

Editorial

Understanding Breast-Cancer-Associated Fibroblasts and Their Epigenetic Activation to Unveil Novel Targets for Breast Cancer Therapy

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The review article ‘Cancer-Associated Fibroblasts: Epigenetic Regulation and Therapeutic Intervention in Breast Cancer’ by Lee et al. [1] discusses the evolving potential of cancer-associated fibroblasts (CAFs) as potential therapeutic targets, particularly in breast cancer. The focus on breast cancer is understandable considering the incidence as well as mortality associated with breast cancer. As per the 2023 statistics in the United States [2], breast cancer is expected to lead all cancers among women in terms of incidence, accounting for almost one-third of all new cancer diagnoses. While this information is scary, a positive note is that even though the incidence rate has been increasing for the past several years, mainly due to more aggressive screenings, the mortality rate has not increased at the same pace [3], suggesting an improvement in the overall management of the disease. However, the number of breast-cancer-associated deaths are still too high, both in the US and worldwide, and more robust targets for therapy need to be identified and characterized.

Among the many aspects of a growing tumor, the tumor microenvironment (TME) has gained a lot of interest recently because it represents a close and protective niche of many different cell types that protect tumor cells from therapies [4] and support their growth, proliferation and invasion. The molecular biomarkers of focus in the article by Lee et al. [1] are fibroblasts, specifically CAFs, which represent a major component on TME. CAFs are specialized, activated fibroblasts that support tumor growth; they have considerable heterogeneity and plasticity [5]. Though it is widely believed that CAFs originate from ‘normal’ tissue-resident fibroblasts, there are examples of alternate origins of CAFs as well [6]. CAFs can be identified based on their spindle-shaped morphology. However, this morphology can be confused with the cancer cells undergoing epithelial-mesenchymal transition (EMT); the lack of endothelial, epithelial, and leukocyte markers’ expressions, in addition to the absence of specific cancer mutations, helps to identify CAFs [1,6]. One way that CAFs support tumor growth is through the release of growth factors, cytokines and chemokines [7–9]. It has been estimated that up to 70% of a breast tumor could be just CAFs, or more specifically, ‘breast cancer-associated fibroblasts (BCAFs)’ [1,10]. The crosstalk between BCAF and breast tumor cells is bi-directional. As discussed, BCAF secrete multiple factors to aid tumor progression. On their part, tumor cells release factors such as platelet-derived growth factors (PDGFs) and transforming growth factor-beta (TGF-β), which help the activation of BCAF [11].

The article by Lee et al. [1] discusses in detail the heterogeneity of BCAF because this is one area of research on CAFs that has attracted a lot of attention. The heterogeneity is apparent in the diverse functionality of different BCAF subtypes. Interestingly, there seems to be a direct effect of BCAF–breast-cancer cells’ bidirectional crosstalk on CAF heterogeneity as well, as supported by the observations that breast cancer with different mutations



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can activate BCAFs differentially, giving rise to BCAFs with diverse functionalities [12]. As an example, BCAFs activated by aggressive breast tumors with high migration capability can make breast cancer cells with relatively lower migration ability more invasive [1,12]. The article [1] discusses the different classifications and subtypes of CAFs. It discusses pro- vs. anti-tumorigenic CAFs. It also discusses four BCAF subtypes depending on the differential expression of specific biomarkers [13] and four BCAF subtypes depending on the different precursor cells that activated the BCAFs initially [14]. The article [1] also provides an overview of genetic changes that are apparent in the activation of CAFs from their precursor normal fibroblasts.

After the initial discussions on CAFs' origin, heterogeneity, etc., the discussion moves to the epigenetic regulation of BCAFs. It is mentioned that the epigenetic regulation of normal fibroblasts leading to activated fibroblasts can be reversible or irreversible. A reversible activation leads to normal activated fibroblasts, whereas CAFs are the result of irreversible activation [1,15]. It is also pointed out that exosomes may play a role in the epigenetic activation of BCAFs through shuttling factors that can bring about epigenetic changes [16]. Among the specific epigenetic events, the changes in DNA methylation and the resulting impact are discussed. For example, differential promoter methylation can impact gene expressions and contribute to tumors supporting and the acquisition of pro-tumorigenic phenotypes of CAFs [17]. Specific examples of several genes are provided; additionally, the emerging concept that the available literature has primarily focused on over-expressed genes in activated CAFs because of their hypomethylation in CAFs, or conversely their hypermethylation in normal fibroblasts, is discussed. The observations might be biased towards oncogenic genes that are overexpressed in CAFs, as compared to their precursors. The article by Lee et al. [1] also discusses the post-translational modifications of histone protein in BCAFs. These modifications represent another major epigenetic event [18] with evidence of differential histone methylation leading to the secretion of A disintegrin and metalloproteinase with thrombospondin motifs 1 (ADAMTS1) and the inhibition of the repressor Enhancer of zeste homolog 2 (EZH2) [19]. Finally, the discussion on the epigenetic regulation of BCAFs moves to microRNAs (miRNAs). As pointed out in the article, this regulation represents one of the most well-studied epigenetic regulations of BCAFs. A few miRNAs are identified in the discussion and, among them, the miR-200 family of miRNAs stands out as a prominent one. miR-200 is a tumor suppressor the miRNA family that negatively correlates with EMT and breast cancer aggressiveness [20]. As expected, this miRNA family is downregulated in BCAFs [21]. The authors summarize a number of miRNAs in a tabular form. It is concluded that while some miRNAs, such as, miR-31, miR-221, miR-9, miR-21, miR-155, miR-143 and miR-378e, are upregulated in BCAFs, other miRNAs, such as, miR-200 family miRNAs, miR-320, let-7g, miR-31, miR-26b and miR-148a, are downregulated in BCAFs [1].

With the identification of mechanisms that lead to the activation of BCAFs, it makes sense to exploit the information to validate novel targets for therapy. This is covered by Lee et al. [1] in the last section of their article. They point out the clinical trials evaluating epigenetic drugs such as DNA methyltransferases inhibitors (DNMTi) and histone deacetylases inhibitors (HDACi) [22]. Several DNMTis and HDACis are discussed, with the eventual recognition that some of these inhibitors have been tested in phase 1 and phase 2 clinical trials [1,23]. Further, even though most of the *in vitro* work on the epigenetic regulation of BCAFs has focused on miRNAs, there is a clear lack of evaluation of miRNAs as targets of therapy in clinics; this is primarily due to the lack of miRNA-targeted therapies that can be tested in clinics in patients with breast cancer. The factors released by BCAFs within the TME also present as attractive targets of therapy. Additionally, a better understanding of tumor-suppressive CAF phenotypes can potentially help identify novel targets for therapy.

In summary, the article by Lee et al. presents a comprehensive overview of CAFs, specifically BCAFs, with discussion on BCAFs' origins/heterogeneity, bi-directional communications with breast tumor cells, their epigenetic regulation and the identification of

key factors in their activation that have the potential to be further developed into valid targets for therapy.

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