breast cancer: a systematic analysis. Arch. Pathol. Lab. Med. 145 (7), 887–890. doi:10.5858/arpa.2020-0378-OA

Weirinda, M. M., Lee, S. K., and Monrose, D. G. (2021). miRNAs in osteosclerotic biology. Bone 143, 115757. doi:10.1016/j.bone.2020.115757

Who Classification of Tumours Editorial Board, Who Classification of Breast Tumours (2019). WHO classification of Tumours, Vol. 2 (Lyon, France: World Health Organization).

Wu, R. W., Lian, W. S., Chen, Y. S., Kuo, C. W., Ke, H. C., Hsieh, C. K., et al. (2019). MicroRNA-29a counters glucocorticoid induction of bone loss through repressing TNSFI1b modulation of osteoclastogenesis. Int. J. Mol. Sci. 20 (20), E5141. doi:10.3390/ijms20205141

Xu, J., Huang, Q., Wang, L., Ma, X., Deng, Q., Kumar, M., et al. (2018). miR-21 depletion in macrophages promotes tumoral polarization and enhances PD-1 immunotherapy. Oncogene 37 (23), 3151–3165. doi:10.1038/s41388-018-0178-3

Xu, Z., Gu, J., Zhang, S., Zhang, and Fang, W. (2019). "Leiomysarcoma with osteoclast-like (LMS-OGC) giant cells the breast: a report of a rare case," in Thorac. cancer. 10: © 2019 the authors (Thoracic Cancer published by China Lung Oncology Group and John Wiley & Sons Australia, Ltd.), 2054

Zhang, H., Yu, Y., Wang, J., Han, Y., Ren, T., Huang, Y., et al. (2021). Macrophages-derived exosomal IncRNA LIFR-AS1 promotes osteosarcoma cell progression via miR-29a/NFIA axis. Cancer Cell Int. 21 (1), 192. doi:10.1186/s12935-021-01893-0

Zhou, S., Yu, L., Zhou, R., Li, X., and Yang, W. (2014). Invasive breast carcinomas of no special type with osteoclast-like giant cells frequently have a luminal phenotype. Virchows Arch. 460 (6), 681–688. doi:10.1007/s00428-014-1573-y