THE ROLE OF TUMOR MICROENVIRONMENT AND IMPACT OF CANCER STEM CELLS ON BREAST CANCER PROGRESSION AND GROWTH

Nenad Markovic[1,4], Ana Lukovic[4], Nebojsa Arsenijevic[3], Srdjan Ninkovic[1,4] and Biljana Ljujic[2]

[1]*University of Kragujevac, Serbia, Faculty of Medical Sciences, Department of Surgery*
[2]*University of Kragujevac, Serbia, Faculty of Medical Sciences, Department of Genetics*
[3]*University of Kragujevac, Serbia, Faculty of Medical Sciences, Department of Microbiology and Immunology, Center for Molecular Medicine and Stem Cell Research*
[4]*Clinical center "Kragujevac", Kragujevac, Serbia, Clinic for General and Thoracic Surgery*
[5]*University of Kragujevac, Serbia, Faculty of Medical Sciences, PhD student*

Received: 06.07.2017.
Accepted: 09.06.2018.

Corresponding author:

Ana Lukovic, MD

University of Kragujevac, Faculty of Medical Sciences, 34000 Kragujevac, Serbia

E-mail: analukovic91@gmail.com

sciendo

UDK: 618.19-006.6-092
Eabr 2023; 24(2):85-92
DOI: 10.2478/sjecr-2018-0018

ABSTRACT

Breast cancer is not only a mass of genetically abnormal tissue in the breast. This is a well-organized system of a complexheterogeneous tissue. Cancer cells produce regulatory signalsthat stimulate stromal cells to proliferate and migrate; then,stromal elements respond to these signals by releasing components necessary for tumor development that provide structuralsupport, vasculature, and extracellular matrices. Developing tumors can mobilize a variety of cell types from both local anddistant niches via secret chemical factors derived from cancercells themselves or neighboring cells disrupted by growing neo-plasm, such as fibroblasts, immune inflammatory cells, and endothelial cells. CSCs are a group of very few cells that are tumorigenic (able to form tumors) and are defined as those cellswithin a tumor that can self-renew and lead to tumorigenesis.BCSCs represent a small population of cells that have stem cellcharacteristics and are related to breast cancer. There are different theories about the origin of BCSCs. BCSCs are responsiblefor breast carcinoma metastasis. Usually, there is a metastaticspread to the bones, and rarely to the lungs and liver. A phenomenon that allows BCSCs to make the transition from epithelialto mesenchymal expression and thus avoid the effect of cytotoxic agents is the epithelial-mesenchymal transition (EMT).During this process, cells change their molecular characteristicsin terms of loss of epithelial characteristics taking the mesenchymal phenotype. This process plays a key role in the progression,invasion, and metastasis of breast tumors.

Keywords: *Cancer stem cell, tumor microenvironment, breast cancer stem cell, resistance to conventional therapy.*

INTRODUCTION

The tumor is a tissue mass resulting from its abnormal growth. Conventionally, this mass is classified as a benign, malignant, and so-called tumor in situ. Until the formationof a tumor, cells go through specific stages of metaplasia and dysplasia. However, not always do metaplasia and dysplasia finally result in the creation of a neoplasm (1-3).

Analogously to the above, breast cancer is formed as a result of breast tissue cells' abnormal growth. Breast cancer is not only a mass of genetically abnormal tissue in thebreast. This is a well-organized system of complex heterogeneous tissue (4). This heterogeneity in the tissue and understanding of cancer as a heterogeneous disease that helpsto understand disease progression and treatment failure (5). Cancer cells produce regulatory signals that stimulate stromal cells to proliferate and migrate; then, stromal elements respond to these signals by releasing componentsnecessary for tumor development that provide structuralsupport, vasculature, and extracellular matrices (6). It isincreasingly appreciated that tumor stroma crosstalk is animportant event for cancer initiation, growth, and progression (7). Developing tumors can mobilize a variety of celltypes from both local and distant niches through production of chemical factors derived from cancer cells themselves or neighboring cells disrupted by growing neoplasm,such as fibroblasts, immune and inflammatory cells, andendothelial cells. This assortment of cells and moleculestogether comprises the tumor microenvironment (TME)(8). TME is composed of extracellular matrix (ECM) andmany distinct cell types, including carcinoma associated fibroblasts (CAFs), tumor associated macrophages(TAMs-M2), cancer stem cells (CSCs), mesenchymal stemcells (MSCs), myofibroblasts, smooth muscle cells, endothelial cells and their precursors, pericytes, neutrophils, eosinophils, basophils, mast cells, T and B lymphocytes, natural killer cells (NK), and antigen presenting cells (APC) such as macrophages and dendritic cells (Figure 1). These non-tumor cells have important roles not only in tumor initiation, progression, and metastasis but also in therapeutic resistances (9-11).

In breast cancer, the most frequent component of tumor stroma is CAFs. There are many hypotheses about theorigin of CAFs (12). The dominant role of CAF in tumor tissues is to increase the expression of matrix metalloproteinase-14 (MMP14) and MMP9 activity, which promote tumor invasion and metastasis (13, 14). Besides the origin,these cells differ by expressing different surface markers which are mainly dependent on the tissue origin. In breastcancer, important CAF markers are fibroblast activation protein (FAP) and a combination of platelet-derived growthfactor-α and β receptor (PDGFR- α and β) and α-smooth muscle actin (α-SMA) (13). However, some studies have demonstrated that CAF can promote tumor progressionin other ways. It has been demonstrated that CAF-derivedCCL2 increases number of breast cancer stem cells (CSCs)which promotes metastasis (15).

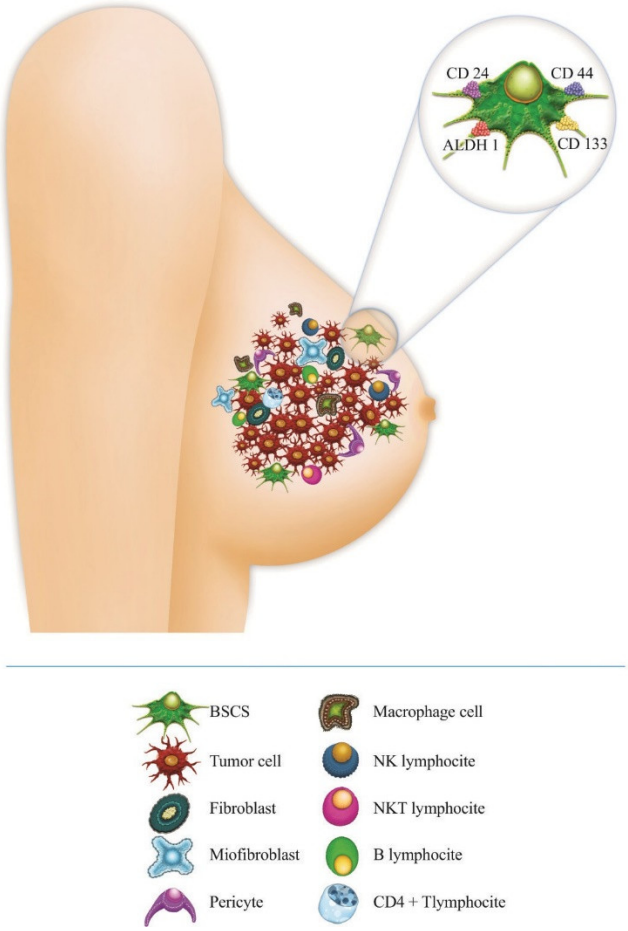
Many immune cells, such as macrophages, NK cells, regulatory T cells (Tregs), myeloid-derived suppressor cellshave also been implicated in breast cancer development (16). Macrophages can alter their polarization state from M1 to M2 (17). "Alternatively-activated" M2 macrophages produce anti-inflammatory cytokines like IL-10 and express non-inflammatory chemokines CCL17, CCL18, CCL22, CCL24 and have pro-tumorigenic functions (18). TAMs are mostly M2 macrophages populations that are either tissue-resident or derived from peripheral reservoirssuch as the bone marrow and spleen. The role of TAMsin breast cancer is to promote immunosuppression, neo angiogenesis, and tumor cell migration and invasion (19). TAMs accumulate in regions of hypoxia which regulate theexpression of M2-related genes that promote angiogenesis. By the production of vascular endothelial growth factor A (VEGF-A) and placental growth factor (PlGF), TAMs induce neo angiogenesis. TAMs-derived epidermal growth factor (EGF) and proteases, such as cysteine cathepsins, promote tumor progression and invasion (16). Studies show that a decrease of mammary tissue macrophages andan increase of TAMs in patients with breast cancer is a badprognostic sign (20).

The suppression and evasion of the host immune system during the progression of tumors can be achieved even through inhibition of effector immune cells or via stimulation of immunosuppressive cells. Myeloid-derived suppressor cells (MDSCs) and Treg cells suppress host immune system and contribute to tumorigenesis through enhancement of tumor immune evasion (21). MDSCs are immature myeloid cells witch derange tumor- associated antigen presentation, the polarization of macrophage, andthe activation of cytotoxic T cells and NK cells. Besides these functions, it has been shown that Treg cells can produce VEGF-A and induce neo angiogenesis. A high number of Treg cells in TME reduces the survival rate of breastcancer patients (17).

NK cells are important immune cells in anticancer immune response. NK cells control tumor initiation; however, they undergo crucial alterations during cancer progression (22). In tumor microenvironment, different factors have aneffect on the phenotype and function of these cells (23). There are two subpopulations of NK cells in tumor stroma, tumor-infiltrating natural killer cells (TINKs) and tumor-associated natural killer cells (TANKs). TINKs and TANKs have changed cytokine expression and increased levels of pro-angiogenic factors important for neo angiogenesis andtumor progression (19).

Myofibroblasts are cells with the characteristics of myoblasts and fibroblasts which have an important role inbreast cancer progression and invasion. Genes expressed in tumor myofibroblasts encode chemokines CXCL12 andCXCL14, important in breast cancer progression. CXCL12have a role in the earlier stages of breast tumorigenesis, while CXCL14 probably participate in inflammation (24).

Figure 1. Tumor microenvironment



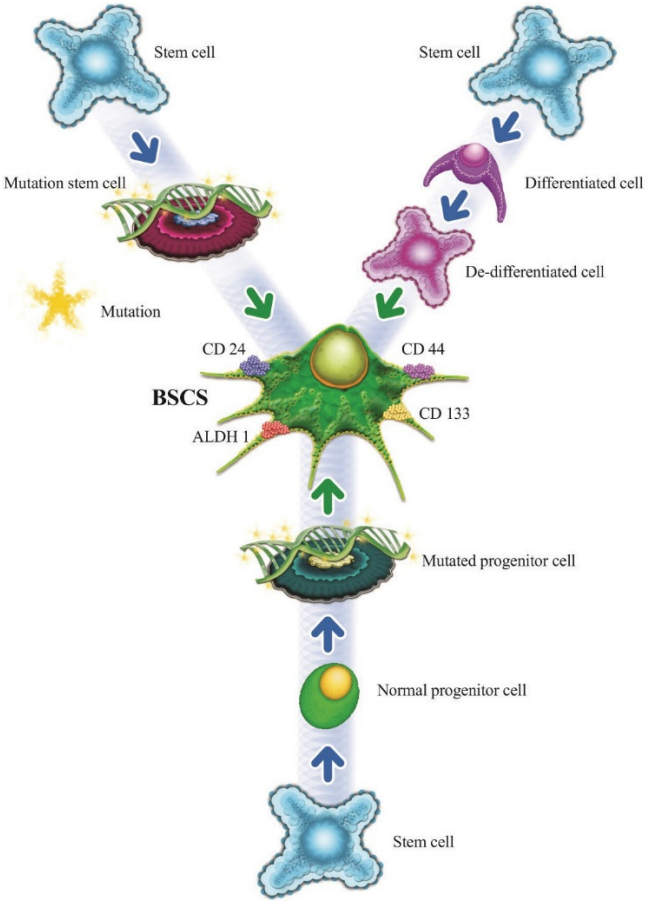
MSCs are multipotent cells that are capable of modulating tumor microenvironment and have an immunomodulatory function. Through these functions, these cells supportbreast cancer growth and progression (25). The immuno-modulatory function is dominantly immunosuppressive and includes changing of immune responses from Th1 to Th2 orinduction of Treg production and proliferation (26).

BCSCs have been associated with tumor initiation, progression, metastasis and resistance to conventional therapy. These small subpopulation of cells inside the tumor mass can be influenced by the other components of tumor microenvironment through complex interactions.T cells and CAFs from breast cancer microenvironment can both induce and inhibit BCSCs. Treg produce factors such as VEGF and TGF-β which promote cancer stemnessand BCSC expansion and effects of the tumor microvasculature and angiogenesis (27). Loss of Tissue Inhibitor of Metalloproteinases (TIMP) by CAF through activation of Notch signalling pathway and upregulation of typical BC- SCs markers increase the formation of distant metastasis. BCSCs express reduced levels of NK ligands what is BC- SCs mechanism for immune escape and also is connected with metastatic spread (28). Other cells including TAMs, MSCs and endothelial cells have effects on BCSCs throughnetworks of cytokines and growth factors. Notch, Hedgehog, Wnt, PI3K, NF-κB, and Jak/STAT stem cell regulatorypathways in breast cancer are most often dysregulated by signals from the tumor microenvironment. Notch signalling pathway regulate the selfrenewal of BCSCs. TAM-derived factors promote BCSCs selfrenewal and maintenance, as well, BCSC-derived factors induce protumor signals in TAMs (29).

Although, breast cancer tissue is composed from desciped cells, it has been noted that different tumor cells may show differences that can be reflected in the cell morphology, gene expression, metabolism, movement, proliferation,and metastasis (30). This functional heterogeneity among cancer cells has led to the creation of at least two models, which have been put forward to account for heterogeneityand differences in tumor-regenerative capacity: the cancerstem cell (CSCs) and clonal evolution models (31).

Under normal conditions, it has been observed that stem cells create a new stem cell and a progenitor cell. In this situation, the progenitor cell provides a differentiated cell. Either by the influence of direct mutations or the effect of external factors, cells enter into a dedifferentiation process and lose their specificity and, as such, these cells can lead to the formation of cancer stem cells (32). Also, certain cells may end the differentiation process before the progenitor cell whose mutations can still lead to CSCs (Figure 2).

Figure 2. Mechanisms of BCSCs occurrence.



It has been proven for many tumors that de novo mutations and events lead to the formation of BCSCs. Biological characteristics of BCSCs are various. Thanks to the specific mutation within tumor cells and its nature, BCSCs frequency, cell-surface phenotype, and drug sensitivity may vary. Also, tumor progression will depend on the tumor itself, i.e. its pathogenesis or a decisive challenge for chemotherapy, which is responsible for BCSCs biology (33).

BCSCs are a group of very few cells that are tumorigenic (able to form tumors) and are defined as those cells within a tumor that can self-renew and lead to tumorigenesis. There are two models of tumors development and growth described so far. One of these models is BCSCs model and it postulates a hierarchical organization of cells such that only a small subset is responsible for sustaining tumorigenesis and establishing the cellular heterogeneity inherent in the primary tumor (34).

On the other hand, the clonal evolution model claims that all cells within a tumor do their bit in varying degrees to maintain a tumor (35). In this model, a number of genetic and epigenetic changes occur over time, leading to the result that the most aggressive cancer cells are ultimately liable for breast tumor progression. The initial tumor cell evolution may occur by two methods: linear and branched expansion (36).

BREAST CANCER STEM CELLS (BCSCS)

Previous studies results demonstrated that the processes of breast tumors initiation, progression, and proliferation occur thanks to the small group of BCSCs which is able to self-renew and differentiate (37). Features of BCSCs are the result of the impact of complex molecular mechanisms or microenvironment (38, 39). Cytokines and their impact on the BCSCs microenvironment are responsible for tumor heterogeneity and the so-called plasticity of BCSCs (40).

BCSCs represent a small population of cells that have stem cell characteristics and are related to breast cancer. There are different theories about the origin of BCSCs. One of them states that improper regulation or mutations may lead to the transformation of normal stem cells into breast cancer stem cells (BCSCs) (41). According to another, the "misplacement somatic stem cell" theory, BCSCs may originate from misplacement of somatic stem cells *de novo* (42). Evidence shows that somatic cells can be considered the BCSC origin. There are studies that suggest there are intratumoral lineages differentiated from common progenitor cells (43). BCSCs were isolated from breast tumor tissue and the cell was characterized as CD44+/CD24/low Lin phenotype (44). CD44 is a cell surface glycoprotein and a specific receptor for hyaluronan. It is a crucial element for breast cancer adhesion, motion, migration, and invasion, and its interaction with osteopontin causes tumor progression. It has an important role in cell proliferation and tumor angiogenesis (38, 39). CD24, a second-surface glycoprotein expressed at low levels, increases tumor's ability to grow and metastasize (38). However, one report shows that CD44+CD24- is not expressed in all breast cancer cell populations (45). The results of some studies show that CSCs is to identify the presence of very important ALDH markers (46). Aldehyde dehydrogenase 1 (ALDH 1) consists of a family of cytosolic enzymes involved in the oxidation of intracellular aldehydes and oxidizes retinol to retinoic acid during stem cells differentiation. ALDH1 plays a role in stem cells differentiation and its activity forecasts poorer clinical outcomes (47). The other markers that have been used to identify BCSCs include CD133, CD49fhi, and CD61 (48, 49). Although the list of CSCs markers grows, some researchers do not consider these markers suitable for identifying CSCs.

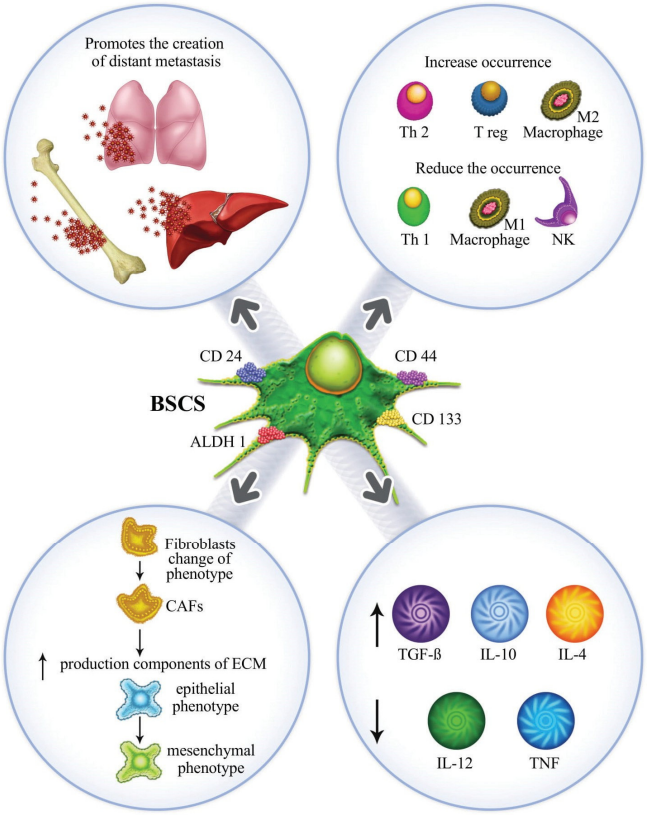
The immediate environment of the BCSCs consists of a group of various cells and molecules which together forming a BCSCs niche. This niche provides adequate physical and chemical conditions for the development of tumors (include fibroblast stimuli, immune cells, autocrine signals, and extracellular matrix (ECM) components, oxygen pressure, nutrients, and PH (50). Those cells produce cytokines such as interleukin IL-1, IL-6, and IL-8, CXCL12, CCL2, and growth factors such as platelet-derived growth factor (PDGF), TGF-β, TNF-α, EGF, vascular endothelial growth factor, and FGF that are responsible for tumor growth and progression (51). This is supported by studies which have shown that blockage of the IL-6 receptor can inhibit tumor metastasis and growth (52). The other results show that blockage of TGF-β with IL-8 inhibition increases the number of BC-SCs in triple negative breast cancer and prevents tumor formation in preclinical models (53). This and other studies show that the cell niche content can and should be an important point for a breast cancer targeted therapy (54). BCSCs are responsible for breast carcinoma metastasis.

Usually, there is a metastatic spread to the bone, and rarely to the lungs and liver (55). The basic molecules of the breast cancer target tissue are hyaluron and osteopontin that exhibit binding sites for the CD44 molecules in BCSCs (bone, brain, liver, and lung, bone marrow endothelium). Osteopontin is associated with a higher incidence of tumor metastasis and invasion (56).

A phenomenon that allows BCSCs to make the transition from epithelial to mesenchymal expression and thus avoid the effect of cytotoxic agents is called *epithelial-mesenchymal transition EMT* (57). During this process, cells change their molecular characteristics in terms of loss of epithelial characteristics taking a mesenchymal phenotype. This process plays a key role in the progression and invasion of metastasis breast tumors. Throughout EMT, some changes occur such as the shutdown of transcription and regulation of epithelial markers such as E-cadherin, and the appearance of mesenchymal markers such as vimentin, fibronectin, and N-cadherin. This leads to destabilization of structures and functions in these cells (58). This transformation leads to cancer cells migration and invasion. It has been found that malignant cells with mesenchymal characteristics are more resistant to therapy and EMT provides an increase in the number of cancer stem-like cells (59). BCSCs are also responsible for a large

number of breast cancer subsets and have a great clinical significance (60). Itis necessary to take into account the fact that tumor is heterogeneous and that the characteristics of BCSCs in one region may be an inadequate predictor for the outcome of the whole breast cancer (61). The results of many studies suggest the need for testing BCSCs as a prognostic factor for different types of breast cancer outcome (Figure 3).

Figure 3. Impact of BCSCs on tumor microenvironment and on progression and invasion of metastasis



RESISTENCE OF BCSCS TO CONVENTIONAL THERAPY

Recent studies suggested that BCSCs posses inbred chemo-and radiation-therapy resistance mechanisms which allow them survive. Resistance of BCSCs to conventional therapy is providen by several mechanisms such as DNA damage repair, cell cycle checkpoint proteins activation, activation of self-renewal pathways or avoidance of apoptosis (62). Radiation induce cell death through DNA damage. All cells respond to DNA damage by activationof detection and repair mechanisms which includes ATM (ataxia telangiectasia mutated) and the checkpoint kinases,Chk1 and Chk2, initiating cell cycle arrest, repair of DNA or apoptosis. BCSCs use these mechanisms more rapidly than non-stem cancer cells and avoid radiation-induced cell death (63). Other potential radioresistance mechanisms is activation of Wnt/β-Catenin signalling pathway which promotes DNA damage tolerance. Jagged- 1 expression and the Notch signalling pathway have also been implicated as playing roles in radioresistance. In the mammary gland, Wnt/β-catenin, Notch and Hedgehog (Hh) signalling pathways induce stem cell self- renewal and theyare potential targets for therapy (64).

ATP-binding cassette (ABC)-G2 transporters, such as breast cancer resistance protein (BRCP-ABCG2) and MDR-associated protein-1 (ABCB1/MDRR1), class ofdrug transporters are often the cause of multidrug resistance. These transporters are expressed on normal stem cells and cancer stem cells and they are capable of pumpingout of these cells different substances, including cytotoxic drugs (65). Some clinical studies have been shown that another possible reason for chemotherapy and radiation therapy resistance can be high expression of CD44 and lowexpression of CD24 on breast cancer cells (66).

However, the clinical relevance of BCSCs in human breast cancer is still under debate. Also, the question arisesas to whether there are any differences between BCSCs and tumor-initiating cells.

CONCLUSION

Role of BCSCs is remarkable in tumor progression and metastasis. Extensive interactions among cancer stem cells, their microenvironments, and other present cells initiate a cascade of growth factors and inducing elements, which in turn influence cancer stem cell role in breast cancer. This population is resistant to conventional therapies due to enhanced membrane transport by specific protein transporters, specific mechanisms of DNA repair, and ROS scavenging systems, and the ability to detoxify cytotoxic drugs. Transcriptional factors, signalling pathways, and tumor suppressor genes act to maintain and amplify a state of stability. More studies are needed to investigate each of these aspects of BCSCs. And finally, the BCSCs as a key point of breast cancer should be subjected to a study in order to individualize the therapy directed to the system of a given breast cell carcinoma.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

FUNDING

None.

REFERENCES

1. Birbrair A, Zhang T, Wang ZM, Messi ML, Olson JD, Mintz A, et al. Type-2 pericytes participate in normal and tumoral angiogenesis. Am J Physiol Cell Physiol. 2014;1;307(1):C25-38.
2. Trends and Controversies in Multi-Disciplinary Care of the Breast Cancer Patient Laura S. Dominici, Monica Morrow, Elizabeth Mittendorf, Jennifer Bellon, Tari A. King Curr Probl Surg. Author manuscript; available in PMC 2017 Dec 1.Published in final edited form as: Curr Probl Surg. 2016;53(12): 559–595.
3. Colin C, Schott AM. Re: Breast tissue composition and susceptibility to breast cancer. J Natl Cancer Inst. 2011;103(1):77.
4. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials.Lancet. 2005;365(9472):1687-717.
5. Pleyer L, Valent P, Greil R. Mesenchymal Stem and Progenitor Cells in Normal and Dysplastic Hematopoi- esis-Masters of Survival and Clonality? Int J Mol Sci. 2016;17(7): E1009.
6. Cuiffo BG, Karnoub AE. Mesenchymal stem cells in tumor development: emerging roles and concepts. Cell Adh Migr. 2012;6(3):220-30.
7. Pietras K, Ostman A. Hallmarks of cancer: interactions with the tumor stroma. Exp Cell Res. 2010;316(8):1324- 31.
8. Koontongkaew S. The tumor microenvironment contribution to development, growth, invasion and metastasis of head and neck squamous cell carcinomas. J Cancer. 2013;4(1):66-83.
9. Zeisberg EM, Potenta S, Xie L, Zeisberg M, Kalluri R. Discovery of endothelial to mesenchymal transition as a source for carcinoma-associated fibroblasts. Cancer Res. 2007;67(21):10123-8.
10. D'souza N, Burns JS, Grisendi G, Candini O, Veronesi E, Piccinno S, et al. MSC and Tumors: Homing, Differentiation, and Secretion Influence Therapeutic Potential. Adv Biochem Eng Biotechnol. 2013;130:209-66.
11. Marusyk A, Polyak K. Tumor heterogeneity: causes and consequences. Biochim Biophys Acta. 2010;1805(1):105-17.
12. Place AE, Jin Huh S, Polyak K. The microenvironment in breast cancer progression: biology and implications for treatment. Breast Cancer Res. 2011;13(6):227.
13. Mao Y, Keller ET, Garfield DH, Shen K, Wang J. Stroma Cells in Tumor Microenvironment and Breast Cancer. Cancer Metastasis Rev. 2013; 32(1-2): 303-315.
14. Hu M, Peluffo G, Chen H, Gelman R, Schnitt S, Polyak K. Role of COX-2 in epithelial–stromal cell interactions and progression of ductal carcinoma in situ of thebreast. Proc Natl Acad Sci U S A. 2009;106(9):3372-7
15. Tsuyada A, Chow A, Wu J, Somlo G, Chu P, Loera S, et al. CCL2 mediates crosstalk between cancer cells and stromal fibroblasts that regulates breast cancer stem cells. Cancer Res. 2012;72(11): 2768-79.
16. Nielsen SR, Schmid MC. Macrophages as Key Drivers of Cancer Progression and Metastasis. Mediators Inflamm. 2017;2017:9624760.
17. DF Quail and JA Joyce. Microenvironmental regulation of tumor progression and metastasis. Nat Med. 2013;19(11): 1423-1437.
18. Biswas SK, Allavena P, Mantovani A. Tumor-associated macrophages: functional diversity, clinical significance, and open questions. Semin Immunopathol. 2013 ;35(5):585-600.
19. Bussard KM, Mutkus L, Stumpf K, Gomez-Manzano C, Marini FC. Tumor-associated stromal cells as key contributors to the tumor microenvironment. Breast Cancer Res. 2016;18(1):84.
20. DeNardo DG, Brennan DJ, Rexhepaj E, Ruffell B, Shiao SL, Madden SF, et al. Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. Cancer Discov. 2011;1(1):54-67.
21. Lindau D, Gielen P, Kroesen M, Wesseling P, Adema GJ. The immunosuppressive tumour network: myeloid-derived suppressor cells, regulatory T cells and natural killer T cells. Immunology. 2013;138(2):105-115.
22. Mamessier E, Sylvain A, Thibult ML, Houvenaeghel G, Jacquemier J, Castellano R, et al. Human breast cancer cells enhance self tolerance by promoting evasion from NK cell antitumor immunity. The Journal of clinical investigation. 2011;121(9):3609.
23. Stabile H, Fionda C, Gismondi A, Santoni A. Role of Distinct Natural Killer Cell Subsets in Anticancer Response. Front Immunol. 2017;8:293.
24. Allinen M, Beroukhim R, Cai L, Brennan C, Lahti- Domenici J, Huang H, Porter D, Hu M, Chin L, Richardson A, Schnitt S, Sellers WR, Polyak K. Molecular characterization of the tumor microenvironment in breast cancer. Cancer Cell. 2004;6(1):17-32.
25. Ljujic B, Milovanovic M, Volarevic V, Murray B, Bugarski D, Przyborski S, et al. Human mesenchymal stem cells creating an immunosuppressive environment and promote breast cancer in mice. Sci Rep. 2013; 3:2298.
26. Patel SA, Meyer JR, Greco SJ, Corcoran KE, Bryan M, Rameshwar P. Mesenchymal stem cells protect breast cancer cells through regulatory T cells: role of mesenchymal stem cell-derived TGF-beta. J Immunol. 2010;184(10):5885-94.
27. Yu X, Li H, Ren X. Interaction between regulatory T cells and cancer stem cells. Int J Cancer. 2012;131(7): 1491-8.
28. Albini A, Bruno A, Gallo C, Pajardi G, Noonan DM, Dallaglio K. Cancer stem cells and the tumor microenvironment: interplay in tumor heterogeneity. Connect Tissue Res. 2015;56(5):414-25.
29. Sainz B Jr, Carron E, Vallespinós M, Machado HL. Cancer Stem Cells and Macrophages: Implications in Tumor Biology and Therapeutic Strategies. Mediators Inflamm. 2016;2016:9012369.

30. Reya T, Morrison SJ, Clarke MF, Weissman IL.Stem cells, cancer, and cancer stem cells. Nature. 2001;414(6859):105-11.
31. Lee G, Hall RR 3rd, Ahmed AU. Cancer Stem Cells: Cellular Plasticity, Niche, and its Clinical Relevance. J Stem Cell Res Ther. 2016;6(10): 363.
32. Visvader JE, Lindeman GJ. Cancer stem cells: current status and evolving complexities. Cell Stem Cell. 2012;10(6):717-28.
33. Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J, Endesfelder D, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012;366(10):883-892.
34. Barabé F, Kennedy JA, Hope KJ, Dick JE. Modeling the initiation and progression of human acute leukemia in mice. Science. 2007;316(5824):600-4.
35. Hartwig FP, Nedel F, Collares T, Tarquinio SB, Nör JE, Demarco FF. Oncogenic somatic events in tissue- specific stem cells: a role in cancer recurrence? Ageing Res Rev. 2014;13:100-6.
36. Lin CY, Barry-Holson KQ, Allison KH. Breast cancer stem cells: are we ready to go from bench to bedside? Histopathology. 2016;68(1):119-37.
37. Reya T, Morrison SJ, Clarke MF, Weissman IL.Stem cells, cancer, and cancer stem cells. Nature. 2001;414(6859):105-11.
38. Plaks V, Kong N, Werb Z. The cancer stem cell niche: how essential is the niche in regulating stemness of tumor cells? Cell Stem Cell. 2015;16(3):225-38.
39. Brooks MD, Burness ML, Wicha MS. Therapeutic Implications of Cellular Heterogeneity and Plasticity in Breast Cancer. Cell Stem Cell. 2015;17(3):260-71.
40. Wang RA, Li ZS, Zhang HZ, Zheng PJ, Li QL, Shi JG, et al. Invasive cancers are not necessarily from preformed in situ tumours - an alternative way of carcinogenesis from misplaced stem cells. J Cell Mol Med. 2013;17(7):921-6.
41. Gorden BH, Kim JH, Sarver AL, Frantz AM, Breen M, Lindblad-Toh K, et al. Identification of three molecular and functional subtypes in canine hemangiosarcoma through gene expression profiling and progenitor cell characterization. Am J Pathol. 2014;184(4):985-995.
42. Herrera-Gayol A, Jothy S. Adhesion proteins in the biology of breast cancer: contribution of CD44. Exp Mol Pathol. 1999;66(2):149-56.
43. Rangaswami H, Bulbule A, Kundu GC. Osteopontin: role in cell signalling and cancer progression. Trends Cell Biol. 2006;16(2):79-87.
44. Götte M, Yip GW. Heparanase, hyaluronan, and CD44 in cancers: a breast carcinoma perspective. Cancer Res. 2006;66(21):10233-7.
45. Kida K, Ishikawa T, Yamada A, Shimada K, Narui K, Sugae S, et al. Effect of ALDH1 on prognosis and chemoresistance by breast cancer subtype. Breast Cancer Res Treat. 2016;156(2):261-9.
46. Pattabiraman DR, Weinberg RA. Tackling the cancer stem cells - what challenges do they pose? Nat Rev Drug Discov. 2014;13(7):497-512.
47. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell. 2007;1(5):555-67.
48. Cariati M, Naderi A, Brown JP, Smalley MJ, Pinder SE, Caldas C, et al. Alpha-6 integrin is necessary for the tumourigenicity of a stem cell-like subpopulation within the MCF7 breast cancer cell line. Int J Cancer. 2008;122(2):298-304.
49. Vaillant F, Asselin-Labat ML, Shackleton M, Forrest NC, Lindeman GJ, Visvader JE. The mammary progenitor marker CD61/beta3 integrin identifies cancer stem cells in mouse models of mammary tumorigenesis. Cancer Res. 2008;68(19):771-7.
50. Borovski T, De Sousa E Melo F, Vermeulen L, Medema JP. Cancer stem cell niche: the place to be. Cancer Res. 2011;71(3):634-9.
51. Brooks MD, Wicha MS. Tumor twitter: cellular communication in the breast cancer stem cell niche. CancerDiscov. 2015;5(5):469-71.
52. Korkaya H, Kim GI, Davis A, Malik F, Henry NL, Ithimakin S, et al. Activation of an IL6 inflammatory loop mediates trastuzumab resistance in HER2+ breast cancer by expanding the cancer stem cell population. Mol Cell. 2012;47(4):570-84.
53. Hosford SR, Miller TW. Clinical potential of novel therapeutic targets in breast cancer: CDK4/6, Src, JAK/STAT, PARP, HDAC, and PI3K/AKT/mTOR pathways. Pharmgenomics Pers Med. 2014;7:203-15.
54. Brown LF, Berse B, Van de Water L, Papadopoulos-Sergiou A, Perruzzi CA, Manseau EJ, et al. Expression and distribution of osteopontin in human tissues: widespread association with luminal epithelial surfaces. Mol Biol Cell. 1992;3(10):1169-80.
55. Chakraborty G, Jain S, Kundu GC. Osteopontin promotes vascular endothelial growth factor-dependent breast tumor growth and angiogenesis via autocrine and paracrine mechanisms. Cancer Res. 2008;68(1):152-61.
56. Matysiak M, Kapka-Skrzypczak L, Jodłowska-Jędrych B, Kruszewski M. EMT promoting transcription factors as prognostic markers in human breast cancer. Arch Gynecol Obstet. 2017;295(4):817-825.
57. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell. 2009;139(5):871-90.
58. Smith BN, Burton LJ, Henderson V, Randle DD, Morton DJ, Smith BA, et al. Snail promotes epithelial mesenchymal transition in breast cancer cells in part via activation of nuclear ERK2. PLoS One. 2014;9(8):e104987.
59. Wei H, Fu P, Yao M, Chen Y, Du L. Breast cancer stem cells phenotype and plasma cell-predominant breast cancer independently indicate poor survival. Pathol Res Pract. 2016;212(4):294-301.
60. Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J, Endesfelder D, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012 Mar 8;366(10):883-892.

61. Azad N, Rojanasakul Y, Vallyathan V. Inflammation and lung cancer: roles of reactive oxygen/nitrogen species. J Toxicol Environ Health B Crit Rev. 2008;11(1):1-15.
62. Al-Ejeh F, Smart CE, Morrison BJ, Chenevix-Trench G, Lopez JA, Lakhani SR, et al. Breast cancer stem cells: treatment resistance and therapeutic opportunities. Carcinogenesis. 2011 May;32(5):650-8.
63. Eyler CE, Rich JN. Survival of the fittest: cancer stem cells in therapeutic resistance and angiogenesis. J Clin Oncol. 2008;26(17):2839-45.
64. Morrison BJ, Schmidt CW, Lakhani SR, Reynolds BA, Lopez JA. Breast cancer stem cells: implications for therapy of breast cancer. Breast Cancer Res. 2008;10(4):210.
65. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer. 2005 Apr;5(4):275-84.
66. Creighton CJ, Li X, Landis M, Dixon JM, Neumeister VM, Sjolund A, Rimm DL, Wong H, Rodriguez A, Herschkowitz JI, Fan C, Zhang X, He X, Pavlick A, Gutierrez MC, Renshaw L, Larionov AA, Faratian D, Hilsenbeck SG, Perou CM, Lewis MT, Rosen JM, Chang JC. Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. Proc Natl Acad Sci U S A. 2009 Aug 18;106(33):13820-5.