Cutting Edge Diagnostic Methods for Early Diagnoses of Breast Cancer

Ekta A. Andriyas[1], Abhishek Verma[2], Arun Kumar Saxena[3], Munzali Hamisu Umar[4], Imran Hussain[5]

[1]Department of Medical Lab Technology, Era University, Lucknow, Uttar Pradesh, India
[2]Department of Medical Lab Technology, School of Allied Health Sciences, Galgotias University, NOIDA, UP
[3,4,5]Department of Medical Laboratory Sciences, Integral University, Lucknow. Uttar Pradesh, **India**

Abstract:
Breast cancer has a lengthy history; the Egyptians were the first to record cases of the disease over 3500 years ago, around 1500 B.C. Worldwide, breast cancer is the most common cancer and the leading cause of cancer-related deaths in women. Asia is the region responsible for 43% of newly diagnosed cases and 50% of all breast cancer deaths globally. Lung cancer is no longer the primary cause of cancer incidence worldwide; breast cancer in women has surpassed it entirely, accounting for 11.7% of all cancer cases with a projected 2.3 million new cases in 2020. With a prevalence incidence of 25.8 instances per 100,000 people, breast cancer is the most frequent cancer in India. In 2020, it will be responsible for 13.6% of all new cancer cases and around 13.3 fatalities per 100,000 people. In 2020, there was 13.3 fatalities per 100,000 persons. When breast cancer is discovered at stage IV as opposed to stage I, the survival rate drops by 2.7 times, and in 2020, about 90,408 Indian women lost their lives as a consequence. In developing nations, breast cancer remains the leading cause of cancer-related mortality for women. The consensus on the causes of cancer is that smoking and alcohol consumption are to blame. However, the reality remains that it is diverse, and if identified early on, it can be treated favorably with a better-than-expected prognosis and even a lower death rate. Additionally, a wealth of scientific evidence supports the notion that 30% of cancers are curable if identified early and treated promptly, 40% are preventable, and the remaining 30% of advanced cancers are treated with palliative care due to the necessity of effective pain relief measures. According to the American Cancer Society, 281,550 women are expected to receive a breast cancer diagnosis in 2021, and 43,600 women are expected to lose their lives to the disease in the US. Early identification of diseases is essential for Individuals with smaller tumors at the time of diagnosis have a much lower chance of dying and a greater survival rate, indicating an effective therapy and expected prognosis. Female breast cancer has surpassed lung cancer as the most commonly diagnosed cancer, with an estimated 2.3 million new cases (11.7%), followed by lung (11.4%), colorectal (10.0 %), prostate (7.3%), and stomach (5.6%) cancers. Lung cancer remained the leading cause of cancer death, with an estimated 1.8 million deaths (18%), followed by colorectal (9.4%), liver (8.3%), stomach (7.7%), and female breast (6.9%) cancers.

Introduction

Breast cancer has a lengthy history; the Egyptians were the first to record cases of the disease over 3500

years ago, around 1500 B.C. [1].Worldwide, breast cancer is the most common cancer and the leading cause of cancer-related deaths in women [2-4]. Asia is the region responsible for 43% of newly diagnosed cases and 50% of all breast cancer deaths globally [2].Lung cancer is no longer the primary cause of cancer incidence worldwide; breast cancer in women has surpassed it entirely, accounting for 11.7% of all cancer cases with a projected 2.3 million new cases in 2020. With a prevalence incidence of 25.8 instances per 100,000 people, breast cancer is the most frequent cancer in India. In 2020, it will be responsible for 13.6% of all new cancer cases and around 13.3 fatalities per 100,000 people [5].In 2020, there was 13.3 fatalities per 100,000 persons. When breast cancer is discovered at stage IV as opposed to stage I, the survival rate drops by 2.7 times, and in 2020, about 90,408 Indian women lost their lives as a consequence [6]. In developing nations, breast cancer remains the leading cause of cancer-related mortality for women. The consensus on the causes of cancer is that smoking and alcohol consumption are to blame. However, the reality remains that it is diverse, and if identified early on, it can be treated favorably with a better-than-expected prognosis and even a lower death rate. Additionally, a wealth of scientific evidence supports the notion that 30% of cancers are curable if identified early and treated promptly, 40% are preventable, and the remaining 30% of advanced cancers are treated with palliative care due to the necessity of effective pain relief measures [7,8]. According to the American Cancer Society, 281,550 women are expected to receive a breast cancer diagnosis in 2021, and 43,600 women are expected to lose their lives to the disease in the US. Early identification of diseases is essential for Individuals with smaller tumors at the time of diagnosis have a much lower chance of dying and a greater survival rate, indicating an effective therapy and expected prognosis [9]. Female breast cancer has surpassed lung cancer as the most commonly diagnosed cancer, with an estimated 2.3 million new cases (11.7%), followed by lung (11.4%), colorectal (10.0 %), prostate (7.3%), and stomach (5.6%) cancers. Lung cancer remained the leading cause of cancer death, with an estimated 1.8 million deaths (18%), followed by colorectal (9.4%), liver (8.3%), stomach (7.7%), and female breast (6.9%) cancers. Therefore, the main goals of all imaging modalities are accurate lesion assessment and early diagnosis of breast cancer. Currently, early detection of breast cancer and prompt treatment following diagnosis are the two main pillars that must be addressed for the illness to be managed effectively and preserve lives.

1. Methodology

1.1. Varieties of research:

A search of the literature was done to find pertinent western and Indian papers on the management of Breast Malignancy as well as internationally published unique guidelines.

The review covered both primary and secondary research articles. Studies conducted by healthcare groups and consensus declarations were also included. Further research was done on any supplementary material by looking through the references of pertinent papers.

1.2. Population types:

Older people and young adults were included in the research projects.

1.3. Sources of information:

Using a variety of search terms, including "breast Cancer," "Novel Diagnostics Tools," "western guidelines," "Indian guidelines," and "Prevalence of Breast Cancer in India," a literature search was conducted in PUBMED, Google Scholar, Cochrane Library, and Science Direct to find pertinent papers.

1.4. Research selection, data synthesis, and data extraction:

Only papers that dealt explicitly with the Novel Diagnostic Techniques in Diagnosis of Breast Cancer

and the related guidelines were deemed acceptable. Only studies written in English were included in our search. Moreover, there were no time filters added to this search. When duplicates were eliminated, publications that met the requirements were initially scrutinized based on their titles, and then their abstracts. After being determined to be eligible, full texts of papers were retrieved and scrutinized for inclusion in the study. The study team had discussions to settle any differences. Information on the present laws of many nations, together with comparison data, were taken from the chosen articles for the narrative.

2. Breast Cancer Classification –

➢ **The classification of breast cancer is based on two criteria –**
- Molecular Classification
- Histological Classification

➢ **Molecular classification is further subdivided in the following subtypes –**
- Luminal A -   ER +   PR + HER2 –
- Luminal B -   ER +   PR+   HER2+
- Basal   -   ER -   PR-   HER2-
- HER2   -   ER-   PR-   HER2 +
- Caludin   -   Adhesion Molecule and Tight junctions

BRCA 2 Mutation

BRCA 1 Mutation

HER2 Positive - Poor Prognosis
HER2 Negative - Good Prognosis

➢ **Histological Classification**
- Invasive   -   Ductal CA   Lobular CA
- Non- Invasive   -   Ductal CA (in situ) Lobular CA (in situ) Paget's Disease
- Ductal CA Subtypes   –   Tubular   Medullary   Inflammatory
- Mucinious**(in situ – When CA has not penetrated the BM)**

3. Techniques for Diagnosis or Detection of Breast Cancer

The secret to a successful breast cancer treatment is an early diagnosis. T1 tumours with a diameter of less than 2 cm have a 10-year survival rate of about 85%, but T3 tumours, which are mostly the consequence of a delayed diagnosis, have a 10-year survival rate of fewer than 60% [10] .

3.1 Molecular Image-Guided Sentinel Node Biopsy

A novel, minimally invasive technique called sentinel lymph node biopsy (SLNB) can detect metastases in patients with early-stage breast cancer. To determine the best course of treatment, SLNB is typically carried out by the nodal metastatic status [11]. The SLNB approach has a well-established reputation for having much lower post-operative problems than traditional axillary lymph node dissection [12, 13]. Because of this, a good diagnosis and course of therapy for breast cancer depend on efficient SLNB control. Precise SLNB guiding can reduce the number of invasive procedures required and identify sentinel lymph node localization to ascertain whether multiple-basin drainage is taking place. This will enhance the accuracy of staging for women with invasive breast cancer [14].

3.2 Role of Macromolecules in Breast Cancer Diagnosis

3.2.1 DNA

One of the most significant molecular changes in carcinogenesis is alteration of DNA methylation. Blood samples from females with breast cancer showed methylation promoters of Adenomatous polyposis coli (APC) and retinoic acid receptors-2 (RARb2) in 93.4% and 95.6% of the samples, respectively, but not in healthy individuals [15].When it came to identifying low-grade tumors, triple-negative breast cancer (NBC), and early breast cancer, all methylation variants outperformed the traditional markers CEA and CA 15-3. using a BeadChip for human methylation research. According to Yang et al., breast cancer is associated with hypomethylation of hyaluronoglucosaminidase 2 (HYAL2) and S100 calcium-binding protein P (S100P) in peripheral blood [16].Both genes with lower methylation have been proven to be possible biomarkers circulating in blood, for the diagnosis of breast cancer, specifically in adolescent girls in the initial stages [17].

3.2.2 Protein

Circulating protein has been regarded as the biological marker of choice second on the list for identifying and analyzing breast cancer (BC). Many protein biomarkers in blood that can be utilized as useful diagnostic biomarkers in the detection of breast cancer have been discovered as a result of systemic and thorough analysis of the blood proteomics pathologically and physiologically by mass spectrometry and blood proteomics. Trefoil factor (TFF) 1, TFF2, and TFF3 have been identified as potentially useful biomarkers for breast cancer screening because they can express particular proteins in the blood of individuals with breast cancer differently than healthy cells can [18].

3.2.3 Autoantibodies

Another method for diagnostic biomarkers is autoantibodies, which have the advantage of having several targets, quick turnaround times, and low-tech hardware [19].The body's cells produce the related antigens for these antibodies, which are produced moderately in healthy cells and excessively in malignant cells. By utilizing toll-like receptors (TLRs) for the innate response, the immune system ascertains this expression and halts the growth of tumours. An integral membrane protein called MUC1 has been connected to tumour aggressiveness and is overexpressed in 90% of adenocarcinomas [20].Targeting oncogenic and tumour suppressor proteins using antibodies is thought to be a significant diagnostic indicator for effective breast cancer identification. The possibility that the medical diagnosis of BC might potentially be made by detecting auto-immunoglobulins has been suggested by the presence of autoantibodies before the medical diagnosis of the disorder in paraneoplastic syndrome and systemic autoimmune diseases [21].As a result, extensive research has revealed the detection of tumor-associated antigens by autoantibodies that were detected in the patient blood sample. The integration of modern proteomics, advanced genomics, high-throughput technology, and traditional immunological approaches has significantly aided advancement in this area [21].

3.2.4　Micro RNA (miRNA)

miRNAs are short (20–25 nucleotide) single-stranded non-coding RNAs that, through mRNA expression or mRNA breakdown, inhibit the post-transcriptional expression of particular genes. Numerous studies have been conducted on the identification and stability of miRNAs in circulation as well as their application in therapeutic and diagnostic procedures [22].miRNAs are soluble and detectable in malignant cells [23], in blood, plasma and even in saliva, which shall be used as a biological marker in early diagnosis of Breast Cancer [24]. The apoptotic cells release miRNAs [23]. Moreover, unregulated miRNA is accountable for tumour growth, development, invasion, cell death, and proliferation, just like

oncogenes (oncomiRNAs) and tumour suppressors. Many potential possibilities, including miR-221, miR-21, and miR-145, have recently been detected in the blood serum or plasma of people with BC. The diagnostic susceptibility of blood-based identities containing miR-221 and/or miR-21 has been demonstrated to be higher than that of CEA and CA 15-3 for all stages of cancer. These identities are better at differentiating between patients with benign tumours and healthy individuals, as well as the TNBC subtype from the healthy subjects [25].Exosomal miR-21 expression has also been shown to be elevated in Breast cancer patients' plasma-derived exosomes. The exosomal miR-21 and miR-1246 form modest diagnostic [26]. Numerous additional miRNAs have been found to overexpress in breast cancer; these include miR-29a, miR 146a, miR-373, miR589, miR-221/222 cluster, miR-9, miR10b, miR-96, miR-181, miR-375, and miR-520c. These miRNAs are associated with breast cancer treatment, diagnosis, and prognosis [27].

3.2.5 Circular RNAs (CircRNAs)

Recently discovered non-coding RNAs known as circular RNAs (CircRNAs) are categorized as minuscule endogenous RNAs having a wide distribution, many shapes, and numerous regulatory uses [28]. Sanger et al. 1976 discovered CircRNAs in the viroids initially. NumerousCircRNAs have been discovered thus far in a wide range of cell lines and animals, including fish, mice, insects, worms, plants, protozoa, and humans [29].With advancements in high-throughput sequencing and bioinformatics technology, researchers are beginning to pay more attention to CircRNAs. It has been determined that CircRNAs are appropriate biological markers for the diagnosis of hepatocellular carcinoma, gastric cancer, and other cancers because of their tissue specificity and durability. There is now evidence that CircRNAs can contribute to cancer, opening up new possibilities for the discovery of diagnostic biomarkers. [30,31].Lu et al. used the CircRNAs microarray method to analyze the CircRNAs expression pattern in breast cancer and normal tissues. They found that the levels of hsa circ 103110, hsa circ 104689, and hsa circ 104821 were upregulated in breast cancer cells, with area under the curve (AUC) values of 0.63 (0.52–0.74), 0.61 (0.50–0.73), and 0.60 (0.49–0.71), respectively. Conversely, hsa circ 006054, hsa circ 100219, and hsa circ 406697 were downregulated, with area under the curve (AUC) values of 0.71 (0.61–0.81), 0.78 (0.69–0.88), and 0.64 (0.52–0.75), respectively. Consequently, combining hsa circ 406697, hsa circ 100219, and hsa circ 006054 produced fruitful diagnostic outcomes. Similarly, Yinetal 2017 discovered that, in comparison to stable controls, 19circRNAs were elevated and 22 were downregulated in the plasma of breast cancer patients. [32. 33]

3.3 Exosomes

Exosomes are nanosized (30–100 nm) extracellular membrane-bound vesicles that are actively released by cancer cells and surrounding cells in the tumour microenvironment (TME) [34].They consist of a wide range of components, including DNA, sugars, proteins, peptides, lipids, mRNAs, miRNAs, and other forms of non-coding RNAs, and are encased in a lipid bilayer made up of phosphoglycerides, ceramides, sphingolipids, and cholesterols [35].Like miRNAs, exosomes can be found in a range of human physiological fluids, such as blood, sweat, urine, and breast milk [36].Exosomes can influence the growth of tumours, angiogenesis, immunosuppression, and metastasis by encouraging intermodulation between tumour cells and stromal cells that are either healthy or cancerous [37].Promising materials for cancer detection include fibronectin, developmental endothelial locus-1 protein (Del-1), and the surface proteins found on circulating extracellular vesicles (EVs). Extracellular matrix protein fibronectin binds to multiple integrins to activate multiple signalling proteins, including

as FAK, Src, and Akt. During every stage of breast cancer, fibronectin levels increased significantly ($p < 0.0001$) and then returned to normal following tumour removal. When it came to fibronectin identification outside of cellular vesicles, clinical diagnostic effectiveness outperformed that of plasma. This highlights the role that extracellular matrix proteins play in breast cancer. The high level of Del-1 in patients' circulating exosomes (p 0.0001) led to a very successful diagnosis in separating early-stage breast cancer patients from the control group. [38].

Conclusion

The second most common disease in women to be recorded, breast cancer has a significant yearly death rate across the globe. Its fatality rate can be somewhat controlled by early diagnosis and prognosis. Its diagnosis is not attributed to a single biomarker, but rather to a collection of several diagnostic biomarkers that are important for its identification, course of treatment, and prognosis. Numerous macromolecules, such as circular RNA, miRNA, DNA, protein, exosomes, and antibodies, have been identified as important diagnostic indicators. The detection of cancer may be aided by the identification of these macromolecules. There are two main methods for identifying breast cancer: DNA methylation and miRNA profiling.

References

1. Ebeid, N.I. Egyptian Medicine in the Days of the Pharaohs; General—Egyptian Book Organization: Cairo, Egypt, 1999; ISBN 9789770164228.
2. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Pineros M: Global Cancer Observatory: Cancer Today. International Agency for Research on Cancer, Lyon; 2019.
3. Hortobagyi GN, de la Garza Salazar J, Pritchard K, et al.: The global breast cancer burden: variations in epidemiology and survival. Clin Breast Cancer. 2005, 6:391-401. 10.3816/cbc.2005.n.043
4. Varughese J, Richman S: Cancer care inequity for women in resource-poor countries . Rev Obstet Gynecol. 2010, 3:122-32.
5. Park JE: Textbook of Preventive and Social Medicine, 25th ed. BanarsidasBhanot, Jabalpur; 2019.
6. GLOBOCAN, The Global Cancer Observatory, Fact Sheet, India . (2020). Accessed: June 1, 2023: https://www.uicc.org/news/globocan-2020-new-global-cancer-data.
7. Lal S, Pankaj A: Textbook Of Community Medicine Preventive And Social Medicine With Recent Update, 3rd ed. CBS Publishers & Distributors Private Limited, Kochi; 2018.
8. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F: Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021, 71:209-49. 10.3322/caac.21660.
9. Duncan, W.; Kerr, G.R. The curability of breast cancer. Br. Med. J. 1976, 2, 781–783. [CrossRef] [PubMed]
10. Sant, M.; Allemani, C.; Berrino, F.; Coleman, M.P.; Aareleid, T.; Chaplain, G.; Coebergh, J.W.; Colonna, M.; Crosignani, P.; Danzon, A.; et al. Breast carcinoma survival in Europe and the United States. Cancer 2004, 100, 715–722. [CrossRef] [PubMed]
11. Lyman, G.H.; Somerfield, M.R.; Bosserman, L.D.; Perkins, C.L.; Weaver, D.L.; Giuliano, A.E. Sentinel Lymph Node Biopsy for Patients With Early-Stage Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline Update. J. Clin. Oncol. 2016, 35, 561–564. [CrossRef] [PubMed]

12. Cox, C.E.; Kiluk, J.V.; Riker, A.I.; Cox, J.M.; Allred, N.; Ramos, D.C.; Dupont, E.L.; Vrcel, V.; Diaz, N.; Boulware, D. Significance of sentinel lymph node micrometastases in human breast cancer. J. Am. Coll. Surg. 2008, 206, 261–268. [CrossRef] [PubMed]

13. Chen, S.L.; Iddings, D.M.; Scheri, R.P.; Bilchik, A.J. Lymphatic mapping and sentinel node analysis: Current concepts and applications. CA A Cancer J. Clin. 2006, 56, 292–297. [CrossRef] [PubMed]

14. Kang, J.; Chang, J.H.; Kim, S.M.; Lee, H.J.; Kim, H.; Wilson, B.C.; Song, T.-K. Real-time sentinel lymph node biopsy guidance using combined ultrasound, photoacoustic, fluorescence imaging: In vivo proof-of-principle and validation with nodal obstruction. Sci. Rep. 2017, 7, 45008. [CrossRef]

15. Swellam, M., Abdelmaksoud, M. D. E., Sayed Mahmoud, M., Ramadan, A., Abdel Moneem, W., and Hefny, M. M. (2015). Aberrant Methylation ofAPCandRARβ2genes in Breast Cancer Patients. Iubmb Life 67 (1), 61–68. doi:10.1002/iub.1346

16. Yang, W., Gong, P., Yang, Y., Yang, C., Yang, B., and Ren, L. (2020). Circ-ABCB10 Contributes to Paclitaxel Resistance in Breast Cancer through Let-7a-5p/ DUSP7 axis. Cmar Vol. 12, 2327–2337. doi:10.2147/cmar.s238513

17. Bleyer, A., and Welch, H. G. (2012). Effect of Three Decades of Screening Mammography on Breast-Cancer Incidence. N. Engl. J. Med. 367 (21), 1998–2005. doi:10.1056/nejmoa1206809.

18. Ishibashi, Y., Ohtsu, H., Ikemura, M., Kikuchi, Y., Niwa, T., Nishioka, K., et al. (2017). Serum TFF1andTFF3butNotTFF2AreHigherinWomenwithBreast Cancer Than in Women without Breast Cancer. Sci. Rep. 7 (1), 4846–4910. doi:10.1038/s41598-017-05129-y

19. Soler, M., Estevez, M.-C., Villar-Vazquez, R., Casal, J. I., and Lechuga, L. M. (2016). Label-free Nanoplasmonic Sensing of Tumor-Associate Autoantibodies for Early Diagnosis of Colorectal Cancer. AnalyticaChim. Acta 930, 31–38. doi:10.1016/j.aca.2016.04.059

20. Zaenker, P., Gray, E. S., and Ziman, M. R. (2016). Autoantibody Production in Cancer-The Humoral Immune Response toward Autologous Antigens in Cancer Patients. Autoimmun. Rev. 15 (5), 477–483. doi:10.1016/j.autrev. 2016.01.017

21. Fernández Madrid, F. (2005). Autoantibodies in Breast Cancer Sera: Candidate Biomarkers and Reporters of Tumorigenesis. Cancer Lett. 230 (2), 187–198.

22. Schwarzenbach, H., Hoon,D.S.B.,andPantel,K. (2011).Cell-free Nucleic Acids as Biomarkers in Cancer Patients. Nat. Rev. Cancer 11 (6), 426–437. doi:10.1038/ nrc3066

23. Shen, J., Stass, S. A., and Jiang, F. (2013). MicroRNAs as Potential Biomarkers in HumanSolid Tumors. Cancer Lett. 329 (2), 125–136. doi:10.1016/j.canlet.2012. 11.001

24. Mitchell, P. S., Parkin, R. K., Kroh, E. M., Fritz, B. R., Wyman, S. K., PogosovaAgadjanyan, E. L., et al. (2008). Circulating microRNAs as Stable Blood-Based Markers for Cancer Detection. Proc. Natl. Acad. Sci. 105 (30), 10513–10518. doi:10.1073/pnas.0804549105.

25. Gao, J., Zhang, Q., Xu, J., Guo, L., and Li, X. (2013). Clinical Significance of Serum miR-21 in Breast Cancer Compared with CA153 and CEA. Chin. J. Cancer Res. 25 (6), 743–748. doi:10.3978/j.issn.1000-9604.2013.12.04

26. Hannafon, B. N., Trigoso, Y. D., Calloway, C. L., Zhao, Y. D., Lum, D. H., Welm, A. L., et al. (2016). Plasma Exosome microRNAs Are Indicative of Breast Cancer. Breast Cancer Res. 18 (1), 90–14. doi:10.1186/s13058-016-0753-x

27. Polytarchou, C., Iliopoulos, D., and Struhl, K. (2012). An Integrated Transcriptional Regulatory Circuit that Reinforces the Breast Cancer Stem Cell State. Proc. Natl. Acad. Sci. 109 (36), 14470–14475. doi:10.1073/pnas. 1212811109

28. Ashwal-Fluss, R., Meyer, M., Pamudurti, N. R., Ivanov, A., Bartok, O., Hanan, M., et al. (2014). circRNA Biogenesis Competes with Pre-mRNA Splicing. Mol. Cel. 56 (1), 55–66. doi:10.1016/j.molcel.2014.08.019

29. Wang, P. L., Bao, Y., Yee, M.-C., Barrett, S. P., Hogan, G. J., Olsen, M. N., et al. (2014). Circular RNA Is Expressed across the Eukaryotic Tree of Life. PloS one 9 (3), e90859. doi:10.1371/journal.pone.0090859

30. Simon, R. (2015). Sensitivity, Specificity, PPV, and NPV for Predictive Biomarkers. J. Natl. Cancer Inst. 107 (8). doi:10.1093/jnci/djv153

31. Zhu, J., Ye, J., Zhang, L., Xia, L., Hu, H., Jiang, H., et al. (2017). Differential Expression of Circular RNAs in Glioblastoma Multiforme and its Correlation with Prognosis. Translational Oncol. 10 (2), 271–279. doi:10.1016/j.tranon. 2016.12.006

32. Lü, L., Sun, J., Shi, P., Kong, W., Xu, K., He, B., et al. (2017). Identification of Circular RNAs as a Promising New Class of Diagnostic Biomarkers for Human Breast Cancer. Oncotarget 8 (27), 44096.

33. Maselli, A., Parlato, S., Puglisi, R., Raggi, C., Spada, M., Macchia, D., et al. (2019). Autoantibodies Specific toERα Are Involved in Tamoxifen Resistance in HormoneReceptorPositive Breast Cancer. Cells 8 (7). doi:10.3390/cells8070750

34. Taylor, D. D., and Gercel-Taylor, C. (2013). The Origin, Function, and Diagnostic Potential of RNA within Extracellular Vesicles Present in Human Biological Fluids. Front. Genet. 4, 142. doi:10.3389/fgene.2013.00142

35. Moreno-Gonzalo, O., Villarroya-Beltri, C., and Sã¡nchez-Madrid, F. (2014). Post translational Modifications of Exosomal Proteins. Front. Immunol. 5, 383. doi:10.3389/fimmu.2014.00383

36. Vlassov, A. V., Magdaleno, S., Setterquist, R., and Conrad, R. (2012). Exosomes: Current Knowledge of Their Composition, Biological Functions, and Diagnostic and Therapeutic Potentials. Biochim. Biophys. Acta (Bba)- Gen. Subjects 1820 (7), 940–948. doi:10.1016/j.bbagen.2012.03.017

37. Alderton, G. K. (2012). Exosomes Drive Premetastatic Niche Formation. Nat. Rev. Cancer 12 (7), 447. doi:10.1038/nrc3304

38. Moon, P.-G., Lee, J.-E., Cho, Y.-E., Lee, S. J., Chae, Y. S., Jung, J. H., et al. (2016). Fibronectin on Circulating Extracellular Vesicles as a Liquid Biopsy to Detect Breast Cancer. Oncotarget 7 (26), 40189–40199. doi:10.18632/oncotarget.9561