Breast Cancer Biomarkers From Peripheral Blood Cells

Subjects: Oncology

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While tissue-specific biomarkers, including immune-cell infiltration of the tumor, atypical cells, changes in tumor gene expression, and other malignant changes can serve as reliable cancer biomarkers, they have certain limitations. For instance, the invasiveness of biopsy acquisition makes tissue-specific biomarkers ill-fitted for the real-time monitoring of treatment response. Additionally, while tumor-infiltrating lymphocytes (TILs) may be powerful prognostic biomarkers and have significant predictive value in identifying patients with the highest likelihood of responding to therapy, they are not useful for the early detection of cancer or for cancer screening in people with no symptoms. Less invasive and more easily accessible methods of biological sample acquisition, such as blood collection, can make early detection more feasible and may increase acceptance among patients, thereby leading to potentially faster diagnosis. Need to focus on circulating blood cell transcriptome as a source of breast cancer (BC) biomarkers.

immune biomarker | triple negative breast cancer | peripheral blood | transcriptome | circulating blood cell | peripheral blood mononuclear cell (PBMC) | cytokine | immune breast cancer subtypes

1. Transitioning toward Less Invasive Immune-Related Biomarkers for Cancer Detection and Prognosis

While tissue-specific biomarkers, including immune-cell infiltration of the tumor, atypical cells, changes in tumor gene expression, and other malignant changes can serve as reliable cancer biomarkers, they have certain limitations. For instance, the invasiveness of biopsy acquisition makes tissue-specific biomarkers ill-fitted for the real-time monitoring of treatment response. Additionally, while TILs may be powerful prognostic biomarkers and have significant predictive value in identifying patients with the highest likelihood of responding to therapy [1], they are not useful for the early detection of cancer or for cancer screening in people with no symptoms. The currently used diagnostic tools such as biopsies and mammography are uncomfortable for patients, can be costly, and may be appropriate only for tumors that have developed to a specific extent [2]. For instance, breast cancer may exist for a while before it is detected by mammography, since the sensitivity of BC screen-testing depends on tumor size, increasing from 26% at 5 mm to 91% at 10 mm tumor size [3]. Furthermore, mammographic sensitivity for BC declines significantly for women with dense breast tissue [4]. Since patient survival rates increase substantially if cancer is identified at the early stages, high sensitivity and specificity of early cancer detection remain among the

most important and challenging issues [5]. For early cancer detection, acquiring tissue by invasive means can be difficult to justify, as it comes with certain risks and may not be a good choice for weak patients. Less invasive and more easily accessible methods of biological sample acquisition, such as blood collection, can make early detection more feasible and may increase acceptance among patients, thereby leading to potentially faster diagnosis.

2. Circulating Blood Cell Transcriptome as a Source of Less Invasive Breast Cancer Biomarkers

Tumor development and survival involve active crosstalk between cancer cells, normal stromal cells, adjacent tissues, and host immune defense system [2][6][7]. Primary tumors release a range of signaling molecules into their surroundings. Circulating blood cells monitor the physiological state of the body and respond to tumor signals with phenotypic and functional changes, rendering them able to either kill cancer cells or to promote cancer proliferation and metastasis [8][9][10]. While peripheral blood diagnostic and prognostic biomarkers include changes in peripheral blood counts, alterations in gene expression, DNA methylation, miRNA profiles, and other changes [11][12], need to focus on transcriptomic changes in peripheral blood cells (PBCs) of BC patients.

Tumor signals trigger distinctive changes in the transcriptome of circulating blood cells [13][14][15][16]. The resulting gene expression signatures can be clinically useful for the detection and characterization of primary tissue tumors, for cancer prognosis, and for monitoring or predicting the efficacy of therapies [2][6][16][17][18][19]. In peripheral blood cells of BC patients, Dumeaux et al. identified deregulated processes that reflect a deficit in immune functions. They proposed a signature of 50 genes associated with systemic immunosuppression which indicate the presence of BC and classify women with changes other than breast carcinoma (i.e., population-based controls, gastrointestinal and brain cancer patients) as negative [20]. Underexpression of several immune pathways, such as antigen processing and presentation (e.g., downregulation of MHC I molecules), decreased CD4 (which is involved in helper T cell (Th) activation), and impairment of natural killer (NK) cell-mediated immunity was observed [20]. Likewise, in a gene expression study of peripheral blood mononuclear cells (PBMCs), a subset of circulating blood cells comprising immune cells (i.e., monocytes, lymphocytes, NK and dendritic cells), the observation that NK-cell activity is decreased in BC patients compared to controls was confirmed [6]. This was evidenced by lower expression of activating receptors NKp30, NKp46 and 2B4 in BC, although the percentage of NK cells and the proportion of the primary NK cell subsets were similar in both groups [6]. Furthermore, Suzuki et al. described the upregulation of cell differentiation pathways of specific subsets of helper T cells (Th17, Th22, Th9) in PBMCs of BC patients compared to healthy subjects, as well as upregulation of TLR3- and TLR4-induced TICAM1-specific signaling pathway [21]. In colorectal cancer, the subsets Th17, Th22 and Th9 and their cytokines have been reported to contribute to its development [22].

Cytokines are key signaling molecules of intercellular communication in the immune system, known to mediate either stimulatory or inhibitory tumor responses. Dysregulation of circulating cytokines has been identified as an important dissimilarity between BC and healthy subjects [19]. Tumor-elicited cytokines are generated in the tumor microenvironment (TME), from which they spread into the circulation. They can be detected in blood during cancer

and have a high potential as biomarkers, particularly for monitoring the severity of cancer and efficacy of drug intervention [23]. Apart from cytokines produced in the TME, peripheral immune cells of cancer patients (even those with localized tumors) can also show dysregulated immune cytokine signaling signatures [23]. For instance, compared to healthy controls, peripheral nucleated blood cells of BC patients have been reported to overexpress a number of proinflammatory factors, such as CXCL1, CXCL2, CXCR4, CCL3, CCL4, IL-8, and others [17]. Cytokines and other biomediators that are upregulated in PBCs of BC patients have been suggested to stimulate the innate peripheral blood immune cells (e.g., phagocytes, granulocytes) to infiltrate TME [17].

Peripheral blood cells of BC patients also show changes in several universal cell programs (i.e., cell metabolism, growth, and proliferation) compared to healthy subjects [20][24]. Deregulation of genes involved in ribosome production and translation control, as well as of various metabolic processes, such as lipid and steroid metabolism, was reported in the peripheral blood of BC patients [15][25].

3. PBMC Gene Expression Biomarkers for Classification of Novel BC Subtypes

Considering that PBMCs comprise blood immune cells that mediate the host immune response to tumor cells, peripheral blood profiling can be useful for evaluating the host's reaction against cancer and offers the possibility for minimally invasive early cancer detection (even before the development of clinical symptoms), thereby distinguishing BC and healthy subjects [26][27][28]. It can also be valuable for predicting tumor progression and for the prognosis of clinical outcome [24][29][30].

In addition to differentiating healthy subjects from those with BC, several studies have attempted to identify the differences in PBMC gene expression within breast cancer to distinguish BC subtypes (**Table 1**) [6][17][21][29]. Important common observations that emerged were that PBMC transcriptomes in BC patients correlate poorly with classical BC subtypes and show substantial heterogeneity [16][21][29]. For instance, a study by Ming et al. involving RNA sequencing of PBMCs from 33 treatment-naïve BC patients (16 luminal A, 6 luminal B, 3 HER2-positive, and 8 TNBC) revealed that the established BC subtypes based on ER, PR, and HER2 were not associated with transcriptome-wide PBMC gene expression profiles [29]. This is not entirely unanticipated, considering that PBMC gene expression represents the immune reaction of blood mononuclear cells to the presence of tumor cells. Similar findings—substantial heterogeneity of peripheral blood leucocyte transcriptomes independent of histological type—were reported for lung cancer patients, where despite distinct origins of different histological types, there were no marked differences in influence on the peripheral immune system [31]. In breast cancer, Ming et al. further used unsupervised cluster analysis of PBMC gene expression, which identified two new BC subtypes, each comprising patients from all the established subtypes [29]. The difference between the novel subtypes was their distinctive immune response to tumor, including the activation of immune cells, the regulation and response of the innate and adaptive immune system, and the production of specific antibodies. Important distinct patterns included osteoclast differentiation (which is associated with metastasis) and the interleukin-10 signaling pathway (which is associated with inflammatory processes and tumor immunology). The novel subtypes also had different neutrophil-to-lymphocyte ratios, which indicate the inflammation level [29]. Interestingly, one of the subtypes was enriched in a

28-gene signature (including IFNGR1, IFNGR2, IL1A, IL1B, TLR2, TLR4, FOSL1, and CSF1), which had the ability to predict clinical outcome. Its tissue expression was associated with lower risk of recurrence and better survival of BC, including the basal-like breast cancer subtype, which is enriched in TNBC [29].

Table 1. Breast cancer immune subsets according to immunological properties of peripheral blood cells.

Study Cohort	Methodology	Novel BC Immune Profiles Based on PBMC Transcriptome	Reference
33 BC (8 TNBC, 16 luminal A, 6 luminal B, 3 HER2-positive)	RNA-seq	Two new BC subtypes differing in the activation of immune cells, regulation and response of innate and adaptive immune system, and antibody production; distinct immune cell proportions (lymphocytes and neutrophils); distinct immune patterns, with altered pathways including myeloid leukocyte activation, osteoclast differentiation and interleukin-10 signaling. Twenty-eight-gene signature enriched in one subtype: TYROBP, IFNGR1, GAB2, TNFRSF1A, PTGS2, NFKB2, NFKBIA, SIRPB1, NFKBIB, RELB, IL1A, IL1R1, IL1B, TLR4, TLR2, FCGR2A, IFNGR2, FCGR3B, JUNB, FOSL1, JUN, SOCS3, SIRPA, CR1, LILRB3, LILRA2, LILRA6, CSF1	[29]
13 BC (4 ER+/HER2-, 2 ER+/HER2+, 3 ER-/HER2+, 4 ER-/HER2-) and 3 healthy subjects	RNA-seq	Two new BC subsets differing in B-cell receptor immunological pathways (Bcl-XL, EGR1, p70 S6 kinase 1, Bcl-10, calcineurin A (catalytic), SOS, calmodulin, SHIP, PI3K reg class IA, IKK-alpha, and TAK1 (MAP3K7)) and CRTH2 signaling in Th2 cells (Bcl-XL, calcineurin A (catalytic), calmodulin, PKC, Apaf-1, and G-protein). Additionally, based on the subset of immune activation- and immune checkpoint-related genes, 4 immunological subgroups suggested: (1) monocyte-activating (CD14, CD40, CD80, Siglec14, NRP1, and TIM3) (included 3 of 4 ER-HER2- patients), (2) lymphocyte retention (CD8A, CD4, CD248, IDO1, and IDO2 (included all healthy controls), (3) T-cell inhibitory (PD-L1, PD1, CTLA4, FOXP3, and CCR3), (4) other	[21]
23 BC (14 TNBC and 9 luminal-A)	Pan-Cancer Immune Profiling Panel, 770 genes	Among all BC patients, a distinct group of 3 patients in the TNBC cohort showed changes in transcripts predominantly involved in inflammation; upregulated: IL1R2, THBS1, CD163, FLT3, MFGE8, IFNGR1, IL1RAP, CXCR4, TXNIP, TFRC, CD1D, CCND3, MAP2K1, HMGB1; downregulated: CLEC4C, TLR7, LTB, IL21R, IFIH1, PIK3CD	[6]

Similarly, RNA sequencing of PBMCs from 13 BC patients and 3 healthy volunteers identified BC and healthy subjects as two distinct clusters, whereas the BC group formed two distinct subsets that did not correlate with the classical BC subtypes [21]. These two subsets differed mainly in B-cell receptor immunological pathways and chemoattractant receptor-homologous molecule on Th2 (CRTH2) signaling in Th2 cells. Based on further analysis of immune-activating and immune-inhibitory gene expression patterns in PBMCs, the authors suggested four

immunological subgroups; all healthy subjects were in the “lymphocyte retention group” (upregulated CD8A, CD4, CD248, IDO1, IDO2), while BC patients were in “monocyte activating” (upregulated CD14, CD40, CD80, Siglec14, NRP1, TIM3), “T-cell inhibitory” (upregulated PD-L1, PD1, CTLA4, FOXP3, CCR3), or “unknown” group. With regard to TNBC patients, three out of four ER-HER2- patients were in the monocyte-activating group.

Overall, the above findings indicate that PBMC transcriptome in BC patients is influenced by the presence of cancer, not by BC subtypes [21][29]. This is in line with a study by Foulds et al., which combined analyses of PBMC transcriptome and blood immune cell profiling in TNBC and luminal A patients [6]. The overall PBMC immune expression profiles of the two BC subtypes were similar. However, 21% of TNBC patients had a clearly distinct immune expression pattern, enriched in transcripts involved in inflammation. From the coupled immune-cell and transcriptomic profiling, the authors concluded that the peripheral blood immunome in BC is influenced by the presence and stage of cancer, not by molecular subtypes (i.e., is disease-related rather than molecular subtype-specific) [6].

Finally, based on their observations, Ming et al. suggested that PBMC transcriptome-based subtyping could serve as a novel and independent classification for BC patients [29]. However, so far, PBMC transcriptome studies have not converged on a select number of common pathways characteristic for specific PBMC immune breast cancer subtypes, highlighting the need for further studies on larger cohorts.

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