Chapter 8
Cancer Stem Cells: An Ever-Hiding Foe

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Contents

Abstract Cancer stem cells are a population of cells enable to reproduce the original phenotype of the tumor and capable to self-renewal, which is crucial for tumor proliferation, differentiation, recurrence, and metastasis, as well as chemoresistance. Therefore, the cancer stem cells (CSCs) have become one of the main targets for anticancer therapy and many ongoing clinical trials test anti-CSCs efficacy of plenty of drugs. This chapter describes CSCs starting from general description of this cell population, through CSCs markers, signaling pathways, genetic and epigenetic regulation, role of epithelial-mesenchymal transition (EMT) transition and autophagy, cooperation with microenvironment (CSCs niche), and finally role of CSCs in escaping host immunosurveillance against cancer.

Keywords Cancer stem cells · Metastasis · Chemoresistance · Epithelial-mesenchymal transition · Niche

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Abbreviations

AKT	protein kinase B
ALDH1	aldehyde dehydrogenase-1
CAFs	cancer-associated fibroblasts
CSCs	cancer stem cells
CTCs	circulating tumor cells
CXCR	C-X-C motif chemokine receptor
DKK1	Dickkopf-related protein 1
ECM	extracellular matrix
EGF	epidermal growth factor
EMT	epithelial–mesenchymal transition
EpCAM	epithelial cell adhesion molecule
ERK	extracellular-signal-regulated kinase
FAK	focal adhesion kinase
HDAC	histone deacetylase
HGF	hepatocyte growth factor
Hh	hedgehog signaling
HIF-1α	hypoxia-inducible factor-1α
IL	interleukin
JAK	Janus kinase
Klf4	Krüppel-like factor-4 transcription factor
LIF	leukemia-inhibiting factor
MAPK	mitogen-activated protein kinases
MDSCs	myeloid-derived suppressor cells
MET	mesenchymal-to-epithelial transition
MMPs	metalloproteinases
MSCs	mesenchymal stem cells
mTOR	mammalian target of rapamycin
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NK	natural killer cells
NKG2D	activating receptor of NK cells
NOTCH	neurogenic locus notch homolog protein
NRF2	nuclear factor erythroid-2-related factor-2
NUMB	protein numb homolog
Oct4	octamer-binding transcription factor-4
OXPHOS	oxidative phosphorylation
PD-L1	programmed death ligand 1 (also called B7-H1)
PGE_2	prostaglandin E_2
PI3K	phosphatidylinositol 3-kinase/phosphatase
PTEN	phosphatase and tensin homolog
ROS	reactive oxygen species
Sox2	sex-determining region-Y box-2 transcription factor
STAT	signal transducer and activator of transcription

TAMs	tumor-associated macrophages
TAZ	transcriptional coactivator with PDZ-binding motif
TGF-β	transforming growth factor-β
TLR	toll-like receptor
TNF-α	tumor necrosis factor-α
Tregs	T regulatory cells
VEGF	vascular-endothelial growth factor
YAP	Yes-associated protein
ZEB1	zinc finger E-box-binding homeobox-1

8.1 Introduction: Cancer Stem Cells: Definition and General Description

Cancer stem cells (CSCs), alternatively known as tumor-initiating or tumor-propagating cells (TICs, TPCs), are a population of cells enable to reproduce the original phenotype of the tumor, but more importantly capable to self-renewal, which is crucial to tumor proliferation, differentiation, recurrence, and metastasis, as well as chemoresistance (Irani 2019; Irani and Dehghan 2017, 2018; Irani and Jafari 2018; Wang 2019). Tumor cells are considered to be CSCs while possessing simultaneously all following features: have specific surface markers, are able to form floating spheres in serum-free medium, and form tumors when transplanted into laboratory animals (Choudhury et al. 2019). From mechanical perspective, CSCs are softer and more deformable cells than both nonmalignant and normal malignant cells (Vander Linden and Corbet 2019; Helmlinger et al. 1997, 2002; Vaupel et al. 1981). CSCs were first identified in 1997 in acute myeloid leukemia (Bonnet and Dick 1997) followed by identification in many solid tumors including prostate, ovarian, breast, pancreatic, colon, head and neck, lung, liver cancer, and glioblastoma (reviewed in: Nazio et al. 2019). Population of CSCs may be divided based on their cell cycle behavior and chemoresistance into two subpopulations: proliferating and quiescent. These two subpopulations occupy different niches inside tumor and exclusively quiescent CSCs are characterized by autophagic state (Marcucci et al. 2017, 2019; Liu et al. 2013). The proliferative CSCs possess acquired chemoresistance in response to treatment, as well as intrinsic chemoresistance to some drugs that have not been used before. The proliferative CSCs could be killed by chemotherapeutics; however, the demanded dose of antimitotic drug is higher compared to normal tumor cells. Otherwise, quiescent CSCs are capable to survive even high doses of antimitotic drugs, thus promoting tumor relapse (Wang 2019; Lee et al. 2019; Batlle and Clevers 2017; Schmidt and Efferth 2016; Naik et al. 2016).

Regarding the origin of CSCs there are two possible mechanisms—differentiation from progenitor or normal stem cells or from normal cancer cells, which acquire stemness characterization via epithelial-mesenchymal transition process (EMT) (Marcucci et al. 2019; Mani et al. 2008). Nowadays, an EMT process is not viewed

as a "switch" from epithelial to mesenchymal state of the cell. Instead, it is perceived as a continuum of states from a fully epithelial/proliferative to fully mesenchymal/invasive phenotype comprising a spectrum of intermediate hybrid states. CSCs could represent any of these final or intermediate phenotypic states (Tam and Weinberg 2013). According to the hierarchical model of tumor growth, only CSCs exhibit self-renewal capacity, while other tumor cells possess only limited proliferative potential. Alternatively, stochastic tumor growth model points out that all cancer cells are capable to undergo either self-renewal as CSCs or differentiation into nonproliferating cancer cells depending on genetic and environmental signals (Wang 2019). In different tumors, CSCs indicate astonishing and diversified plasticity allowing to conclude that CSCs hierarchy is not a rigid phenomenon, and non-CSCs cells could be reprogrammed to functional CSCs by various environmental and epigenetic stimuli. A situation met in real tumors seems to be rather a mixture of what is described by pure hierarchical and stochastic models (Chen et al. 2012; Suva et al. 2014). This fact has a profound influence on the anti-CSCs treatment efficacy. If CSCs were strictly defined (as does the hierarchical model) it would be relatively easy to eliminate them. But if stemness were a stochastic and transient feature of competing cancer cells, therapeutic targeting against CSCs would be of a great challenge (Wang 2019; Vlashi and Pajonk 2015).

The capacity of CSCs and non-CSCs populations to interconvert is a unique feature of CSCs, which distinguishes them from normal stem cells. Another difference is based on observation that CSCs are able to form tumors when transplanted into laboratory animals, while normal stem cells cannot do this (Wang 2019). The third main difference involves stem cell niche composition. While normal stem cell niche is tumor suppressive and produces signals arresting cell growth, the CSCs niche produces signals supporting CSCs growth and activation of survival pathways (Khan et al. 2019; Asadzadeh et al. 2019; Batlle and Clevers 2017; Lopez-Lazaro 2015).

Tumor-initiating potential of CSCs could be obtained due to different events occurring in their environment, mainly stressors (hypoxia, pH, drugs, mechanical stress, immunological response), stressor-promoted epigenetic changes (i.e., histone and noncoding RNA modifications), and finally activation of "stemness" signaling pathways (i.e., wingless-related integration site—Wnt, Hedgehog, neurogenic locus notch homolog protein—NOTCH). As the action of these factors could vary between different tumors, and even in different areas of the same tumor, the functions and, to some extent, a phenotype of CSCs could differ spatially and temporally (Takebe et al. 2015; Berabez et al. 2018; Marcucci et al. 2014; Dumont et al. 2008; Wallin et al. 2012; Visvader and Lindeman 2008; Vermeulen et al. 2012; Taniguchi et al. 2019). The CSCs abundance inside tumors could vary from 0,0001–0,1% to as many as 25% of tumor mass depending on method of their identification, and even more on the environment they used to exist in (Capp 2019; Quintana et al. 2008; Rosen and Jordan 2009). According to that functional and phenotypic diversity of CSCs it might be stated that CSCs are a population of cells with both increased gene expression variability and epigenetic plasticity followed by a disturbed interactions with other cells, which disables existence of normal

intercellular interaction network (Capp 2019). From evolutionary perspective, CSCs are a result of an adaptive tumor response sustaining malignant progression shaped by genetic alterations and selective environment (Vander Linden and Corbet 2019).

8.2 Cancer Stem Cells Markers

The CSCs surface markers are not specific for CSCs, but are also expressed on normal stem cells. Moreover, the presence of some surface molecules is not enough to recognize CSCs. They have to indicate precisely defined behavior in in vitro spheroid formation or aldefluor assays to be properly recognized. In vivo limiting dilutions assays and formation of tumors after transplantation to laboratory animals remain the gold standard for CSCs identification. Despite this, several markers have been suggested to identify CSCs, but their precise clinical significance is incomplete as they are applied only as surrogate markers for CSCs identification. The composition of CSCs' surface markers may vary between tumors originating from different tissues. However, there is a group of markers most frequently and reproducibly describing CSCs. Among them CD133, CD44, ALDH1, and CD24 are the most universal and have been most widely studied (Irani 2019). Elevated levels of CD133, glycoprotein known as prominin-1, were noticed in metastatic tumors, correlating to migration, stemness, and tumorigenicity resulting from EMT. Expression of CD133 enhances invasive abilities and chemoresistance of tumor cells. In ovarian cancer, CD133 augmented the adhesion of cancer cells to peritoneal mesothelium, promoting formation of peritoneal implants (Motohara and Katabuchi 2019; Roy et al. 2018). CD44 is a cell surface antigen engaged in cell–cell interactions, migration, and adhesion. Its expression regulates lymphocyte activation and hyaluronic metabolism. It is also responsible for metastatic properties and invasiveness of cancer cells both by regulation of EMT and interaction with hyaluronan acid in extracellular matrix (Irani 2019). In ovarian cancer, peritoneal disseminated implants are enriched in CD44 expression compared to primary tumors, indicating growing aggressiveness (Miranda et al. 2016). CD44 is involved in activation of a variety of receptor tyrosine kinase–induced pathways including hepatocyte growth factor receptor (HGF/c-Met), Src and focal adhesion kinase (Src/FAK), and phosphatidylinositol 3-kinase/phosphatase/protein kinase B (PI3K/AKT), which increase proliferation and survival of cells (Chen and Wang 2019**;** Marjanovic et al. 2013; Matzke et al. 2007; Skupien et al. 2014). Aldehyde dehydrogenase-1 (ALDH1) is a member of protein enzymes involved in cell differentiation, metastasis, detoxification, and drug resistance through the oxidation of intracellular aldehydes (Rodriguez-Torres and Allan 2016). Expression of ALDH1 correlates with migration of cancer cells and unfavorable prognosis for cancer patients (Irani 2019). CD24 is a protein known as heat-stable antigen CD24 engaged in cell adhesion. Lack of or low CD24 expression on CSCs is probably responsible for their increased invasive and metastatic potential and is responsible for worse clinical prognosis (Jaggupilli and Elkord 2012; Taniuchi et al. 2011). In breast cancer, CD44+/CD24−/low phenotype characterizes

mesenchymal and quiescent, while ALDH1+ cells characterize epithelial and proliferative CSCs, respectively (Zhou et al. 2019). The other cancer-specific CSCs markers are CD26, CD29, CD49f, CD117, CD166, EpCAM, CK17, CXCR4 (Organista-Nava et al. 2019; Motohara and Katabuchi 2019). It is noteworthy that at the beginning of cervical cancerogenesis, oncogenic human papilloma virus targets exclusively CD133 + CD44+ CD49f + CD17+ cells considered to be stem cells for cervical epithelium. Through the action of viral E6 and E7 proteins, these cells acquire stemness features of CSCs (Organista-Nava et al. 2016, 2019; Hou et al. 2015). In ovarian cancer, EpCAM/Bcl-2 signaling pathway prevents platinum-dependent apoptosis of cancer cells, resulting in chemoresistance. EpCAM expression is increased in tumors of chemo-resistant patients and correlates with unfavorable outcome. Tyrosine kinase receptor CD117 is responsible for tumor formation, chemoresistance, and poor prognosis in ovarian cancer patients (Motohara and Katabuchi 2019).

Apart from surface markers, there is a group of transcription factors, which by altered expression could characterize CSCs cells. Among them, Oct4, Sox2, Klf4, c-Myc (so-called Yamanaka factors), and Nanog are the best described intracellular CSCs markers (Vlashi and Pajonk 2015; Yamanaka and Blau 2010). Octamer-binding transcription factor-4 (Oct4) is involved in embryonic development and cellular pluripotency. Its function is to stabilize the higher-order structure of chromatin in the Nanog locus (Levasseur et al. 2008). Cytoplasmic expression of Oct4 regulates EMT transformation and is recognized predictor of adverse clinical outcome in cancer. Sex-determining region-Y box-2 transcription factor (Sox2) makes complex with Oct4 and is essential for embryonic and acquired pluripotency and self-renewal of cells. Deregulated Sox2 expression was noticed in several malignant tumors and linked to risk of cancer recurrence and poor prognosis (Takahashi and Yamanaka 2006; Vlashi and Pajonk 2015). Krüppel-like factor-4 transcription factor (Klf4) targets genes involved in cell cycle control and inhibits proliferation by maintaining cell arrest in the G1/S and G2/M checkpoints. In most circumstances, Klf4 acts as cancer suppressor (Chen et al. 2003). Another transcription factor with changed expression in CSCs is c-Myc belonging to Myc regulatory gene and proto-oncogene family. c-Myc is a downstream target for leukemia inhibitory factor/signal transducer and activator of transcription (LIF/STAT3) signaling pathway and amplifies expression of other Yamanaka factors to induce cellular pluripotency (Takahashi and Yamanaka 2006). Nanog is a homeobox protein family transcription factor engaged in upregulation of embryonic stem cells pluripotency and co-operating with Oct4 and Sox2. Nanog is highly expressed in CSCs and its expression correlates negatively with patient's outcome (Chen and Wang 2019; Chiou et al. 2008; Habu et al. 2015). All mentioned above transcription factors augment CSCs maintenance and self-renewal, tumor formation, and chemoresistance. Exclusive increased expression of these transcription factors in CSCs is determined by the fact that they are all substrates for 26S proteasome activity but are spared from degradation as proteasome activity is not present in CSCs cells (Vlashi and Pajonk 2015).

8.3 Cancer Stem Cells Signaling Pathways

CSCs survival depends on the activation of intracellular signaling pathways responsible for stemness. The most important pathways engaged in CSCs function are Wnt/β-catenin, Hedgehog, Hippo/Yes-associated protein (YAP), NOTCH, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and hypoxia-inducible factor-1α (HIF-1α). Wnt/β-catenin is canonical and conservative signal pathway necessary for initiation and regulation of cell self-renewal, growth, migration, survival, and participation in organogenesis. Disturbed Wnt/β-catenin signaling was observed in many malignancies and is indispensable feature of CSCs stemness. In breast cancer, Wnt/β-catenin signaling is associated with epithelial ALDH1+ CSCs populations and their expansion. Inhibition of Wnt/β-catenin signals in CSCs enables entering by them quiescence state and inhibits CSCs metastatic potential. Wnt/β-catenin pathway enhances chemoresistance of breast cancer cells and correlates with poor clinical outcome (Sulaiman et al. 2018, 2019; Forget et al. 2007; Dey et al. 2013; Tzeng et al. 2015; Pohl et al. 2017; Jang et al. 2015; Yang et al. 2016; Khan et al. 2019). In ovarian cancer, CD117 overexpression upregulates ATP-binding cassette G2 (ABCG2) drug resistance system through the Wnt/β-catenin pathway, thereby increasing chemoresistance of ovarian tumor in a hypoxic microenvironment (Chau et al. 2013).

The Hedgehog (Hh) signaling is extremely important for interactions between CSCs and cancer-associated fibroblasts (CAFs) being a key component of tumor CSCs niche. It was observed in breast cancer, that CSCs secrete sonic hedgehog homolog (Shh), a ligand for Hh, which in turn activates CAFs to secrete factors for self-renewal and expansion of CSCs. Hh-signaling takes also part in EMT transition of CSCs and formation of cell-signaling surface structures called primary cilia (De Angelis et al. 2019; Guen et al. 2017). In ovarian cancer, activation of Hh-signaling pathway is connected to formation of CSCs spheroids and chemoresistance (Ray et al. 2011; Song et al. 2018; Park et al. 2010).

The Hippo/YAP pathway is essential signaling component in regulation of tissue growth and organ size as well as stemness maintenance. Trigger regulatory signals are very interesting as they consist of cell density, stiffness of extracellular matrix, shear stress, and nutrient abundance. YAP overexpression promotes cell proliferation, metastasis, and chemoresistance in breast and ovarian cancers. It also enhances CSCs through upregulation of stemness regulatory genes via YAP/transcriptional coactivator with PDZ-binding motif (TAZ)/interleukin (IL)-6/SRF pathway (Kim et al. 2015; Halder and Johnson 2011). YAP/TAZ (transcriptional coactivator with PDZ-binding motif) activity correlates with poor prognosis in breast cancer (Zanconato et al. 2019).

NOTCH signaling is a conservative cell-to-cell communication pathway responsible for cell proliferation, differentiation, and tissue angiogenesis. Deregulated NOTCH signaling is critically involved in maintenance of cellular stemness and migration of CSCs and inside hypoxic niche conditions acts together with HIF-1α pathway. Its function in CSCs dormancy was also reported (Capulli et al. 2019;

Venkatesh et al. 2018; Khan et al. 2019). NOTCH signaling enables increased expression of intracellular CSCs markers including Oct4, Nanog and Klf4. NOTCH pathway is activated in recurrent ovarian cancer and correlates with poor survival (Park et al. 2010).

NF-κB signaling takes place in multiple processes including proliferation, inflammation, angiogenesis, and migration. NF-κB pathway is a common target for different signals like cytokines, infective agents, DNA damage, stress, and hypoxia. NF-κB pathway co-operates with other signaling pathways like Wnt/β-catenin, PI3K, and Janus kinase (JAK)/STAT3 especially in promotion of pro-inflammatory tumor environment and chemoresistance. Breast cancer CSCs showed increased expression of NF-κB (Xia et al. 2014; Gallo et al. 2018; Yamamoto et al. 2013).

HIF-1α transcription factor signaling is one of the key pathways engaged in promotion of CSCs. The areas of hypoxic tumor and hypoxic niche for CSCs make this pathway one of the most important ways to perform cancer cells proliferation, dormancy, and chemoresistance. Through HIF-1α, hypoxia regulates EMT transition and promotes epithelial CSCs (Xia et al. 2014; Wong et al. 2011; Shiraishi et al. 2017).

Function of several other signaling pathways in maintenance of CSCs has been described, including JAK/STAT pathway, TGF-β-signaling pathway or PI3K/phosphatase, and tensin homolog (PTEN) pathway (Roca et al. 2019).

8.4 Genetic and Epigenetic Regulation of Cancer Stem Cells

Cancer stem cells have increased ability to DNA repair what helps them to resist hypoxic conditions and drug toxicity produced by hostile environment or cancer treatment, respectively. Therefore, mutations and epigenetic changes of *BRCA* genes play important role in maintenance of CSCs population. *BRCA1* expression was found to be enhanced in CD133+ lung cancer CSCs and highly aggressive pancreatic cancer cells (Desai et al. 2014; Mathews et al. 2011). BRCA proteins activate JAK/STAT and NOTCH pathways, as well as regulate Hh-signaling, while loss of *BRCA1* expression activates the PI3K-signaling pathway in CSCs cells, respectively (Gorodetska et al. 2019). Defective genes (i.e., *CTNNB1, PTC, SMO, NOTCH, k-Ras, b-Raf, MEK*) cause improper function of Wnt/β-catenin, Hedgehog, NOTCH, RAS/MEK, or PI3K signaling pathways in ovarian cancer CSCs (Suster and Virant-Klun 2019; Testa et al. 2018). Additionally, genes responsible for cell-cycle regulation and activation of apoptosis are also frequently mutated in CSCs cells (Lee et al. 2019; Karimi-Busheri et al. 2010). Studies devoted to ovarian cancer indicated that besides changes of *BRCA* and *TP53* gene expression, ovarian cancer CSCs showed deregulation of genes responsible for function of centrosome, cell membrane receptors, and cell cycle, like *NAB1, PROS1, GREB1, KLF9* (Suster and Virant-Klun 2019; Huang et al. 2014). Another group of genes involved in CSCs maintenance are *HOX* genes, which in physiological conditions regulate

morphogenesis and organogenesis of the embryo through changes of cell proliferation, differentiation, migration and apoptosis (Smith et al. 2019; Hombria and Lovegrove 2003). In cancer, *HOX* genes could play a role of both stimulators and suppressors of oncogenesis. Aberrant *HOX* function in cancer may cause dedifferentiation of cells and increase of their plasticity inducing the population of CSCs cells (Bhatlekar et al. 2018; Ben Khadra et al. 2014). Epigenetic deregulation of *HOX* genes can support CSCs self-renewal, death evasion, metastasis potential, EMT transition, and chemoresistance (Bhatlekar et al. 2018; Haria and Naora 2013; Jin et al. 2012).

Epigenetic changes of gene expression are the most important genetic factor shaping CSCs' behavior and responsible for CSCs plasticity. Accumulative evidence shows that noncoding RNAs play a key role in these mechanisms through modification of target genes locally and in distant places (i.e., premetastatic or metastatic niche) when being transported to them via exosomes (Irani 2019).

Small single-strand noncoding regulatory micro RNAs (miRNAs) by changing the expression of target genes are capable to act both as stimulators and suppressors of CSCs stemness, self-renewal, proliferation, migration, and chemo- and radioresistance. The main way to perform biological functions by miRNAs is epigenetic modification of signaling pathways in CSCs. miRNAs could modulate the DNA repair genes, like *RAD51*, apoptosis regulator *MCL1*, F2R like thrombin or trypsin receptor 3 (*F2RL3*), and Poly(ADP-Ribose) polymerase 1 (*PARP1*) (Schulz et al. 2019; Gong et al. 2015). Function of CSCs could be suppressed by orchestrated influence of many miRNAs affecting transduction of cellular signals: miR-200c and miR-145 (protein containing a disintegrin and metalloprotease (ADAM) pathway), miR-494 (polycomb complex protein BMI-1 pathway), miR-195-5p and miR-34 (NOTCH pathway), miR-99a (mammalian target of rapamycin (mTOR) pathway), miR-519d and miR-128 (caspases). Conversely CSCs' functions are stimulated by other miRNAs: miR-19 and miR-501-5p (via Wnt/β-catenin pathway), miR-21 and miR221/222 (PTEN pathway), miR-483-5p (cyclin D1 pathway), miR-196b-5p (STAT3 pathway), and miR-494-3p (NOTCH1 pathway) (reviewed in Khan et al. 2019). Some miRNAs have ability to regulate function of many different signaling pathways or target genes, while other miRNAs are able to regulate only one pathway or gene, respectively. For instance, miR-372/373 studied in colorectal cancer CSCs is capable to modulate as many as eight pathways including Nanog, Hedgehog, NF-κB, mitogen-activated protein kinase (MAPK), vitamin-D receptor (VDR), JAK/STAT, TGF-β, PI3K/Akt (Khan et al. 2019; Wang et al. 2018a, b, c; Xu et al. 2018). Similarly miR-128 studied in lung cancer CSCs regulates AKT/extracellular-signal-regulated kinase (ERK), p38, PI3K/Akt, vascular-endothelial growth-factor (VEGF), IL-6/JAK/STAT signal pathways (Kwon et al. 2018; Yang et al. 2017; Jiang et al. 2016; Hu et al. 2014). In prostate cancer, miR-302/367 targets four genes (encoding Oct4, Sox2, Nanog, Klf4) and two signaling pathways (BMI-1, large tumor suppressor kinase-2 (LATS2) /YAP) (Guo et al. 2017a, b). On the contrary, some miRNAs are specific regulators of one pathway in CSCs, like miR-138 in lung cancer, which regulates TGF-β pathway, or miR-92a in ovarian cancer regulating Wnt/β-catenin pathway (Zhang et al. 2018;

Chen et al. 2017). There are miRNAs, which function as CSCs modulators and are represented in many cancers (like miR-200c or miR-21), as well as miRNAs, which have been described exclusively in one type of cancer (reviewed in Khan et al. 2019). The most critical miRNAs for acquisition of stemness properties by cancer cells in most circumstances differ between different cancers. miR-21 is of greatest importance for induction of stemness in colorectal and head/neck cancer (Ju 2011; Yu et al. 2013), miR-218 in lung cancer (Yang et al. 2017), miR-221/222 in breast cancer (Li et al. 2017), miR-383 in prostate cancer (Guo et al. 2017a, b), and finally miR-744 in pancreatic cancer (Zhou et al. 2015).

Circular RNAs (circRNAs) are noncoding stable RNAs that act as "sponges" to bind and regulate function of miRNAs, and could be found both intracellularly and inside exosomes. CircGprc5a and circ-ITCH are examples of circRNAs capable of stimulation of self-renewal of CSCs. CircGprc5a modifies function of retinoic acid–induced protein-3 gene (*GPRC5A*) enhancing stemness of CSCs in bladder cancer, while circ-ITCH functions as a "sponge" for miR-214, which modulates stemness by Wnt/β-catenin signaling pathway (Feng et al. 2019; Gu et al. 2018; Qi et al. 2015). Hsa_circ_0020397 through binding with miR-138 regulates proliferation functions of telomerase reverse transcriptase in CSCs. Another circRNA, hsa_circ_0005075 produces "sponge" for miR-93 followed by mesenchymal-to-epithelial transition (MET) and inhibition of CSCs differentiation. CircUBAP2 enhances the expression of antiapoptotic Bcl-2 in CSCs by formation of "sponge" with miR-143. In laryngeal cancer migration of CD133 + CD44+ CSCs could be induced via EMT caused by upregulation of STAT signaling pathway by hg19_circ_0005033 circRNA (Zhang et al. 2017; Shang et al. 2016; Vadde et al. 2015; Wu et al. 2018). CircRNAs could also influence interactions between CSCs and microenvironment by causing anoikis of CSCs deprived of attachment to components of extracellular matrix (ECM) (Agliano et al. 2017).

The function of CSCs could be also regulated by long noncoding RNAs (lncRNAs) defined as RNA transcripts exceeding 200 nucleotides but not translated to proteins. They participate in the regulation of gene transcription, as well as in posttranslational and epigenetic regulation. Epigenetic regulation by expression of *HOX*-derived lncRNAs can influence CSCs function. The *HOTAIR* gene encodes lncRNA that supports CSCs phenotype and EMT transition in breast and colon cancer CSCs. HOTTIP, another lncRNA originating from HOX cluster, stimulates pancreatic CSCs functions by regulation of Wnt signaling (Padua Alves et al. 2013; Zhang et al. 2014; Fu et al. 2017). Highly upregulated lncRNA of transcription factor 7 seen in liver cancer CSCs activates Wnt/β-catenin signaling and leads to tumor propagation (Toh et al. 2017; Wang et al. 2015).

Another mechanism of epigenetic regulation in CSCs is dependent on methylation of both histones and non-histone proteins. Methylation is associated with either activation or repression of regulated gene. Methylation of histone H3 lysine 4 (H3K4), H3K36, and H3K79 results in gene activation, whereas methylation of H3K9, H3K27, and H4K20 produces a gene repression. Methylation concerns also DNA, when methyl groups are transferred from S-adenosyl methionine (SAM) to CpG groups of gene promoters and regulatory regions. Hypermethylation of DNA in

cancer results in silencing of tumor suppressor or differentiation genes and may contribute to formation of CSCs (Kouzarides 2007; Esteller 2007). Aberrant Wnt/β-catenin activation in CSCs could result from methylation of promoters for Wnt inhibitors and negative regulators, namely, secreted frizzled-related protein 1 (SFRP-1), and Dickkopf-related protein 1 (DKK1), as was found in breast and colon cancers (Klarmann et al. 2008; Suzuki et al. 2004; Koinuma et al. 2006). Disturbed histone H3K16 and H3K27 modifications could also inhibit the expression of Wnt antagonists (Hussain et al. 2009). Disturbed methylation of *Shh* gene promoter results in upregulation of Hh-signaling pathway in breast and gastric cancers (Cui et al. 2010; Wang et al. 2006). Methylation of H3K27 histone causes silencing of miR-200c and miR-205 expression, thus activating EMT transition and CSCs phenotype (Tellez et al. 2011). Histone methylation is also responsible for increased expression of ATP-binding cassette (ABC) family of transmembrane transporters responsible for chemoresistance of CSCs (To et al. 2008). Dysregulated function of histone acetyltransferases (HAT) and deacetylases (HDAC) is also connected to cancer progression. HDAC1 and HDAC7 enzymes promote stemness in CSCs of breast and ovarian cancer. Knockdown of HDAC function resulted in arrest of growth and entering apoptosis in many cancers (Roca et al. 2019; West and Johnstone 2014; Cai et al. 2018). Enhanced histone acetylation of jagged canonical NOTCH ligand-2 gene (*JAG2*) promoter in multiple myeloma affects NOTCH pathway activity in CSCs (Ghoshal et al. 2009).

8.5 Cancer Stem Cells and EMT Transition

Epithelial-to-mesenchymal transition is a process, which occurs in three different types: type-1 EMT during embryogenesis, type-2 EMT during wound healing and regeneration, and type-3 EMT in cancer (Hass et al. 2019; Kalluri and Weinberg 2009). Type-3 EMT facilitates cells' metastasize potential and promotes CSCs motility and invasion. EMT changes cell apico-basal polarity, cytoskeleton remodeling, cell morphology, cell–matrix interaction, attenuates cell–cell adhesion, and facilitates cell migration (Jolly and Celià-Terrassa 2019; Savagner 2015). Acquisition of mesenchymal phenotype by CSCs enables them to migrate into surrounding tissues ("invasive front"), microvasculature (lymphatic and blood microvessels), and to distant localizations. Moreover, this enhances their survival and chemoresistance, thus promoting tumor recurrence. In the target organs (metastatic niches), CSCs go through MET transition gaining again epithelial phenotype. MET transition augments intercellular contact, proliferation, and differentiation of metastatic tumors (Ishiwata 2016). "Invasive front" of the tumor is defined as an interface between growing tumor and surrounding stroma. The components of the "invasive front" are extracellular matrix, cells (including lymphocytes, tumor-associated macrophages—TAMs, fibroblasts, myeloid progenitor cells) and blood and lymphatic vessels. At the "invasive front" TAMs initiate EMT and promote CSCs via activation of TGF-β, Wnt/β-catenin, and RAS/ERK signaling pathways (Clark

and Vignjevic 2015, Shiga et al. 2015, Lee et al. 2018). In breast cancer epithelial state, CSCs are proliferative, localized inside the tumor, and marked as ALDH1+ E-cadherin ^{high} vimentin ^{low} zinc finger E-box-binding homeobox-1 (ZEB1) ^{low}. Mesenchymal-state CSCs are quiescent, localized at the "invasive front" and marked as CD44 + CD24- E-cadherin ^{low} vimentin ^{high} ZEB1 ^{high} (De Angelis et al. 2019; Liu et al. 2014). In the primary tumor, the cells undergoing EMT adopt mesenchymal phenotype, then migrate to distant organs where they produce metastases, and finally revert into epithelial phenotype.

EMT transition is also a way by which differentiated cancer cells could possess stemness and become CSCs (Brabletz et al. 2005). The CSCs arisen in this process could have a phenotypic heterogeneity. They either show "pure" epithelial (E) or mesenchymal (M) phenotypes, or alternatively they show hybrid E/M phenotype combining both epithelial and mesenchymal features in different proportions. These hybrid E/M state cells are highly tumorigenic, and display stemness features like self-renewal and plasticity (Suster and Virant-Klun 2019). The epithelial, hybrid, and mesenchymal states are interchangeable in response to signals coming from intrinsic (i.e., tumor niche) and extrinsic (i.e., chemotherapy) sources. Their response depends also on the history of previous signals—"cellular memory" (Elowitz et al. 2002; Chang et al. 2006). Hybrid E/M phenotypes are sustained by "stability factors," like protein numb homolog (NUMB), transcription factor Ovo-like-2 (OVOL2), grainyhead-like protein-2 homolog (GRHL2), and nuclear factor erythroid-2-related factor-2 (NRF2), as well as by TGFβ- and NOTCH signaling (Bocci et al. 2019; Matsumura et al. 2019; Boareto et al. 2016). Hybrid E/M cells behave as aggressive CSCs, and their function is regulated between different interim E/M phenotypes by Wnt, NOTCH, and NF-κB signaling (Colacino et al. 2018; Kroger et al. 2019). Subset of CSCs of intermediate E/M phenotype shows probably the highest level of adaptation to secondary localizations and sometimes are called circulating CSCs (CTCs) (Agnoletto et al. 2019; Tam and Weinberg 2013). They have been identified in several metastatic cancers, including breast, lung, gastric, colon, and hepatocellular cancer (Vishnoi et al. 2015; Koren et al. 2016; Nel et al. 2014; Katoh et al. 2015; Li et al. 2014). CTCs are also described by increased expression of ALDH1, and associated with high tumor grade, poor outcome, and high level of expression of multi-drug resistance proteins (Aktas et al. 2009; Ginestier et al. 2007; Gradilone et al. 2011). Transcriptional and epigenetic regulation of EMT involves *CDH1* (for E-cadherin) gene promoter and downstream NF-κB pathway targets (Markopoulos et al. 2019; Jing et al. 2011). Epigenetic regulation of EMT embraces H3K27me3 histone methylation and changes in miR-200 and miR-34 expression (zinc finger transcription factors ZEB/miR-200 and SNAIL/miR-34 regulatory loops), which additionally governs the EMT-dependent activation of CSCs (Polyak and Weinberg 2009; Brabletz and Brabletz 2010). The presence of balanced interactions between feedback regulatory loops of p53- and NF-κB-dependent miRNA regulations is crucial for EMT and CSCs behavior (Markopoulos et al. 2018). Inflammatory environment in tumors created by TAMs, CAFs, MDSCs, cytokines (IL-1, IL-6, TNFα, TGFβ), and chemokines (IL8) participates in EMT and promotion of CSCs and depends on

TGF-β and NF-κB signaling. Besides those two pathways, Hedgehog, Wnt/β-catenin, and NOTCH pathways also regulate EMT (Iliopoulos et al. 2009; Hass et al. 2019).

8.6 Cancer Stem Cells and Tumor Microenvironment Niche

CSCs niche is a specialized tumor microenvironment taking part in origination and regulation of CSCs. Components of CSCs niche provide both nutrients and signals needed for the effective function of CSCs. In cancer, functionally understood niche is composed of CAFs, mesenchymal stem cells (MSCs), immune cells including tumor-associated macrophages (TAMs), non-CSCs cancer cells, adipocytes, components of extracellular matrix, blood and lymphatic vessels, cytokines, chemokines, and growth factors. CSCs niche enhances cell differentiation, accumulation of genetic mutations and epigenetic signals, resistance to apoptosis and toxic agents. The proper function of CSCs niche demands interchange of signals between CSCs and niche microenvironment (Kubo et al. 2016; Quante et al. 2011).

One of the most important cellular components of CSCs niche is CAFs, which regulate EMT transition, secrete proangiogenic factors, produce cytokines (IL-6, LIF, TGF-β), chemokines (IL-8, CXCL12, CXCL1), prostaglandins (PGE), and growth factors (HGF, VEGF). CAFs are situated mainly at the tumor "invasive front" (Zhang and Peng 2018; Guo et al. 2017a, b). Observation in breast cancer prooved that CSCs stemness and EMT transition was regulated by CAFs-derived exosomes containing regulatory molecules like miR-21, miR-378e, miR-143 and lnc RNA h19 (Huang et al. 2019; Ren et al. 2018; Donnarumma et al. 2017). They also activate the NF-κB, STAT, and NOTCH pathways in CSCs, thus supporting their drug resistance (Lee et al. 2019; Boelens et al. 2014). Exosomes containing miR-105 derived from cancer cells are a signal that force CAFs to reciprocally support CSCs. Cancer-associated fibroblasts have been classified functionally into different subpopulations. Inflammation in cancer niche is very important phenomenon and is dependent on the "inflammatory" iCAFs function. iCAFs inflammasome pathway is regulated by NOD-LRR-and pyrin domain-containing protein-3 (NLRP3), IL-6/STAT3/PTEN/NF-κB, TGF-β/SMAD and IL-1 mediated signals, and supports tumor progression by creation of immune-suppressive environment (Ershaid et al. 2019; Yan et al. 2018; Iliopoulos et al. 2011). In breast cancer, IL-6 secreted by CAFs regulates stemness mainly in CSCs of mesenchymal phenotype, while IL-8 stimulates mainly epithelial ALDH1+ CSCs (Chan et al. 2019; Chang et al. 2014; Ginestier et al. 2010). CAFs are even able to travel with CSCs to distant localizations to produce metastases. During chemo- or radiotherapy, cancer cell niche is enriched in CAFs by action of IL-8-CXCL1-pathway. Chemotherapy-recruited CAFs produce several CXCL chemokines, which further stimulate expansion of CSCs (Duda et al. 2010; Chan et al. 2016; Ginestier et al. 2010). In breast cancer, they are CXCL12 (stromal-cell-derived factor-1—SDF-1) and CCL2 (monocyte chemoattractant protein-1), which act on cancer cells, and activate prostemness

pathways, mainly Wnt/β-catenin, PI3K/AKT and NOTCH. Also in breast cancer, it was discovered that high mobility group box-1 (HMGB1) protein secreted by autophagic CAFs enhances stemness of breast CSCs via toll-like receptor-4 (TLR-4) (Tsuyada et al. 2012; Todaro et al. 2014; Zhao et al. 2017). The direct cell-cell contact between CAFs and CSCs is also an indispensable component of niche properties. CD44 and CD10/GPR77 membrane molecules are both engaged in this kind of interaction (Su et al. 2018).

Mesenchymal stem cells are a population of multipotent mesenchymal stromal cells capable to generate different cell types. They are described functionally by ability to migration into sites of inflammation, tissue injury, and cancer where they suppress immune response. MSCs are recruited into the tumors via TGF-β- and CXCL12-dependent ways (Quante et al. 2011). Inside tumors MSCs participate in regulation of EMT phenomenon, angiogenesis, and chemoresistance, as well as are able to differentiate into CAFs (Ma et al. 2014; Ishihara et al. 2017; Chang et al. 2015). They activate stemness in CSCs by secretion of IL-6, IL-8, CCL2, CCL5, PGE-2, metalloproteinase inhibitor-2 (TIMP-2), VEGF, fibroblast growth factor (FGF), and JAG1. Similar to CAFs, MSCs are multiplied in radio- or chemotherapy-treated tumors and through secretion of CXCL12 chemokine and activation of STAT3 signaling, they augment CSCs stemness and resistance to therapy. In breast and ovarian cancer, interaction with MSCs upregulated the PI3K/AKT pathway and MDR proteins in CSCs, resulting in resistance to trastuzumab and paclitaxel/carboplatin, respectively (Lee et al. 2019; Kalluri and Zeisberg 2006; Rafii et al. 2008; Chan et al. 2016; Park et al. 2009; Wang et al. 2018a, b, c). MSCs cell possess a unique possibility to fuse with cancer cells to form so-called hybrid cancer cells. This cell population although not very numerous has been identified in several cancers and contributes to cancer plasticity, genetic variability, and metastases (Melzer et al. 2018; Pawelek and Chakraborty 2008).

Provascular signals from tumor niche trigger neovascularization. CSCs participate in this phenomenon by "vasculogenic mimicry" where CSCs and cancer cells form vascular-like channels to supply nutrition during prevascular phase of tumor growth. Later on CSCs could differentiate to epithelial and vascular smooth muscle–like cells, creating the "mosaic pattern" of vascularization. CSCs are also able to secrete HIF-1α and VEGF in response to exogenic expression of these factors (Maniotis et al. 1999; Ping and Bian 2011). Epithelial cells of the niche vessels secrete many factors maintaining CSCs stemness phenotype, including IL-1, IL-3, IL-6, granulocyte colony–stimulating factor (G-CSF), and granulocyte-macrophage colony–stimulating factor (GM-CSF) (Barbato et al. 2019; Pirtskhalaishvili and Nelson 2000; Butler et al. 2010). Secretion of TNF-α by endothelium upregulates NF-κB signaling and stimulates chemoresistance of CSCs (Tang 2012).

Inflammation inside tumor is directly connected to EMT transition, thus influencing cancer progression and metastatic potential. It also upregulates significantly resistance of CSCs against host immune surveillance. Several proinflammatory cytokines/chemokines, including TGF-β, TNF-α, IL-1, IL-6, and IL-8, are secreted by the cells occupying CSCs niche and are potent EMT-inducers. Cytokine-triggered signaling pathways activate transcription factors and epigenetic regulation in

CSCs. One of the key regulators of EMT is TGF-β, which has a dual role for cancer progression—cancer suppressor in early cancer and tumor promoter in advanced cancer. Switch to promoter function is connected to initiation of EMT transition and depends on Smad3/Smad4 transcription factors, RAS/RAF/MAPK, and NF-κB signaling (Tian et al. 2013, Balkwill 2009, reviewed in Markopoulos et al. 2019). TGF-β-induced Smad expression is crucial for EMT as it upregulates ZEB and SNAIL zinc finger transcription factors followed by downregulation of miR-200 and miR-34. Complex functionality of TGF-β-induced regulatory loops consists of SNAIL/miR-34- and ZEB/miR-200-dependent switch between EMT and MET CSCs phenotypes, respectively. NF-κB signaling triggered by TGF-β promotes EMT and consequently CSCs motility, stemness, metastasis, and drug resistance (Tian et al. 2013, Markopoulos et al. 2018, reviewed in: Markopoulos et al. 2019). Prolonged stimulation of breast epithelial cells by TGF-β stimulated EMT and caused increase of CSCs-phenotype CD44 + CD24- cells (Bhat et al. 2019; Katsuno et al. 2019). In gastric cancer, *Helicobacter* infection stimulates TGF-β secretion followed by EMT activation, IL-17 secretion by Th17 cells, neutrophil recruitment, and creation of chronic inflammatory reaction sustaining CSCs (Rezalotfi et al. 2019; Lina 2014; Choi et al. 2015). TNF-α is a main proinflammatory cytokine engaged in regulation of differentiation and apoptosis in cancer. Its downward signaling activates NF-κB, caspase, p38, c-Jun N-terminal kinases (JNK), and ERK pathways. Through NF-κB pathway, TNF-α stimulates cytokine and chemokine effectors [IL-6, IL-8, IL-18, inducible nitric synthase (iNOS), cyclo-oxygenase (COX)-2, and lipoxygenase (LOX)], which link inflammation to cancer progression. TNF-α could negatively affect growth of early tumors; however, it promotes survival, angiogenesis, and EMT in advanced tumors. TNF-α co-operates strongly with TGF-β to accelerate the process of EMT (Balkwill 2009; Aggarwal et al. 2012; Brenner et al. 2015; Onder et al. 2008; Bates and Mercurio 2003). Breast cancer cells exposed to TNF-α have been enriched by CSCs CD44 + CD29+ cells (Weitzenfeld et al. 2016). IL-1 is another proinflammatory cytokine exerting effects on CSCs residing in niche. IL-1-dependent NF-κB signaling upregulates stemness-promoting genes, like proto-oncogene polycomb ring finger gene (*BMI1*) and nestin gene (*NES*). In head and neck cancer, CSCs IL-1 activates EMT by downregulation of E-cadherin gene (*CDH1*) expression, while in breast cancer, CSCs activate EMT by IL-1/IL-1R/β-catenin pathway, which additionally leads to estrogen receptor *ESR1* gene silencing and tamoxifen resistance (Mantovani et al. 2018; Soria et al. 2011; Li et al. 2012; Charuworn et al. 2006; Jiménez-Garduño et al. 2017). IL-6 is another proinflammatory cytokine activated in tumor microenvironment by TGF-β, TNF-α and IL-1, NF-κB and STAT3 transcription factors, and RAS/RAF/MEK and PI3K signaling pathways. Through the upregulation of NF-κB and STAT3 transcription factors, IL-6 increases expression of miR-21, miR-181b-1, and Let-7; enhances cancer-associated inflammation; and activates EMT. IL-6-induced EMT induces invasion and migration of cancer cells via activation of metalloproteinases (Chang et al. 2014; Ancrile et al. 2007; Chou et al. 2005). In breast cancer, IL-6 was shown to stimulate CSCs stemness by increase of CD44 and Oct4 expression. It is also capable of autocrine augmentation of self-secretion in CSCs via JAG1/NOTCH3

signaling, thus stimulating self-renewal and proliferation of CSCs (Kim et al. 2013; Sansone et al. 2007; Al-Hajj et al. 2003). Breast cancer ALDH1+ CSCs were shown to have higher expression of IL-8 receptor and alpha-chemokine receptor *CXCR* gene. IL-8 signaling was connected to increased CSCs activity both in HER2-positive and triple negative breast cancers (Dominguez et al. 2017; Singh et al. 2013; Charafe-Jauffret et al. 2009).

Metabolic reprogramming of CSCs is one of the key factors influencing their stemness, migratory potential, and chemoresistance. CSCs show unique adaptation to variable levels of tissue oxygenation found inside the tumors and are capable of functioning using both aerobic glycolysis and oxidative phosphorylation (OXPHOS) (Nazio et al. 2019; Peixoto and Lima 2018; Menendez et al. 2013; Pacini and Borziani 2014). Generally, in normoxic and most hypoxic conditions, CSCs rely on OXPHOS, which is more energetically efficient process. In this situation, the maintenance of CSCs stemness depends on increase of antioxidant defense against reactive oxygen species (ROS) derived by enhancement of OXPHOS rate and mitophagy, which through degradation of defective mitochondria prevents apoptosis of CSCs (Nazio et al. 2019, Held and Houtkooper 2015, Peiris-Pagès et al. 2016, Snyder et al. 2018, reviewed in Jagust et al. 2019). ROS balance and resistance to ROS inducers (like chemo- and radiotherapy) are regulated in CSCs by c-Myc, p53, HIF-1α, NF-κB, and NRF2 pathways. HIF-1α via signaling pathway reduces ROS production and protects CSCs from their adverse effects. ALDH1, the CSCs marker, directly reduces ROS and produces antioxidants, as well as facilitates resistance to paclitaxel (Takahashi and Yamanaka 2006). In hypoxic conditions of the niche, CSCs can switch from OXPHOS to aerobic glycolysis. Although it is usually less efficient in production of energy, in cancer cells, it could achieve levels of energy comparable to OXPHOS. Besides this, it was found that even in hypoxic environment, cancer cells use simultaneously OXPHOS and glycolytic metabolic pathways (reviewed in Jagust et al. 2019). Hypoxia-activated cascade of cellular pathways dependent on HIF-1α helps to endure hostile conditions by reprogramming CSCs, which can finally enter the state of quiescence. Genes and transcription factors responsible for CSCs pluripotency were demonstrated to be engaged in switch from OXPHOS to glucose-dependent metabolism (reviewed in Jagust et al. 2019). CAFs and other cells of CSCs niche support CSCs metabolic reprogramming and help to remove lactates in so-called reverse Warburg effect. (Nazio et al. 2019, Yoshida 2017, reviewed in Jagust et al. 2019). CSCs are generally situated inside or close to hypoxic areas inside tumors; however, in some brain tumors, CSCs reside in well-oxygenated perivascular niches (Gilbertson and Rich 2007). Tumors possessing high expression of HIF-1α have been associated with higher mortality and resistance to chemotherapeutics. In breast cancer, HIF-1α was correlated to MDR proteins expression (Semenza 2014; Cao et al. 2013). The presence of HIF-1α expression enhances activation of EMT and stemness activators like Wnt/β-catenin, Hedgehog, NOTCH pathways and CD133, Nanog and Sox2 in CSCs markers (Liu et al. 2014; Majmundar et al. 2010). Tumor environment is described by acidosis, resulting from glycolytic activity and mitochondrial respiration–derived carbon dioxide hydration. Acidosis seems to be a triggering

and a maintenance factor for CSCs stemness. Acidic conditions stabilize HIF-1α, change histone epigenetic regulation, and downregulate von Hippel-Lindau (VHL) tumor suppressor molecule. They also stimulate MSCs, increase expression of transcription factors Oct4 and Nanog in CSCs, and secretion of VEGF and IL-8 in CSCs niche (Vander Linden and Corbet 2019; Schornack and Gillies 2003; Corbet and Feron 2017; Hjelmeland et al. 2011; Mekhail et al. 2004). Acidosis drives energy gain into OXPHOS mechanism and changes lipid metabolism. It augments drug resistance by direct influence on cell membrane integrity, efficacy of membrane transporters, cancer cell dormancy, and autophagy (reviewed in Vander Linden and Corbet 2019).

Dysregulation of lipid metabolism is observed in the most aggressive tumors. Lipid desaturation plays important role in self-renewal and tumorigenicity of CSCs through the changes of lipid composition of cell membrane and Wnt/β-catenin signaling. Monounsaturated fatty acids/Stearoyl-CoA desaturase-1 (SCD-1) converses fatty acids into monosaturated fatty acids. Upregulation of monounsaturated fatty acids/Stearoyl-CoA desaturase-1 (SCD-1) enhances tumor proliferation, while inhibition of SCD-1 results in decrease of ALDH1, Nanog and Oct4 activity, and restores chemoresistance in lung CSCs (Begicevic et al. 2019; Kim and Ntambi 1999; Colacino et al. 2016; Noto et al. 2013). Lipids can also function as second messengers of signal transduction in CSCs via NOTCH, AKT, and NF-κB pathways (reviewed in: Jagust et al. 2019). Lipids are also an important substrate for energy supply; therefore, blockade of fatty acid synthase (FASN) inhibits CSCs growth (Wang et al. 2013). In breast cancer, JAK/STAT3 pathway was found to regulate lipid metabolism in CSCs, thus stimulating their stemness.

Adipocytes from cancer microenvironment (cancer-associated adipocytes—CAAs) are capable to provide lipids for CSCs. Increased lipid uptake results in lipid droplet accumulation inside CSCs. High concentration of lipid droplets is correlated with tumor aggressiveness and poor survival. Fatty acids stored up in CSCs serve as energetic reserve for the cells during the periods of metabolic restrictions and are then mobilized during a lipophagy process (Lue et al. 2017). Breast adipocytes via secretion of leptin and IL-8 could participate in lipid metabolism of CSCs using the same STAT3 signaling pathway. Adipocytes and adipose progenitor cells being component of breast cancer niche and secreting GM-CSF and metalloproteinase 9 are also capable of stimulating breast cancer CSCs (Reggiani et al. 2017; Wang et al. 2018a, b, c; Al-Khalaf et al. 2019). In ovarian cancer, omental implants are an example of another niche, in which adipocytes play important role in nesting and proliferation of CSCs (Nieman et al. 2011).

In ovarian cancer, ascites represents a unique microenvironment for CSCs and accounts for transcoelomic spread of metastases/implants. During this process, cancer cells go through EMT and, in the form of single cells or cell spheroids containing a lot of CSCs, are transported passively over peritoneal cavity, then homing mesothelium, going through MET, and starting to grow extensively (Bregenzer et al. 2019; Yeung et al. 2015). Ascites also facilitates entry of cancer cells into lymphatic vessels. Pro-inflammatory IL-6 from ascites stimulates stemness in CSCs via JAK/STAT3 and Wnt/β-catenin signaling (Abubaker et al. 2014).

VEGF is also a regulator of peritoneal carcinomatosis, and IL-8 recruits cancer cells into the surface of omentum due to the tropism between CSCs and adipocytes (Winiarski et al. 2013; Nieman et al. 2011). Extracellular vesicles play important role in regulatory network inside ascitic fluid. They are able to transport miRNAs, lipids, cytokines and growth factors, as well as CSCs markers like CD44 or EpCAM molecules (Zong and Nephew 2019; Runz et al. 2007; Gutwein et al. 2005). One of the astonishing mechanisms resulting in enrichment of CSCs in peritoneal implants is response of cancer cells to mechanic stimuli and mechanic stress produced by peritoneal extension due to ascitic fluid. Mechanic stimuli cause activation of mechanotransduction signals involving mainly YAP/TAZ signaling pathway, and accessory NF-κB, ERK, FAK, and Rho/Rho-associated protein kinase (Rho/ROCK) pathways. There a plenty of mechanical stressors that influence behavior of CSCs in ovarian cancer. The first of them are shear and compression produced mainly by ascites build-up and movement, then tension and compression caused by tumor growth against surrounding tissue, and finally stiffness resulting from ECM remodeling and desmoplastic response (reviewed in: Bregenzer et al. 2019). Activation of mechanotransduction signals regulates EMT/MET transition, changes cancer cell shape and morphology, enhances CD133 + CD44 + Oct4+ CSCs population, increases CSCs chemoresistance through upregulation of ABCG2 and P-gp membrane transporting systems, increases angiogenesis via VEGF secretion, and regulates interaction with ECM (reviewed in Bregenzer et al. 2019). Response of CSCs to mechanic stressors in metastatic locations augments ovarian cancer invasiveness, chemoresistance, and stemness of cancer cells.

ECM composition is altered inside tumor niche due to the activity of CAFs and cancer cells themselves. The changes of metalloproteinase activity and VEGF in tumor environment influence ECM behavior and are the source of different changes including desmoplastic reaction. Disturbed ECM and aberrant tumor vasculature results in fluctuations of tumor interstitial fluid pressure that would further influence pathways regulating EMT transition, hypoxia, and chemoresistance. Components of ECM could co-operate with CSCs in different ways. CD44, a marker of CSCs, is a receptor of hyaluronic acid and versican, constituents of ECM. CD133, whose expression is connected to CSCs stemness, could be activated by type I collagen. Both CD44 and CD133 promote attachment of cancer cells to mesothelium on the surface of peritoneum. Mechanic signals are also transmitted to CSCs by syndecan-1 (CD138) and discoidin domain receptor-1 (DDR1) activated by fibronectin and collagen, respectively (reviewed in Choudhury et al. 2019). In breast cancer, tenascin C expressed in ECM supports WnT/β-catenin and NOTCH signaling, thus stabilizing CSCs functions (Oskarsson et al. 2011).

8.7 Cancer Stem Cells and Autophagy

Autophagy is defined as a self-digestion inside auto-phagosomes of proteins, lipids, and damaged cellular organelles followed by recycling of digestion products. In normal conditions, autophagy is a mechanism of controlling cell homeostasis, but

during stress produced by hypoxia, starvation or toxic drugs autophagy is a mode of cell survival. The role of autophagy is ambivalent—it could act as antitumor mechanism, or it could promote tumorigenesis. Protection against tumor initiation depends on ability to control cell homeostasis in chronically inflamed or mutagenic environment. In cancer, autophagy helps to maintain tumor survival and progression despite hostile conditions. Forkhead box family transcription factor-3 (FOXO3) signaling pathway mediates transcription of autophagy-regulating genes including autophagy-related genes (*ATG*), beclin-1 gene (*BECN1*), Unc-51-like autophagy-activating kinase-1 gene (*ULK1*), and gamma-aminobutyric acid receptor–associated protein-like-1 gene (*GABARAPL1*) (Nazio et al. 2019; Van Der Vos and Coffer 2008). Autophagy is linked to EMT and present in cells with mesothelial phenotype. It is also connected to chemoresistance of CSCs. Autophagy protects cancer cells from proapoptotic stimuli and genome instability. It is also capable to modify antitumor immune responses and maturation of some immune cells. During premetastatic latency breast cancer, CSCs indicate dormancy phenotypes supported by several mechanisms including autophagy. Autophagy in dormant CSCs could be regulated by activation of SRC-mediated TNF-related apoptosis-inducing ligand (TRAIL) resistance (in bone metastases), effective DNA repair and p53 function sustained by expression of *ATG7* gene, and by decrease of 6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase-3 (PFKFB3) concentration in the cells (Zhang et al. 2009; Lee et al. 2012; Shinde et al. 2019; Janji et al. 2016).

8.8 Cancer Stem Cells and Immunosurveillance

Cancer CSCs would not be so dangerous if they were not able to escape from immune surveillance. This property is known as immune CSCs resistance, and is based on lower CSCs immunogenicity and ability to manipulate immune system through secretion of suppressor molecules, recruitment of immune-regulatory cells, and decreased expression of cell antigens. CSCs are capable to mimic the function of antigen-presenting cells, however, in altered way as they show an eleveted expression of check point programmed death ligand-1 (PD-L1) and decreased expression of MHC molecules. As a result of defective antigen presentation, inhibition of T cell effectors, stimulation of Tregs, and promotion of tumor tolerance occur. Glioblastoma CSCs have altered expression of PD-L1, galectin-3, and macrophage-inhibitory cytokine-1, thus being able to avert cytotoxic T reactions and phagocytosis (Kim et al. 2016; Downs-Canner et al. 2017). Moreover, upregulated expression of HLA-E class II molecule and simultaneous low expression of MHC class I and NKG2D molecules on glioblastoma CSCs were shown to inhibit cytotoxic T cell and NK effectors (Sultan et al. 2017; Du et al. 2014). In cancer breast cells, PD-L1 overexpression was connected with increased function of Oct4 and Nanog transcription factors promoting stemness (Zhao et al. 2009). Breast CSCs also indicate downregulation of MICA and MICB ligands for NK cell receptor NKG2D that makes them resistant against NK cell–mediated cytotoxicity (Gagliani et al. 2015).

CD95 molecule is a death-promoting factor for regulation of activation-induced death of T lymphocytes and many other types of cells. In gastric cancer, CD95/CD95-ligand signaling promotes EMT and supports maintenance of CSCs population (Badrinath and Yoo 2019; Ceppi et al. 2014).

Immune cells present in CSCs niche comprise mainly of TAMs, MSCs, and MDSCs, which through TGF-β signals stimulate tumor EMT, progression, and metastatic potential. All three cell populations contribute to immunosuppressive environment in tumor and CSCs niche. Through secretion of macrophage inflammatory proteins (MIP1 and MIP2) and PGE, MSCs recruit suppressor M2 macrophages into the tumor (Vasandan et al. 2016). Moreover, TAMs and MSCs stimulate the T regulatory CD4 + CD25 + FoxP3 cells, while MDSCs recruit the T helper IL-17-secreting suppressors (Barbato et al. 2019; Kalluri and Weinberg 2009; Kitamura et al. 2015). In ovarian cancer, IL-17 activates NF-κB and p38-mitogen-activated protein kinase pathways, which increase stemness of cancer cells (Xiang et al. 2015) R. In colon cancer, regulatory T FoxP3 + IL-17+ cells promote expansion of CSCs in hypoxic environment (Sultan et al. 2017; Silver et al. 2016). CSCs cells in glioblastoma and colon cancer were shown to secrete increased levels of immunosuppressive TGF-β and IL-4, respectively (Codony-Servat and Rosell 2015; Viry et al. 2014; Lorin et al. 2013). They could downregulate the intensity of host immune antitumor response. Acidic conditions in CSCs microenvironment and premetastatic niche also decrease antitumor efficacy of T lymphocytes and NK cells, as well as the secretion of IL-2, interferon (IFN) γ, perforin, and granzyme B. Acidosis inhibits also maturation of dendritic cells. Accumulation of H^+ ions and lactate inhibits glycolytic processes in T cells and expression of nuclear factor of activated T cells (NFAT). Acidic conditions help also to deviate TAMs activity into M2 tumorigenic phenotype (Fischer et al. 2007; Gottfried et al. 2006; Dietl et al. 2010; Brand et al. 2016). TAMs present in cancer cells niche produce TNF-α and TGF-β for maintaining CSCs. In breast cancer, TAMs promote CSCs via EGFR/STAT3/Sox2 signaling pathway. The function of FoxP3+ Tregs and PD-1/PD-L1 pathway also support the CSCs population (Zhou et al. 2019; Plaks et al. 2015; Yang et al. 2013; Seo et al. 2013; Malta et al. 2018). Metastatic aggressive foci characterized by high level of autophagy are poorly infiltrated by tumor-infiltrating lymphocytes (TILs) (Zarogoulidis et al. 2016).

8.9 Conclusion

Cancer stem cells have become one of the main targets for anticancer therapy and many ongoing clinical trials test anti-CSCs drugs. However, high plasticity of CSCs gives rise to many doubts concerning efficacy of these drugs. Some treatment options propose multidirectional inhibition of CSCs by simultaneous use of several drugs having different points of action, but results of such trials are still inconclusive. Only time will tell if we can tame and neutralize CSCs successfully, but even today, many are skeptical. And this will not change in the nearest future, I am afraid.

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