# Apoptotic Effects of Adipose-Derived Stem Cell Secretome on Breast Cancer Stem Cells: Literature Review


## Abstract

Adipose-derived stem cells (ASCs) secrete various cytokines, proteins, growth factors, and extracellular vesicles with which they can be used in regenerative medicine, especially in the case of cancer. This has great potential, and can be developed as a new treatment strategy using the secretome of ASCs of global interest as a future medicine. The secretome has hidden effects in non-coding RNAs such as miR-21, miR-24, and miR-26 carried through exosomes secreted by adequate cells. The entire secretome, including ASC-derived exosomes (ASC-exos) in many studies has been shown to have immunomodulatory, proangiogenic, neurotrophic and epithelializing activities and has the potential to be used for neurodegenerative, cardiovascular, respiratory, inflammatory and autoimmune diseases and as a wound healing treatment. Due to limitations in the use of stem cells in cell-based therapy, secretomes with emphasis on exosomes may be a safer alternative future treatment with higher levels of effectiveness and lower side effects. In addition, a great advantage of cell-free therapy is the possibility of biobanking the ASC secretome. In this review, we focus on the current knowledge about the secretome of ASCs that can induce apoptosis of breast cancer cells.



**Keywords:** adipose-derived stem cells, secretome, stem cell therapy, breast cancer stem cells




## Introduction

Mesenchymal stem cells (MSC) are stromal cells that have the ability to self-renew and multilineage differentiation. MSCs can be isolated from various tissues, such as umbilical cord, bone marrow, endometrial polyps, menstrual blood, adipose tissue, etc. This is because the ease of retrieval and quantity obtained make these sources the most practical for experimental and clinical applications. MSCs may be the best choice for experimental or clinical applications in the future. The multipotent nature of MSCs makes them an attractive option for the development of cancer treatments [1].

Mesenchymal stem cells (MSC) are a type of Adipose-Derived Stem Cells that can be used in breast reconstruction. Adipose-Derived Stem Cells (ASC) can also be considered as a cancer therapy because it has autocrine and paracrine factors that can increase the recruitment of endogenous precursors. They secrete many important proteins, including growth factors (GFs) and cytokines [1], as well as extracellular vesicles (EVs) and RNA [3,4,5] to support cell regeneration, proliferation, differentiation, and migration [6]. Many studies have optimized the conditions for ASC stem cell-based therapy by manipulating the route of administration, cell dose and time [1,7] to reach the site of inflammation, but currently the results are not satisfactory [8,9]. Sacerdote et al. Has been studying the use of ASCs for many years and demonstrated that $1 \times 10^6$ human ASCs administered intravenously can have anti-pain effects in a mouse model of neuropathic pain [10]. Therefore, new strategies for ASCs-based treatment are needed, and ASCs secretome may be a promising therapy for medical purposes as a safe therapeutic agent for free cell-based therapy and easy to store for long-term use [11,12].

This review provides information on the current knowledge of the secretome of adipose-derived stem cells, with a special focus on their exosomes. This research uses all available sources such as the PubMed and Google Scholar databases, as well as Clinical Trials. The authors collected data and summarized information from in vitro, in vivo, and clinical trials using adipose-derived stem cell secretomes in the context of use in breast cancer therapy. The literature study is based on keywords such as adipose-derived stem cells, secretome, exosomes, breast cancer therapy and combinations of these keywords.

**Adipose-Derived Stem Cells Characteristics**

Adipose derived stem cells are mesenchymal stem cells (MSC) which can be taken through surgery and direct excision, liposuction of the limbs and extremities. Isolation of ADSCs is performed from lipoaspiration material in which stromal vascular fraction (SVF) cells can be found; from this fraction, different cell types can be isolated after washing, enzymatic digestion and centrifugation of the sample [13]. The types of cells found in SVF include: preadipocytes, fibroblasts, adult mesenchymal stem cells, monocytes, macrophages, lymphocytes and pericytes which are associated with the angiogenesis process[13].

Adipose-derived stem cells are a heterogeneous population and no unique surface markers have yet been described. However, ADSCs express characteristic markers of mesenchymal stem cells established by ISCT and the International Federation for Adipose Therapy and Science (IFATS) and described by CD73(+), CD90(+), CD105(+), and CD36(+) but also CD31 (−), CD45(−), CD11b(−), CD106(−). CD36 expression and lack of CD106 expression distinguish ASCs from BM-MSCs [1]. In addition, ASCs express β-1 integrin (CD29) which participates in angiogenesis and CD44 hyaluronate and osteopontin receptors, which are essential in the development of the extracellular matrix and pathological processes such as neoplasia [1]. There are no consistent data, in terms of whether ASCs isolated from the stromal vascular fraction can express CD34and CD106 or not [1]. It is recognized that cultured MSCs do not express CD34, in contrast to freshly isolated cells. Several studies have shown that CD34 is present at the start of culture in freshly isolated ASCs but after the first and subsequent passages it disappears [69] or remains at low levels [1, 13].

**Adipose-Derived Stem Cells Secretome**

Adipose-derived stem cells produce many molecules responsible for cell signaling, such as cytokines [1,14], growth factors [15,16], morphogens, chemokines [1], and extracellular vesicles [17, 18, 19], which enhances various cellular mechanisms. It has also been shown, in vivo, that ASCs are better at secreting bioactive factors such as nerve growth factor (NGF), hepatocyte growth factor (HGF), monocyte chemotactic protein 1 (MCP-1), granulocyte colony stimulator-macrophage factor (GM- CSF), granulocyte colony-stimulating factor (CSF), interleukin 1 receptor antagonist (IL-1RA), interleukin (IL)-6 and IL-8 versus bone marrow (BM)-MSCs [1]. These bioactive factors play a role in better differentiation, migration, proliferation and autocrine activity compared to BM-MSCs [1].

Adipose-derived stem cells (ASCs) secrete various cytokines, proteins, growth factors, and extracellular vesicles with which they can be used in regenerative medicine, especially in the case of cancer. This has great potential, and can be developed as a new treatment strategy using the secretome of ASCs of global interest as a future medicine. The secretome has hidden effects in non-coding RNAs such as miR-21, miR-24, and miR-26 carried through exosomes secreted by adequate cells. The entire secretome, including

ASC-derived exosomes (ASC-exos) **(Figure. 1)** in many studies has been shown to have immunomodulatory, proangiogenic, neurotrophic and epithelializing activities and has the potential to be used for neurodegenerative, cardiovascular, respiratory, inflammatory and autoimmune diseases and as a wound healing treatment [1].

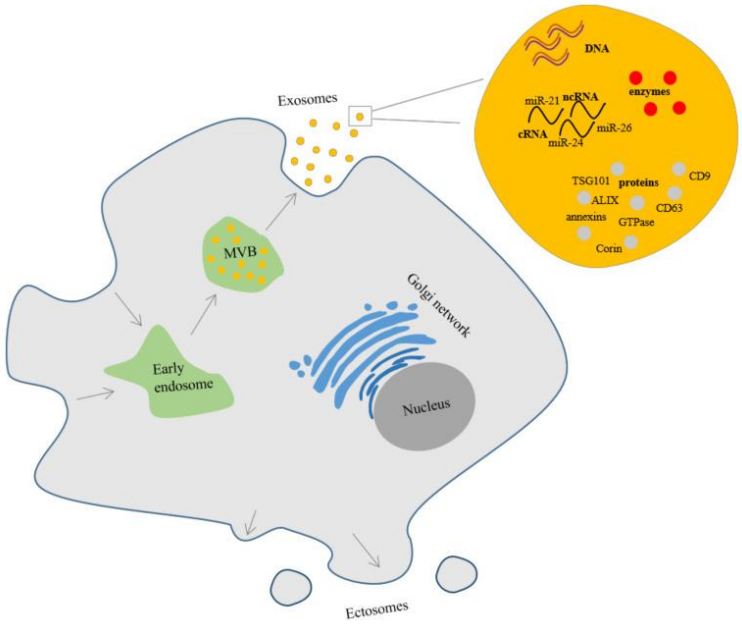



**Figure. 1.** Exosomes secretion in Adipose-derived stem cells

**Role of Pathology in Breast Cancer Stem Cells**

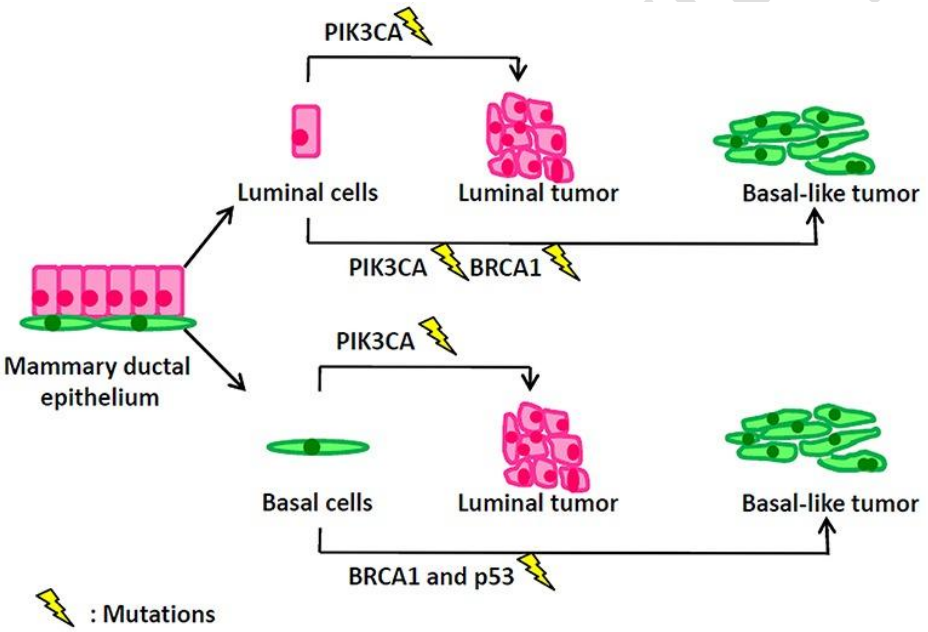
The origins of the BCSC have sparked controversy in the field for years. It remains unclear whether BCSCs originate from multipotent mammary stem cells (MaSC), constitute a unique population of progenitors, result from dedifferentiation of non-stem cells, or arise from a combination of both. A widely accepted opinion is that BCSCs arise from MaSCs and progenitor cells. Lineage tracing studies indicate the presence of unipotent luminal and basal progenitor cells in the developing mammary gland that sustain and support fully differentiated luminal and basal cell lineages, respectively, for long periods of time [20]. It is thought that the accumulation of mutations in these progenitor cells may give rise to BCSC as evidence suggests similar phenotypic features of CD44+/CD24−/low are present in MaSC and BCSC populations [20].

Dedifferentiation of non-stem cells is another theory supported by recent evidence. Environmental exposure to chemotherapy and radiotherapy causes genetic and epigenetic changes in non-stem cells, resulting in the de novo generation of BCSCs [20].

Changes in the tumor microenvironment may also contribute to the dedifferentiation of non-stem cells to a BCSC phenotype and recent evidence suggests that MYC-driven epigenetic reprogramming results in the dedifferentiation of mammary epithelial cells to a BCSC-like phenotype [21]. In summary, it appears that “stemness” in breast cancer is a phenotype that can arise through different mechanisms, either through mutation of tissue stem cells or through the acquisition of a stem-like phenotype by transformed cells, induced by EMT, chemotherapy, or even targeted therapy. For example, Bhola et al. recently demonstrated that in triple-negative breast cancer, resistance to mTOR, and/or PI3K inhibitors arises through Notch-dependent emergence of BCSCs [22].

Historically, in breast cancer, the name “luminal” of the luminal-A/B subtype and the name “basal-like” of the basal-like subtype were derived from transcriptome similarities between the breast tumor and the corresponding normal mammary luminal or basal epithelium. However, the actual cellular origin of luminal and basal breast cancers differs greatly from their naming conventions. Oncogenic events in different types of breast cells lead to different types of breast tumors **(Figure. 2)**.

PIK3CA (α-catalytic subunit of PI3K) mutations occur in 30% of breast cancers, including luminal and basal tumors. However, research by Meyer et al. reported that mutant PIK3CA in mammary luminal progenitors produced heterogeneous tumors with both luminal and basal differentiation [23]. Research by Van Keymeulen et al. also found that expression of mutant PI3KCA in luminal cells—marked with CK8—induced luminal or basal-like breast tumors, whereas its expression in basal cells—marked with CK5—gave rise to luminal tumors [24]. BRCA1 basal-like breast cancer may originate from basal stem cells. However, what is interesting, Molyneux et al. showed that BRCA1 deletion in breast luminal epithelial cells—targeted by Blg—can produce basal-like breast tumors, which phenocopy BRCA1-associated breast cancer in humans, whereas BRCA1 deficiency in basal cells–targeted CK14–can cause BRCA1-associated breast cancer in humans produces




by only [25] malignant adenomyoepithelioma which is a rare form of BRCA1-associated breast cancer in humans. Furthermore, Tao et al. described that luminal CK8+ cells carrying the Etv6-NTRK3 fusion oncogene could induce heterogeneous tumors with expression of both luminal and basal markers [26]. Strong evidence suggests that luminal progenitors may serve as the cellular origin of luminal-like and basal human breast cancers, whereas different genetic mutations—which occur upon transformation of luminal progenitors—may be determinants of luminal-like or basal breast cancers. such as tumor phenotype [27]. Genetic sequencing results have described different mutation profiles between luminal-like tumors and basal-like tumors. Luminal-like tumors show different mutations, such as PIK3CA, GATA3, and FOXA1, whereas basal-like tumors show high levels of p53 and BRCA1 mutations [30]. Using a conditional mouse model, Liu et al. showed that

somatic loss of BRCA1 and p53 did result in the development of basal-like breast cancer [30].

**Figure. 2.** Mutation events in various types of breast cells cause different types of breast tumors

**Potential Apoptosis of Adipose-Derived Stem Cells Secretome in Breast Cancer Stem Cells**

In the study, there was a significant decrease in cell viability, and it remains to be evaluated whether the cells are undergoing the process of apoptosis. In another study, Adipose-derived stem cellsSecretome Formulation (SF) had a dose-dependent apoptotic effect on cancer cells. The TNBC population showed an increase in the number of cancer stem cells with CD44+/CD24− markers, that cancer stem cells with CD44+/CD24− markers were reduced substantially despite the increased presence of a population of cancer stem cells with this phenotype in the chemically determined TNBC cell medium. Clearly, SF has a chilling effect on cancer stem cells. The MDR1+ (multidrug resistance protein 1) and PD-L1+ (programmed death ligand 1) phenotypes were substantially reduced after Secretome Formulation treatment at a dose of 70 mg/ml [31].

To further assess whether SF is synergistic with the conventional chemo drug paclitaxel, it is still necessary to evaluate the viability of TNBC cells in chemically defined breast cancer cell media [31]. Can Secretome Formulation, especially on Adipose-Derived

Stem Cells, be used as an apoptotic agent for other types of breast cancer? So further research needs to be carried out both in vitro and in vivo in the future.

**Conclusion**

Adipose-derived stem cell secretome is considered as a potential agent for breast cancer treatment with its apoptotic function. The properties of ASCs are influenced by the specific contents of their secretome: cytokines, proteins, growth factors, and exosomes with several types of RNA, characterized by broad bioactivities including immunomodulatory, antiapoptotic, angiogenic, vasculogenic, neurogenic, and epithelial activities. Application of bioactive factors without administration of whole cells is a safer and possibly more effective alternative treatment. Currently, numerous in vitro and in vivo studies from the last four years confirm the effectiveness of ASC secretome therapy, mostly with ASC-derived exosomes. Several clinical trials are currently being evaluated for the safety and effectiveness of ASC-derived exosome therapy, but results are not yet available.However, further in vivo and in vitro studies are needed to understand the specific role of the ASC secretome in treating breast cancer through its apoptotic function which can be administered intravenously or locally during breast reconstruction.



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