Cancer Stem Cells, Bone and Tumor Microenvironment: Key Players in Bone Metastases

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Abstract: Tumor mass is constituted by a heterogeneous group of cells, among which a key role is played by the cancer stem cells (CSCs), possessing high regenerative properties. CSCs directly metastasize to bone, since bone microenvironment represents a fertile environment that protects CSCs against the immune system, and maintains their properties and plasticity. CSCs can migrate from the primary tumor to the bone marrow (BM), due to their capacity to perform the epithelial-to-mesenchymal transition. Once in BM, they can also perform the mesenchymal-to-epithelial transition, allowing them to proliferate and initiate bone lesions. Another factor explaining the osteotropism of CSCs is their ability to recognize chemokine gradients toward BM, through the CXCL12–CXCR4 axis, also known to be involved in tumor metastasis to other organs. Moreover, the expression of CXCR4 is associated with the maintenance of CSCs' stemness, and CXCL12 expression by osteoblasts attracts CSCs to the BM niches. CSCs localize in the pre-metastatic niches, which are anatomically distinct regions within the tumor microenvironment and govern the metastatic progression. According to the stimuli received in the niches, CSCs can remain dormant for long time or outgrow from dormancy and create bone lesions. This review resumes different aspects of the CSCs' bone metastastic process and discusses available treatments to target CSCs.

Keywords: bone metastases; cancer stem cells; osteotropism

1. Introduction

Due to advances in early diagnosis and cancer treatments, the survival of cancer patients has improved over the last two decades. As a negative consequence, the probability of cancer patients developing metastasis has increased. Bone is one of the most common sites of metastases, since some tumors show a marked osteotropism [1]. The propensity of some tumor to metastasize to bone depends on many factors such as the expression of adhesive molecules and bone markers on the surface of tumor cells (i.e., vitamin D receptor, RANKL, RUNX2, αVβ3, etc.) [2,3]; the expression of a set of genes associated with bone metastases [4–21]; and the capability of performing the epithelial–mesenchymal transition (EMT) of carcinomas [2,3,22–61]. In the bone microenvironment, cancer cells disrupt the physiological balance between bone resorption and formation, leading to lesions. Indeed, the interaction between cancer and bone cells causes the disarrangement of the bone matrix, disrupting the bone micro-architecture and impairing the bone function [62–64].

Literature reports that bone marrow (BM) is an appealing site for tumor cells and particularly for cancer stem cells (CSCs), which are able to resist treatments, such as chemo-radiotherapy. Indeed, once CSCs reach BM, they can remain dormant or cause bone metastases, long after the primary tumor has been surgically removed from patients [65,66]. CSCs have been isolated from many tumors and have been characterised for the expression of markers according to their different origin: the most

representative markers with their functions are reported in Table 1. In this work, we focus on the dialogue among CSCs, bone and the tumor microenvironment, and the consequent formation of bone metastases.

Table 1. Cancer stem cell markers.

Marker	Family	Function	Tumors	Effects	References
CD44	Cell surface HA-binding glycoprotein	Tissue remodeling, adhesion of cell-matrix, and cell migration	Breast, Prostate, Liver	Aggressive phenotype, Tumor progression, Stemness phenotype, Bone metastasis	[4,67–70]
E-cadherin	Type I transmembrane protein	Maintain normal cell structure, cell polarity and integrity	Prostate, Breast, Brain	Stemness gene expression, Tumor progression, Invasion and metastasis, Theraphetic resistance	[71–73]
CD166	Immunoglobulin superfamily of cell adhesion molecules (Ig-CAMs)	Intercellular adhesion, leukocyte extravasation, T cell activation and proliferation, and stabilization of the immunological synapse	Lung	Cellular proliferation, Stemness phenotype	[74,75]
EpCAM	Epithelial cell adhesion molecule	Wnt-beta-catenin signaling	Liver, Prostate	Tumor progression, Invasion and metastasis, Therapheutic resistance	[76,77]
ABCB5	ATP-binding cassette sub-family B	Drug efflux transporter	Melanoma, Breast, Colorectal, Liver	Tumor progression, recurrence, Therapeutic resistance, Metastasis, Invasion	[78–82]
ABCG2	ATP-binding cassette (ABC)	Drug efflux transporter	Breast, Prostate, Liver	Stem cell phenotype, Proliferation, Migration, Therapeutic Resistance	[83–85]
ALDH	Detoxifying enzyme	Proliferation	Breast, Lung, Brain, Colon, Liver, Prostate, Bladder, Ovarian, Renal	Tumor progression, Self-renewal capacity,	[86–92]
CD133	Transmembrane protein	Proliferation, differentiation and self-renewal	Gastric, Lung, Liver, Colon, Renal, Prostate, Pancreatic	Tumor progression, Stemness gene expression, Bone metastasis	[20,93–97]
CD13	Membrane glycoprotein	Aminopeptidase N	Liver	Invasion, Angiogenesis, Proliferation	[98,99]
CD90	Glycosylphosphatidylinositol-anchored glycoprotein	Cell-cell and cell-matrix interactions	Liver, Breast, Lung	Invasion, Tumor progression	[100–102]
CD105	Type I membrane glycoprotein, TGF beta receptor complex	Angiogenesis, Mesenchymal Stem cell marker	Renal, Breast, Liver	Initiating metastatic process, Stemness gene expression, Migration, Bone metastasis	[50,103–106]

2. Cancer Stem Cells (CSCs) Have a Pivotal Role in Tumor Heterogeneity

Tumor mass is constituted by a heterogeneous group of cells responsible for its formation and maintenance. This cell heterogeneity of many cancer types derives from a hierarchical organization that resembles one of the tissue of origin [80,107]. According to the hierarchical model, in the primary tumor at the apex of the hierarchy there are cells showing stem cell-like properties, the so-called CSCs. The phenotype and function of cancer CSCs appear to be equivalent to normal stem cells, but they harbour oncogenic mutations. CSCs possess high regenerative properties: self-renewal, and the ability to modify the response to stress conditions and thus survive in hostile conditions [108,109]. Date in literature, deriving from mouse models, provides genetic evidence that primary brain, colon and skin cancers comply with the hierarchical organization [110,111]. Also, clinical data confirm this hierarchical model; indeed, cell populations isolated from primary tumors using stem-cell marker genes can generate tumors when transplanted into mice [112–114]. Stem-cell properties are mainly

maintained by four known transcription factors, such as octamer-binding transcription factor 4 (Oct4), Nanog, SRY-Box 2 (Sox2) and Kruppel-like factor 4 (Klf4) [115–118] and by different growth factors, such as epidermal growth factor (EGF) and fibroblast grow factor (FGF) [119,120]. To maintain the pluripotent stem-cell state Oct4 and Sox2 bind together forming a heterodimer, before linking to different sites on Klf4. Then, the Oct4/Sox2/Klf4 complex binds Nanog promoter [121]. Among these transcription factors, Oct4 is the main regulator, indeed the knock out of Oct4 expression dramatically reduces Sox2 and Nanog expression [122]. Moreover, Oct4 controls its expression: its increase inhibits the transcription of the *Nanog* gene and thus reduces Oct4 expression [123]. This work demonstrates that a delicate balance among these transcription factors influence the stem-cell phenotype [124].

Tumor heterogeneity also depends on the presence of various genetically related subclones, which compete with each other thanks to their different characteristics and evolve during tumor progression, allowing the acquisition of genetic instability and somatic mutations that favour one tumor clone over others [125–127]. Genome-sequencing studies have shown more similarities than differences between primary tumors and metastasis, suggesting that most of the genetic mutations required for metastasis are already present in the primary tumors [128,129]. Lastly, the environment where cancer cells reside activates epigenetic mechanisms, such as epigenetic reprogramming, which induces the stem-cell state [130].

3. EMT: A Key Step in CSC-induced Bone Metastases

EMT is a crucial step in tumor progression and has an important role during cancer invasion and metastasis. The importance of EMT for the induction of stem characteristics has been demonstrated in different carcinomas [131,132]. In immortalized human mammary epithelial cells, induction of an EMT results in the acquisition of mesenchymal traits and in the expression of stem-cell markers [133]. Some recent studies in transgenic mouse models showed that CSCs undergo EMT and initiate a tumor process [134,135]. These data were confirmed in clinical studies, showing the presence of tumor cells with CSCs capabilities in BM of breast cancer patients [136]. CSCs exist both in epithelial and mesenchymal states [133,137]: they perform EMT and express epithelial markers in order to promote migration from the primary tumor to secondary organ, such as BM, where they perform a mesenchymal-epithelial transition (MET), expressing markers that allow them to proliferate and initiate secondary lesions. The EMT transition is also a mechanism involved in bone metastasis formation [138]. Different studies reported that growth factors, such as transforming growth factor-β (TGFβ) and insulin-like growth factor (IGF), produced or released from the bone microenvironment, are potent effectors of EMT and can stimulate the formation of bone metastasis [139–141].

CD44 is an adhesion molecule that binds to the extracellular matrix through hyaluronan [142], increases the expression of the receptor activator of nuclear factor (NF)-κB ligand (RANKL) in stromal cells of BM, and promotes osteoclastogenesis [143,144]. CD44 has been recognized as one of the cell surface markers of CSCs in different tumors [145,146], and its expression is linked to an enhanced ability of CSCs to metastasize [147]. For instance, CD44 is significantly expressed by breast cancer cells, where it promotes invasion and adhesion to BM [148], and breast CSCs were initially identified as $CD44^+CD24^-$ cells [70,149]. Clinical studies suggested a positive correlation between CD44 expression and bone metastasis [136,150]. Hiraga et al. demonstrated that breast CSCs' migration, invasion and their ability to form bone metastasis in nude mice were inhibited by the down regulation of CD44 [151]. In a human-in mouse model, we previously showed that breast $CD44^+CD24^-$ CSCs were present in the primary tumor, with a mesenchymal phenotype allowing them to migrate towards bone. After reaching the bone, in order to proliferate they performed a MET transition acquiring an epithelial phenotype; indeed, we detected $CD44^-CD24^+$ cells in bone. Once $CD44^-CD24^+$ cells collected by bone lesions were re-injected in mice, they formed new tumor masses, with a heterogeneous population, constituted mainly by $CD44^+CD24^-$ cells [4]. Our data confirm a previously reported work of Liu et al. demonstrating a transition between epithelial and mesenchymal states of breast CSCs [5]. Also, Chaffer et al. demonstrated that $CD44^{low}$ breast-cancer cells can spontaneously

convert into CD44^{high} CSCs both in vitro and in vivo [130]. The capability of the breast metastatic CD44^{-}CD24^{+} cells isolated from bone to generate new heterogeneous tumors with a high percentage of CD44^{+}CD24^{-} CSCs is consistent with a phenotypic plasticity of these cells, that allow metastatic cells to regain a tumor-initiating capacity. This transition between different states observed in CSCs can be considered a stochastic transition, and can be explained by the quantitative Markov model of cell-state interconversion, where any subpopulation of cancer cells returns to equilibrium, with the phenotypic proportion of the primary tumor over time. Thus, by contrast with the hierarchical model and according to the stochastic one, breast cancers can be constituted by discrete populations that randomly perform transitions between states without increasing their proliferation rate, simply to reach a progressive equilibrium proportion [6,7].

4. Role of CXCL12–CXCR4 Axis in CSC-Tumor Microenvironmet Crosstalk

CSCs seem to be quite pleiotropic in the expression of receptors in response to different microenvironmental stimulation, and thus they can modify their expressions in response to the level of ligand and move towards a secondary organ according to a chemotactic gradient. One of the main chemotactic stimulus involved in the regulation of trafficking of normal and CSCs is represented by the C–X–C motif chemokine 12 ligand (CXCL12) and C–X–C chemokine receptor type 4 (CXCR4) axis [8]. Indeed, CXCR4 is expressed not only in normal stem cells in different organs, but also in several tumors [9]. The CXCR4–CXCL12 axis is involved in cancer-cell-tumor microenvironment interactions and it is one of the mechanism involved in bone metastases formation [10]. Indeed, bone-seeking breast cancer cells express high levels of CXCR4, which is associated to cancer-cell stemness [11,136].

CXCL12 is highly expressed in bone, lymph nodes and lungs, CSCs expressing CXCR4 are attracted by these organs [12,13]. CSCs recognize chemokine gradients toward BM, like the gradient followed by hematopoietic stem cells (HSCs) and leukocytes to migrate to the bone [14]. Indeed, both mesenchymal stromal cells and osteoblasts (OBs), in BM, constitutively express CXCL12 [15], and they help the entrance of CSCs expressing CXCR4 into the bone microenvironment. In non-small cell lung cancer (NSCLC), the expression of the CXCR4 receptor has been associated with the maintenance of stemness of chemoresistant CSCs both in cell lines and primary tumors [16,17]. CXCL12 has been proven to induce EMT in different tumors [18,19], such as NSCLC, where a subset of CD133^{+}CXCR4^{+} EpCAM^{-} CSCs could be directly generated through EMT activation [17]. Indeed, we demonstrated in a humanized murine model that CD133^{+}CXCR4^{+} EpCAM^{-} CSCs were endowed with an enhanced bone tropism [20]. The ability of CD133^{+}CXCR4^{+} CSCs to originate frank metastases when injected in humanized mice implanted with human bone stresses the prominent relevance of a conductive tumor microenvironment (with a pre-metastatic niche) to support tumor metastasis [139,140]. The over-expression of CXCR4 and CXCR7 by breast and prostate cancer cells increases their vascular ability and bone colonization in mouse models [21,152]. CXCR4 is also essential for maintenance of renal cell carcinoma-initiating cells [153].

5. Tumor Microenvironment and CSCs

The tumor microenvironment is composed of non-malignant cells such as endothelial cells, fibroblasts, immune cells and a non-cellular matrix. Both the cellular and non-cellular components form the tumor stroma, which dramatically changes during the tumor progression, influencing tumor growth and chemoresistance [154,155]. The tumor microenvironment releases different factors, it is able to protect CSCs against the immune system, and it maintains CSCs' properties and plasticity [156]. Furthermore, the primary tumor stroma also seems to select for organ-specific seeding traits. For example, cancer-associated fibroblasts (CAFs), in breast-cancer stroma, produce CXCL12 and IGF-1, which select Src hyperactive cancer clones, and promote bone metastases [157,158]; in NSCLC, stimuli from CAFs in the tumor microenvironment could dictate de novo creation of the CD133^{+}CXCR4^{+} CSCs subset, directly linked to EMT induction with the acquisition of increased dissemination [20]. In colorectal cancer, CAF release hepatocyte growth factor, which stimulates the self-renewal of CSCs

through a β-catenin-dependent mechanism [159] and promotes the reprogramming of colorectal cancer progenitors into CSCs [160]. Moreover, CAFs secrete specific cytokines and chemokines upon chemotherapy treatment, such as IL-17A, that stimulates colorectal CSC self-renewal and invasion [161]. This last observation indicates that chemotherapy induces a remodelling of the tumor microenvironment and thus contributes to promote CSC proliferation [162].

6. CSCs' Dormancy in the Niche

CSCs have the capability to grow as spheres, and thus they can easily enter and disseminate in blood circulation. Once they have reached a secondary organ, such as BM, they can remain silent or undergo asymmetric division, allowing the maintenance of the CSC population as well as the expansion of differentiated cancer cells, which represent the full spectrum of the original tumor heterogeneity and maintain secondary lesions [163–165] (Figure 1).
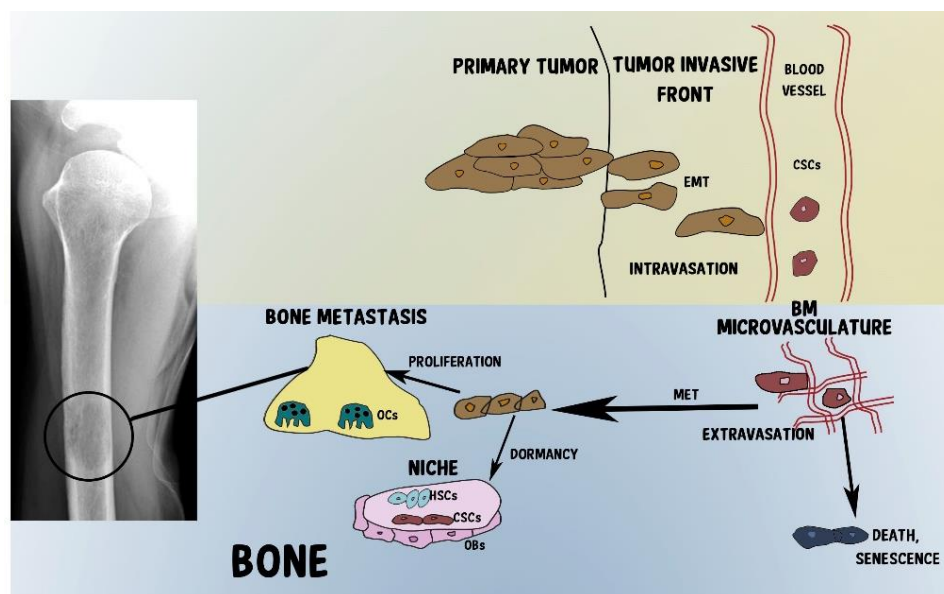

Figure 1. Cancer stem cells' (CSCs) steps toward bone metastasis. At the primary tumor, cancer cells grow and can perform epithelial-to-mesenchymal transition (EMT), which confers them with invasive properties and consequent intravasation in the circulation. Once they have reached the BM microvasculature, CSCs can undergo senescence and cell death, or colonize the BM, regaining their original epithelial phenotype through a mesenchimal-to-epithelial transition (MET). Then CSCs can enter into a quiescent state (dormancy) in the BM niches, where osteoblasts (OBs) contribute to dormancy, or they can proliferate, releasing factors activating osteoclasts (OCs) and forming bone lesions.

CSCs metastasize to the BM, localizing in HSC niches, creating the so-called pre-metastatic niches, which are anatomically distinct regions within the tumor microenvironment that govern metastatic progression [166,167]. OBs are constituent of the niches and maintain HSC by secreting growth factors such as stem-cell factor (SCF), CXCL12 and angiopoietin [168,169]. The importance of OBs in supporting the metastatic niche has been demonstrated in prostate cancer, where in an in vivo model the ablation of OBs significantly decreased the presence of disseminated cancer cells in the BM [170]. Compelling evidence from Shiozawa et al. reported that disseminated prostate cancer cells compete with HSC for niche support, where they can be remobilised in the circulation by the same molecules that induce HSC [170]. OBs also control long-term dormancy and support the cell survival of CSCs [171]: they exert a protective role for CSCs from the immune system, preserve their phenotypic plasticity, and promote their dormancy or metastatic potential.

Dormancy depends on different exogenous factors released by several BM stromal cells and the matrix: for instance, OBs express cadherins which allow bonds with CSCs [172] and the bone matrix [173]; annexin II expressed by OBs and endothelial cells plays a critical role in niche selection [174]. OBs also secrete factors such as growth arrest specific 6 (GAS6), which binds to the receptor tyrosine kinase AXL, activating it and thus promoting tumor cell dormancy [175]. Osteopontin induces mesenchymal stem cells in the tumor microenvironment to differentiate into CAFs, which promote cancer growth and can be educated to release periostin in the metastatic microenvironment [22,23]. Periostin is a critical factor promoting the stemness of CSCs and the CSCs' niche via the IL-6/STAT3 signaling pathway [23,24], and the expression of IL-6 has also been associated with the dormancy phenotype. Recent data reported that dormancy could also be an intrinsic feature of a subpopulation of cancer cells that have arisen in a hypoxic microenvironment, which can remain dormant initially and later on be responsible for disease relapse [25].

Perivascular niches are important for the maintenance of both HSCs [26] and CSCs [27]. Indeed, endothelial cells in the mature microvascolature express thrombospondin-1 and other factors which maintain breast CSCs dormant [28]. On the other hand, endothelial cells in the sprouting neovasculature produce tenascin C, fibronectin and other factors that induce the formation of different perivascular niches and accelerate CSCs' outgrowth [28]. Thus, it is likely that the dynamic of the niche components, rather than spatial localization, regulates the quiescence or the proliferation of CSCs.

7. Outgrowth from Dormancy

CSC-niche crosstalk is fundamental for outgrowth from dormancy and is affected by the bone microenvironment, which can remove crucial signals able to maintain cells dormant in the metastastic niche. Indeed, the balance between dormancy and CSCs' re-activation depends on microenvironmental factors, which contribute to the outgrowth from the dormancy of CSCs, and their interactions with stromal cells in bone [29]. For instance, stromal cells can be activated by dormant breast CSCs to release niche extracellular matrix components, such as Periostin and Tenascin C, which in turn activate the crucial stem-cell signalling pathways such as Wnt, Nanog and Oct4, fostering proliferation and outgrowth from the dormancy of CSCs [23,172]. In the tumor microenvironment, fibronectin and type I collagen were reported to stimulate ERK/MAPK and Src driven proliferation, shifting tumor cells from dormant to proliferative via β1-integrin stimulation [30–32]. Specifically, in the BM, multiple myeloma cell dormancy has been described as a reversible state, which is switched on by OBs and switched off by OCs remodelling the endosteal niche [171]. Indeed, OCs remodel the surface of the endosteal niche releasing dormant myeloma cells and favouring their outgrowth from dormancy [171]. A similar mechanism of OC-mediated escape from dormancy has been reported in breast-cancer cells that aberrantly express the vascular cell-adhesion molecule 1 (VCAM-1), recruit α4β1 OC precursors by engaging in VCAM-1/α4β1 binding to increase Oct activity, and stimulate tumor growth [166]. The physiological bone remodelling, through the release of different molecules, provides a fertile soil for the growth of tumor cells, such as TGF-β, IGF I and II, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and bone morphogenetic proteins (BMPs) [33,34]. It is also responsible for the high extracellular Ca^{2+} concentration, which can bind to calcium-sensing receptors and stimulate PTH-related peptide (PTHrP) in re-activated CSCs, promoting tumor-cell proliferation and survival [35,36]. Moreover, physical factors such as acid pH, hypoxia and high extracellular calcium concentration contribute to CSCs' awakening and, thus, metastasis formation in breast cancer [166]. Proliferating tumor cells can secrete prostaglandins, PTH, activated vitamin D, IL-6 and tumor necrosis factor (TNF), leading to an increase in RANKL expression on OBs and BM stromal cells [34], which stimulates OC numbers, survival and activity. Among the factors and pathways that regulate the progression of CSCs to bone metastases there are also VCAM1, tumor-induced OC miRNAs and Jagged1 [37–39]. In particular, Jagged1 is a ligand of Notch, one of the most important downstream mediators of the pro-metastatic TGF-β, that directly activates OC differentiation and promotes tumor growth, stimulating IL-6 production by OBs [40].

Recent data reported that tumor-derived microvescicles, such as exosomes, are also involved in metastasis formation. They contain integrins, which mediate the homing of metastatic cells to specific secondary organs [41], and they can transfer oncogenic proteins and nucleic acids, modulating the fate of tumor cells and target organs [42–44].

8. Targeting CSCs to Block Bone-Metastasis Formation

Since CSCs play a fundamental role in promoting bone metastases, they represent a target for novel or combined anti-cancer therapies. Indeed, targeting and eliminating CSCs is an approach largely envisioned to avoid cancer dissemination, disease relapse and thus the development of bone metastases. Methods to target CSCs may be represented by drugs inhibiting CSC-specific signalling pathways, compounds targeting alterations in CSC metabolism, methods to induce differentiation or a loss of stemness, and immunotherapy directed at CSC markers [45].

Different groups have focused their efforts on studies aimed at discovering new targeted CSCs therapies. For instance, BR-DIM, a cruciferous vegetable metabolite added to the prostate cancer cell cultures, was proved to inhibit self-renewal ability of CSCs [46], suggesting that this treatment may induce CSC terminal differentiation and prevent therapeutic resistance.

Another successful approach has recently been reported by Cuyas et al. for aggressive forms of breast cancer: they showed that in breast cancer, a dysregulation of the OPG/RANK/RANKL signalling axis is present and is associated with the presence of CSCs' highly expressing RANK, residing in the pre-metastatic niche and able to initiate metastasis including bone lesions [47–49]. Moreover, in these tumors, bone metastases are also promoted by the low levels of OPG and high levels of RANKL. It is known that treatment with denosumab, an anti-RANKL antibody, reduces the mammosphere-forming ability in BRCA1-mutated epithelial cells, in triple negative and in HER2-overexpressing breast-cancer cells, supporting the notion that tumor and cancer initiating cells have hyperactive RANKL signalling in primary and metastatic sites such as bone. Moreover, the combination with metformin, an anti-diabetic molecule, with denosumab, showed a synergistic action of the two drugs in reducing breast CSCs [49].

Our group showed that renal CSCs expressing CD105 and c-MET can directly form osteolytic bone lesions, and a selective c-MET inhibitor treatment abrogated bone metastases' development. Those data suggest that c-MET expression on renal CSCs drives renal cancer progression to bone, thus HGF/c-MET signalling is relevant in the metastatic bone process induced by these CSCs [50]. Based on previous studies on breast cancer reporting that CD44$^+$ CSCs expressed also the oncogene c-MET [114], and its inhibition blocked bone metastases from breast cancer [51], we tested a selective c-MET inhibitor. We demonstrated it was able to inhibit the stimulatory activity of renal CSCs on OCs, likely interfering with paracrine factors produced by CSCs, which promoted OC bone resorption. On the other hand, we observed that it stimulated OBs, suggesting us to conclude that this inhibitor exerted both effects on bone cells and CSCs.

In addition, inhibitors of CXCR4, such as CTCE-9908, a small peptide analogue of SDF-1, have been demonstrated to be effective in preventing metastatic dissemination in different cancer models such as breast, oesophageal and prostate [52–55], by acting both on CSCs and on proliferating tumor cells. In particular in NSCLC, CXCR4 targeting was able to counteract the chemotherapy-induced metastatic spread of chemoresistant fractions of CD133$^+$CXCR4$^+$ CSCs, pointing at combination therapy with CXCR4 inhibitor as an attractive novel strategy to improve neo-adjuvant and adjuvant therapy for these tumors [20]. In a prostate-cancer model, the use of a neutralizing antibody to CXCR4 also reduced the growth of prostate cancer cells injected intra-tibia and the subsequent formation of bone metastases [13].

Other possible pathways of interest include Akt activation and Erk signalling [8,56], which may be up-regulated in CSCs in comparison with the bulk-tumor population and responsible for enhanced CSC survival. In addition, new small molecule inhibitors are under development; they target signalling pathways and transcription factors prevalent in CSCs but not in normal cells [57]. Moreover,

CSC-targeted interventions, combination therapies could also target simultaneously several types of cells and pathways, such as bulk-tumor cells, CSCs in the niche, tumor-associated macrophages, tumor microenvironment or the adaptive immune system [45].

Lastly, CSC markers can be considered as immune targets [58], but examination of CSC markers is needed since some markers may identify both normal stem cells and CSCs, such as Trop2 and α6 integrin [59,60], causing adverse events in clinical trials.

9. Conclusions

The importance of targeting CSCs to avoid tumor metastases has emerged as a prominent concern. Thus, an integrated approach with the development of ex vivo and in vivo models should lead to further characterization of the CSCs. High-resolution imaging technology together with the identification of stromal markers will improve our understanding of CSCs in order to more accurately recapitulate the niche of tumorigenic cells and address novel mechanisms that operate in CSCs, especially during tumor progression. Moreover, continuous investigation of CSC markers is needed, since some markers may identify both normal stem cells and CSCs and cause adverse events in clinical trials. Lastly, many efforts are also advocated on the discovery of novel procedures to isolate, identify and enrich for CSCs, with the final goal of monitoring the disease in terms of prevention of tumor progression and resistance to treatments.

Conflicts of Interest: The authors have no conflict of interest to declare.

References

1. Mundy, G.R. Metastasis to bone: Causes, consequences and therapeutic opportunities. *Nat. Rev. Cancer* **2002**, *2*, 584–593. [CrossRef] [PubMed]

2. Scimeca, M.; Bonfiglio, R.; Montanaro, M.; Bonanno, E. Osteoblast-like cells in human cancers: New cell type and reliable markers for bone metastasis. *Future Oncol.* **2018**, *14*, 9–11. [CrossRef] [PubMed]

3. Stucci, S.; Tucci, M.; Passarelli, A.; Silvestris, F. Avbeta3 integrin: Pathogenetic role in osteotropic tumors. *Crit. Rev. Ncol. Hematol.* **2015**, *96*, 183–193. [CrossRef] [PubMed]

4. D'Amico, L.; Patane, S.; Grange, C.; Bussolati, B.; Isella, C.; Fontani, L.; Godio, L.; Cilli, M.; D'Amelio, P.; Isaia, G.; et al. Primary breast cancer stem-like cells metastasise to bone, switch phenotype and acquire a bone tropism signature. *Br. J. Cancer* **2013**, *108*, 2525–2536.

5. Liu, S.; Cong, Y.; Wang, D.; Sun, Y.; Deng, L.; Liu, Y.; Martin-Trevino, R.; Shang, L.; McDermott, S.P.; Landis, M.D.; et al. Breast cancer stem cells transition between epithelial and mesenchymal states reflective of their normal counterparts. *Stem Cell Rep.* **2014**, *2*, 78–91. [CrossRef] [PubMed]

6. Gupta, P.B.; Fillmore, C.M.; Jiang, G.; Shapira, S.D.; Tao, K.; Kuperwasser, C.; Lander, E.S. Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells. *Cell* **2011**, *146*, 633–644. [CrossRef] [PubMed]

7. Harris, M.A.; Yang, H.; Low, B.E.; Mukherjee, J.; Guha, A.; Bronson, R.T.; Shultz, L.D.; Israel, M.A.; Yun, K. Cancer stem cells are enriched in the side population cells in a mouse model of glioma. *Cancer Res.* **2008**, *68*, 10051–10059. [CrossRef] [PubMed]

8. Darash-Yahana, M.; Pikarsky, E.; Abramovitch, R.; Zeira, E.; Pal, B.; Karplus, R.; Beider, K.; Avniel, S.; Kasem, S.; Galun, E.; et al. Role of high expression levels of CXCR4 in tumor growth, vascularization, and metastasis. *FASEB J.* **2004**, *18*, 1240–1242. [CrossRef] [PubMed]

9. Kucia, M.; Reca, R.; Miekus, K.; Wanzeck, J.; Wojakowski, W.; Janowska-Wieczorek, A.; Ratajczak, J.; Ratajczak, M.Z. Trafficking of normal stem cells and metastasis of cancer stem cells involve similar mechanisms: Pivotal role of the SDF-1-CXCR4 axis. *Stem Cells* **2005**, *23*, 879–894. [CrossRef] [PubMed]

10. Orimo, A.; Gupta, P.B.; Sgroi, D.C.; Arenzana-Seisdedos, F.; Delaunay, T.; Naeem, R.; Carey, V.J.; Richardson, A.L.; Weinberg, R.A. Stromal fibroblasts present in invasive human breast carcinomas promote

tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* **2005**, *121*, 335–348. [CrossRef] [PubMed]

11. Xu, C.; Zhao, H.; Chen, H.; Yao, Q. CXCR4 in breast cancer: Oncogenic role and therapeutic targeting. *Drug Des. Devel. Ther.* **2015**, *9*, 4953–4964. [PubMed]

12. Dontu, G.; Al-Hajj, M.; Abdallah, W.M.; Clarke, M.F.; Wicha, M.S. Stem cells in normal breast development and breast cancer. *Cell Prolif.* **2003**, *36*, 59–72. [CrossRef] [PubMed]

13. Sun, Y.X.; Wang, J.; Shelburne, C.E.; Lopatin, D.E.; Chinnaiyan, A.M.; Rubin, M.A.; Pienta, K.J.; Taichman, R.S. Expression of CXCR4 AND CXCL12 (SDF-1) in human prostate cancers (PCA) in vivo. *J. Cell Biochem.* **2003**, *89*, 462–473. [CrossRef] [PubMed]

14. Muller, A.; Homey, B.; Soto, H.; Ge, N.; Catron, D.; Buchanan, M.E.; McClanahan, T.; Murphy, E.; Yuan, W.; Wagner, S.N.; et al. Involvement of chemokine receptors in breast cancer metastasis. *Nature* **2001**, *410*, 50–56. [CrossRef] [PubMed]

15. Broxmeyer, H.E.; Orschell, C.M.; Clapp, D.W.; Hangoc, G.; Cooper, S.; Plett, P.A.; Liles, W.C.; Li, X.; Graham-Evans, B.; Campbell, T.B.; et al. Rapid mobilization of murine and human hematopoietic stem and progenitor cells with amd3100, a cxcr4 antagonist. *J. Exp. Med.* **2005**, *201*, 1307–1318. [CrossRef] [PubMed]

16. Jung, M.J.; Rho, J.K.; Kim, Y.M.; Jung, J.E.; Jin, Y.B.; Ko, Y.G.; Lee, J.S.; Lee, S.J.; Lee, J.C.; Park, M.J. Upregulation of CXCR4 is functionally crucial for maintenance of stemness in drug-resistant non-small cell lung cancer cells. *Oncogene* **2013**, *32*, 209–221. [CrossRef] [PubMed]

17. Bertolini, G.; Roz, L.; Perego, P.; Tortoreto, M.; Fontanella, E.; Gatti, L.; Pratesi, G.; Fabbri, A.; Andriani, F.; Tinelli, S.; et al. Highly tumorigenic lung cancer CD133+ cells display stem-like featuRes. and are spared by cisplatin treatment. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 16281–16286. [CrossRef] [PubMed]

18. Li, X.; Li, P.; Chang, Y.; Xu, Q.; Wu, Z.; Ma, Q.; Wang, Z. The sdf-1/cxcr4 axis induces epithelial-mesenchymal transition in hepatocellular carcinoma. *Mol. Cell Biochem.* **2014**, *392*, 77–84. [CrossRef] [PubMed]

19. Hu, T.H.; Yao, Y.; Yu, S.; Han, L.L.; Wang, W.J.; Guo, H.; Tian, T.; Ruan, Z.P.; Kang, X.M.; Wang, J.; et al. SDF-1/CXCR4 promotes epithelial-mesenchymal transition and progression of colorectal cancer by activation of the wnt/beta-catenin signaling pathway. *Cancer Lett.* **2014**, *354*, 417–426. [CrossRef] [PubMed]

20. Bertolini, G.; D'Amico, L.; Moro, M.; Landoni, E.; Perego, P.; Miceli, R.; Gatti, L.; Andriani, F.; Wong, D.; Caserini, R.; et al. Microenvironment-modulated metastatic CD133+/CXCR4+/EpCAM- lung cancer-initiating cells sustain tumor dissemination and correlate with poor prognosis. *Cancer Res.* **2015**, *75*, 3636–3649. [CrossRef] [PubMed]

21. Kang, Y.; Siegel, P.M.; Shu, W.; Drobnjak, M.; Kakonen, S.M.; Cordon-Cardo, C.; Guise, T.A.; Massague, J. A multigenic program mediating breast cancer metastasis to bone. *Cancer Cell* **2003**, *3*, 537–549. [CrossRef]

22. Kuo, M.C.; Kothari, A.N.; Kuo, P.C.; Mi, Z. Cancer stemness in bone marrow micrometastases of human breast cancer. *Surgery* **2018**, *163*, 330–335. [CrossRef] [PubMed]

23. Cui, D.; Huang, Z.; Liu, Y.; Ouyang, G. The multifaceted role of periostin in priming the tumor microenvironments for tumor progression. *Cell. Mol. Life Sci.* **2017**, *74*, 4287–4291. [CrossRef] [PubMed]

24. Lambert, A.W.; Wong, C.K.; Ozturk, S.; Papageorgis, P.; Raghunathan, R.; Alekseyev, Y.; Gower, A.C.; Reinhard, B.M.; Abdolmaleky, H.M.; Thiagalingam, S. Tumor cell-derived periostin regulates cytokines that maintain breast cancer stem cells. *Mol. Cancer Res.* **2016**, *14*, 103–113. [CrossRef] [PubMed]

25. Fluegen, G.; Avivar-Valderas, A.; Wang, Y.; Padgen, M.R.; Williams, J.K.; Nobre, A.R.; Calvo, V.; Cheung, J.F.; Bravo-Cordero, J.J.; Entenberg, D.; et al. Phenotypic heterogeneity of disseminated tumour cells is preset by primary tumour hypoxic microenvironments. *Nat. Cell Biol.* **2017**, *19*, 120–132. [CrossRef] [PubMed]

26. Crane, G.M.; Jeffery, E.; Morrison, S.J. Adult haematopoietic stem cell niches. *Nat. Rev. Immunol.* **2017**, *17*, 573–590. [CrossRef] [PubMed]

27. Calabrese, C.; Poppleton, H.; Kocak, M.; Hogg, T.L.; Fuller, C.; Hamner, B.; Oh, E.Y.; Gaber, M.W.; Finklestein, D.; Allen, M.; et al. A perivascular niche for brain tumor stem cells. *Cancer Cell* **2007**, *11*, 69–82. [CrossRef] [PubMed]

28. Ghajar, C.M.; Peinado, H.; Mori, H.; Matei, I.R.; Evason, K.J.; Brazier, H.; Almeida, D.; Koller, A.; Hajjar, K.A.; Stainier, D.Y.; et al. The perivascular niche regulates breast tumour dormancy. *Nat. Cell Biol.* **2013**, *15*, 807–817. [CrossRef] [PubMed]

29. Oskarsson, T.; Batlle, E.; Massague, J. Metastatic stem cells: Sources, niches, and vital pathways. *Cell Stem Cell* **2014**, *14*, 306–321. [CrossRef] [PubMed]

30. Aguirre-Ghiso, J.A.; Liu, D.; Mignatti, A.; Kovalski, K.; Ossowski, L. Urokinase receptor and fibronectin regulate the ERK(MAPK) to p38(MAPK) activity ratios that determine carcinoma cell proliferation or dormancy in vivo. *Mol. Biol. Cell* **2001**, *12*, 863–879. [CrossRef] [PubMed]

31. Barkan, D.; Kleinman, H.; Simmons, J.L.; Asmussen, H.; Kamaraju, A.K.; Hoenorhoff, M.J.; Liu, Z.Y.; Costes, S.V.; Cho, E.H.; Lockett, S.; et al. Inhibition of metastatic outgrowth from single dormant tumor cells by targeting the cytoskeleton. *Cancer Res.* **2008**, *68*, 6241–6250. [CrossRef] [PubMed]

32. Barkan, D.; El Touny, L.H.; Michalowski, A.M.; Smith, J.A.; Chu, I.; Davis, A.S.; Webster, J.D.; Hoover, S.; Simpson, R.M.; Gauldie, J.; et al. Metastatic growth from dormant cells induced by a col-i-enriched fibrotic environment. *Cancer Res.* **2010**, *70*, 5706–5716. [CrossRef] [PubMed]

33. Bussard, K.M.; Gay, C.V.; Mastro, A.M. The bone microenvironment in metastasis; what is special about bone? *Cancer Metastasis Rev.* **2008**, *27*, 41–55. [CrossRef] [PubMed]

34. Roodman, G.D. Mechanisms of bone metastasis. *N. Engl. J. Med.* **2004**, *350*, 1655–1664. [CrossRef] [PubMed]

35. Kim, W.; Takyar, F.M.; Swan, K.; Jeong, J.; VanHouten, J.; Sullivan, C.; Dann, P.; Yu, H.; Fiaschi-Taesch, N.; Chang, W.; et al. Calcium-sensing receptor promotes breast cancer by stimulating intracrine actions of parathyroid hormone-related protein. *Cancer Res.* **2016**, *76*, 5348–5360. [CrossRef] [PubMed]

36. Sanders, J.L.; Chattopadhyay, N.; Kifor, O.; Yamaguchi, T.; Butters, R.R.; Brown, E.M. Extracellular calcium-sensing receptor expression and its potential role in regulating parathyroid hormone-related peptide secretion in human breast cancer cell lines. *Endocrinology* **2000**, *141*, 4357–4364. [CrossRef] [PubMed]

37. Lou, Y.; McDonald, P.C.; Oloumi, A.; Chia, S.; Ostlund, C.; Ahmadi, A.; Kyle, A.; Auf dem Keller, U.; Leung, S.; Huntsman, D.; et al. Targeting tumor hypoxia: Suppression of breast tumor growth and metastasis by novel carbonic anhydrase ix inhibitors. *Cancer Res.* **2011**, *71*, 3364–3376. [CrossRef] [PubMed]

38. Ell, B.; Mercatali, L.; Ibrahim, T.; Campbell, N.; Schwarzenbach, H.; Pantel, K.; Amadori, D.; Kang, Y. Tumor-induced osteoclast mirna changes as regulators and biomarkers of osteolytic bone metastasis. *Cancer Cell* **2013**, *24*, 542–556. [CrossRef] [PubMed]

39. Bednarz-Knoll, N.; Efstathiou, A.; Gotzhein, F.; Wikman, H.; Mueller, V.; Kang, Y.; Pantel, K. Potential involvement of jagged1 in metastatic progression of human breast carcinomas. *Clin. Chem.* **2016**, *62*, 378–386. [CrossRef] [PubMed]

40. Sethi, N.; Dai, X.; Winter, C.G.; Kang, Y. Tumor-derived jagged1 promotes osteolytic bone metastasis of breast cancer by engaging notch signaling in bone cells. *Cancer Cell* **2011**, *19*, 192–205. [CrossRef] [PubMed]

41. Hoshino, A.; Costa-Silva, B.; Shen, T.L.; Rodrigues, G.; Hashimoto, A.; Tesic Mark, M.; Molina, H.; Kohsaka, S.; Di Giannatale, A.; Ceder, S.; et al. Tumour exosome integrins determine organotropic metastasis. *Nature* **2015**, *527*, 329–335. [CrossRef] [PubMed]

42. Dos Anjos Pultz, B.; Andrés Cordero da Luz, F.; Socorro Faria, S.; Peixoto Ferreira de Souza, L.; Cristina Brígido Tavares, P.; Alonso Goulart, V.; Fontes, W.; Ricardo Goulart, L.; José Barbosa Silva, M. The multifaceted role of extracellular vesicles in metastasis: Priming the soil for seeding. *Int. J. Cancer* **2017**, *140*, 2397–2407. [CrossRef] [PubMed]

43. Bigagli, E.; Luceri, C.; Guasti, D.; Cinci, L. Exosomes secreted from human colon cancer cells influence the adhesion of neighboring metastatic cells: Role of microrna-210. *Cancer Biol. Ther.* **2016**, *17*, 1062–1069. [CrossRef] [PubMed]

44. Wang, Z.; von Au, A.; Schnolzer, M.; Hackert, T.; Zoller, M. CD44v6-competent tumor exosomes promote motility, invasion and cancer-initiating cell marker expression in pancreatic and colorectal cancer cells. *Oncotarget* **2016**, *7*, 55409–55436. [CrossRef] [PubMed]

45. Pattabiraman, D.R.; Weinberg, R.A. Tackling the cancer stem cells—-What challenges do they pose? *Nat. Rev. Drug Discov.* **2014**, *13*, 497–512. [CrossRef] [PubMed]

46. Kong, D.; Heath, E.; Chen, W.; Cher, M.L.; Powell, I.; Heilbrun, L.; Li, Y.; Ali, S.; Sethi, S.; Hassan, O.; et al. Loss of LET-7 up-regulates EZH2 in prostate cancer consistent with the acquisition of cancer stem cell signatuRes. that are attenuated by br-dim. *PLoS ONE* **2012**, *7*, e33729. [CrossRef] [PubMed]

47. Sigl, V.; Owusu-Boaitey, K.; Joshi, P.A.; Kavirayani, A.; Wirnsberger, G.; Novatchkova, M.; Kozieradzki, I.; Schramek, D.; Edokobi, N.; Hersl, J.; et al. Rankl/rank control BRCA1 mutation-driven mammary tumors. *Cell Res.* **2016**, *26*, 761–774. [CrossRef] [PubMed]

48. Nolan, E.; Vaillant, F.; Branstetter, D.; Pal, B.; Giner, G.; Whitehead, L.; Lok, S.W.; Mann, G.B.; Rohrbach, K.; Huang, L.Y.; et al. Rank ligand as a potential target for breast cancer prevention in BRCA1-mutation carriers. *Nat. Med.* **2016**, *22*, 933–939. [CrossRef] [PubMed]

49. Cuyas, E.; Corominas-Faja, B.; Martin, M.M.; Martin-Castillo, B.; Lupu, R.; Brunet, J.; Bosch-Barrera, J.; Menendez, J.A. BRCA1 haploinsufficiency cell-autonomously activates rankl expression and generates denosumab-responsive breast cancer-initiating cells. *Oncotarget* **2017**, *8*, 35019–35032. [CrossRef] [PubMed]

50. D'Amico, L.; Belisario, D.; Migliardi, G.; Grange, C.; Bussolati, B.; D'Amelio, P.; Perera, T.; Dalmasso, E.; Dalle Carbonare, L.; Godio, L.; et al. C-MET inhibition blocks bone metastasis development induced by renal cancer stem cells. *Oncotarget* **2016**, *7*, 45525–45537. [CrossRef] [PubMed]

51. Previdi, S.; Abbadessa, G.; Dalo, F.; France, D.S.; Broggini, M. Breast cancer-derived bone metastasis can be effectively reduced through specific c-MET inhibitor tivantinib (ARQ 197) and shRNA c-MET knockdown. *Mol. Cancer Ther.* **2012**, *11*, 214–223. [CrossRef] [PubMed]

52. Huang, E.H.; Singh, B.; Cristofanilli, M.; Gelovani, J.; Wei, C.; Vincent, L.; Cook, K.R.; Lucci, A. A cxcr4 antagonist ctce-9908 inhibits primary tumor growth and metastasis of breast cancer. *J. Surg. Res.* **2009**, *155*, 231–236. [CrossRef] [PubMed]

53. Richert, M.M.; Vaidya, K.S.; Mills, C.N.; Wong, D.; Korz, W.; Hurst, D.R.; Welch, D.R. Inhibition of CXCR4 by ctce-9908 inhibits breast cancer metastasis to lung and bone. *Oncol. Rep.* **2009**, *21*, 761–767. [PubMed]

54. Drenckhan, A.; Kurschat, N.; Dohrmann, T.; Raabe, N.; Koenig, A.M.; Reichelt, U.; Kaifi, J.T.; Izbicki, J.R.; Gros, S.J. Effective inhibition of metastases and primary tumor growth with CTCE-9908 in esophageal cancer. *J. Surg. Res.* **2013**, *182*, 250–256. [CrossRef] [PubMed]

55. Porvasnik, S.; Sakamoto, N.; Kusmartsev, S.; Eruslanov, E.; Kim, W.J.; Cao, W.; Urbanek, C.; Wong, D.; Goodison, S.; Rosser, C.J. Effects of cxcr4 antagonist ctce-9908 on prostate tumor growth. *Prostate* **2009**, *69*, 1460–1469. [CrossRef] [PubMed]

56. Dubrovska, A.; Elliott, J.; Salamone, R.J.; Telegeev, G.D.; Stakhovsky, A.E.; Schepotin, I.B.; Yan, F.; Wang, Y.; Bouchez, L.C.; Kularatne, S.A.; et al. Cxcr4 expression in prostate cancer progenitor cells. *PLoS ONE* **2012**, *7*, e31226. [CrossRef] [PubMed]

57. Li, Y.; Rogoff, H.A.; Keates, S.; Gao, Y.; Murikipudi, S.; Mikule, K.; Leggett, D.; Li, W.; Pardee, A.B.; Li, C.J. Suppression of cancer relapse and metastasis by inhibiting cancer stemness. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 1839–1844. [CrossRef] [PubMed]

58. Deng, Z.; Wu, Y.; Ma, W.; Zhang, S.; Zhang, Y.Q. Adoptive T-cell therapy of prostate cancer targeting the cancer stem cell antigen EpCAM. *BMC Immunol.* **2015**, *16*, 1. [CrossRef] [PubMed]

59. Goldstein, A.S.; Lawson, D.A.; Cheng, D.; Sun, W.; Garraway, I.P.; Witte, O.N. Trop2 identifies a subpopulation of murine and human prostate basal cells with stem cell characteristics. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 20882–20887. [CrossRef] [PubMed]

60. Hofner, T.; Eisen, C.; Klein, C.; Rigo-Watermeier, T.; Goeppinger, S.M.; Jauch, A.; Schoell, B.; Vogel, V.; Noll, E.; Weichert, W.; et al. Defined conditions for the isolation and expansion of basal prostate progenitor cells of mouse and human origin. *Stem Cell Rep.* **2015**, *4*, 503–518. [CrossRef] [PubMed]

61. Owen, K.L.; Parker, B.S. Beyond the vicious cycle: The role of innate osteoimmunity, automimicry and tumor-inherent changes in dictating bone metastasis. *Mol. Immunol.* **2017**. [CrossRef] [PubMed]

62. Sekita, A.; Matsugaki, A.; Nakano, T. Disruption of collagen/apatite alignment impairs bone mechanical function in osteoblastic metastasis induced by prostate cancer. *Bone* **2017**, *97*, 83–93. [CrossRef] [PubMed]

63. Sekita, A.; Matsugaki, A.; Ishimoto, T.; Nakano, T. Synchronous disruption of anisotropic arrangement of the osteocyte network and collagen/apatite in melanoma bone metastasis. *J. Struct. Biol.* **2017**, *197*, 260–270. [CrossRef] [PubMed]

64. Kimura, Y.; Matsugaki, A.; Sekita, A.; Nakano, T. Alteration of osteoblast arrangement via direct attack by cancer cells: New insights into bone metastasis. *Sci. Rep.* **2017**, *7*, 44824. [CrossRef] [PubMed]

65. Ross, J.S.; Slodkowska, E.A. Circulating and disseminated tumor cells in the management of breast cancer. *Am. J. Clin. Pathol.* **2009**, *132*, 237–245. [CrossRef] [PubMed]

66. Pantel, K.; Alix-Panabieres, C. Bone marrow as a reservoir for disseminated tumor cells: A special source for liquid biopsy in cancer patients. *Bonekey Rep.* **2014**, *3*, 584. [CrossRef] [PubMed]

67. Yang, Z.F.; Ngai, P.; Ho, D.W.; Yu, W.C.; Ng, M.N.; Lau, C.K.; Li, M.L.; Tam, K.H.; Lam, C.T.; Poon, R.T.; et al. Identification of local and circulating cancer stem cells in human liver cancer. *Hepatology* **2008**, *47*, 919–928. [CrossRef] [PubMed]

68. Williams, K.; Motiani, K.; Giridhar, P.V.; Kasper, S. CD44 integrates signaling in normal stem cell, cancer stem cell and (pre)metastatic niches. *Exp. Biol. Med.* **2013**, *238*, 324–338. [CrossRef] [PubMed]

69. Ugolkov, A.V.; Eisengart, L.J.; Luan, C.; Yang, X.J. Expression analysis of putative stem cell markers in human benign and malignant prostate. *Prostate* **2011**, *71*, 18–25. [CrossRef] [PubMed]

70. Al-Hajj, M.; Wicha, M.S.; Benito-Hernandez, A.; Morrison, S.J.; Clarke, M.F. Prospective identification of tumorigenic breast cancer cells. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 3983–3988. [CrossRef] [PubMed]

71. Bae, K.; Su, Z.; Frye, C.; McClellan, S.; Allan, R.W.; Andrejewski, J.T.; Kelley, V.; Jorgensen, M.; Steindler, D.A.; Vieweg, J.; et al. Expression of pluripotent stem cell reprogramming factors by prostate tumor initiating cells. *J. Urol.* **2010**, *183*, 2045–2053. [CrossRef] [PubMed]

72. Rhodes, D.R.; Sanda, M.G.; Otte, A.P.; Chinnaiyan, A.M.; Rubin, M.A. Multiplex biomarker approach for determining risk of prostate-specific antigen-defined recurrence of prostate cancer. *J. Natl. Cancer Inst.* **2003**, *95*, 661–668. [CrossRef] [PubMed]

73. Brusgard, J.L.; Choe, M.; Chumsri, S.; Renoud, K.; MacKerell, A.D., Jr.; Sudol, M.; Passaniti, A. RUNX2 and TAZ-dependent signaling pathways regulate soluble E-Cadherin levels and tumorsphere formation in breast cancer cells. *Oncotarget* **2015**, *6*, 28132–28150. [CrossRef] [PubMed]

74. Zhao, M.; Zhang, Y.; Zhang, H.; Wang, S.; Zhang, M.; Chen, X.; Wang, H.; Zeng, G.; Chen, X.; Liu, G.; et al. Hypoxia-induced cell stemness leads to drug resistance and poor prognosis in lung adenocarcinoma. *Lung Cancer* **2015**, *87*, 98–106. [CrossRef] [PubMed]

75. Zhang, W.C.; Shyh-Chang, N.; Yang, H.; Rai, A.; Umashankar, S.; Ma, S.; Soh, B.S.; Sun, L.L.; Tai, B.C.; Nga, M.E.; et al. Glycine decarboxylase activity drives non-small cell lung cancer tumor-initiating cells and tumorigenesis. *Cell* **2012**, *148*, 259–272. [CrossRef] [PubMed]

76. Thalgott, M.; Rack, B.; Maurer, T.; Souvatzoglou, M.; Eiber, M.; Kreß, V.; Heck, M.M.; Andergassen, U.; Nawroth, R.; Gschwend, J.E.; et al. Detection of circulating tumor cells in different stages of prostate cancer. *J. Cancer Res. Clin. Oncol.* **2013**, *139*, 755–763. [CrossRef] [PubMed]

77. Yamashita, T.; Ji, J.; Budhu, A.; Forgues, M.; Yang, W.; Wang, H.Y.; Jia, H.; Ye, Q.; Qin, L.X.; Wauthier, E.; et al. EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. *Gastroenterology* **2009**, *136*, 1012–1024. [CrossRef] [PubMed]

78. Aya-Bonilla, C.A.; Marsavela, G.; Freeman, J.B.; Lomma, C.; Frank, M.H.; Khattak, M.A.; Meniawy, T.M.; Millward, M.; Warkiani, M.E.; Gray, E.S.; et al. Isolation and detection of circulating tumour cells from metastatic melanoma patients using a slanted spiral microfluidic device. *Oncotarget* **2017**, *8*, 67355–67368. [CrossRef] [PubMed]

79. Wilson, B.J.; Schatton, T.; Zhan, Q.; Gasser, M.; Ma, J.; Saab, K.R.; Schanche, R.; Waaga-Gasser, A.M.; Gold, J.S.; Huang, Q.; et al. ABCB5 identifies a therapy-refractory tumor cell population in colorectal cancer patients. *Cancer Res.* **2011**, *71*, 5307–5316. [CrossRef] [PubMed]

80. Schatton, T.; Murphy, G.F.; Frank, N.Y.; Yamaura, K.; Waaga-Gasser, A.M.; Gasser, M.; Zhan, Q.; Jordan, S.; Duncan, L.M.; Weishaupt, C.; et al. Identification of cells initiating human melanomas. *Nature* **2008**, *451*, 345–349. [CrossRef] [PubMed]

81. Setia, N.; Abbas, O.; Sousa, Y.; Garb, J.L.; Mahalingam, M. Profiling of ABC transporters ABCB5, ABCF2 and nestin-positive stem cells in nevi, in situ and invasive melanoma. *Mod. Pathol.* **2012**, *25*, 1169–1175. [CrossRef] [PubMed]

82. Cheung, S.T.; Cheung, P.F.; Cheng, C.K.; Wong, N.C.; Fan, S.T. Granulin-epithelin precursor and ATP-dependent binding cassette (ABC)B5 regulate liver cancer cell chemoresistance. *Gastroenterology* **2011**, *140*, 344–355. [CrossRef] [PubMed]

83. Cojoc, M.; Mäbert, K.; Muders, M.H.; Dubrovska, A. A role for cancer stem cells in therapy resistance: cellular and molecular mechanisms. *Semin. Cancer Biol.* **2015**, *31*, 16–27. [CrossRef] [PubMed]

84. Gottesman, M.M.; Fojo, T.; Bates, S.E. Multidrug resistance in cancer: Role of ATP-dependent transporters. *Nat. Rev. Cancer* **2002**, *2*, 48–58. [CrossRef] [PubMed]

85. Zhou, S.; Schuetz, J.D.; Bunting, K.D.; Colapietro, A.M.; Sampath, J.; Morris, J.J.; Lagutina, I.; Grosveld, G.C.; Osawa, M.; Nakauchi, H.; et al. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nat. Med.* **2001**, *7*, 1028–1034. [CrossRef] [PubMed]

86. Li, T.; Su, Y.; Mei, Y.; Leng, Q.; Leng, B.; Liu, Z.; Stass, S.A.; Jiang, F. ALDH1A1 is a marker for malignant prostate stem cells and predictor of prostate cancer patients' outcome. *Lab. Invest.* **2010**, *90*, 234–244. [CrossRef] [PubMed]

87. Marcato, P.; Dean, C.A.; Pan, D.; Araslanova, R.; Gillis, M.; Joshi, M.; Helyer, L.; Pan, L.; Leidal, A.; Gujar, S.; et al. Aldehyde dehydrogenase activity of breast cancer stem cells is primarily due to isoform ALDH1A3 and its expression is predictive of metastasis. *Stem Cells* **2011**, *29*, 32–45. [CrossRef] [PubMed]

88. Chen, W.; Dong, J.; Haiech, J.; Kilhoffer, M.C.; Zeniou, M. Cancer stem cell quiescence and plasticity as major challenges in cancer therapy. *Stem Cells Int.* **2016**, *2016*, 1740936. [CrossRef] [PubMed]

89. Van den Hoogen, C.; van der Horst, G.; Cheung, H.; Buijs, J.T.; Lippitt, J.M.; Guzmán-Ramírez, N.; Hamdy, F.C.; Eaton, C.L.; Thalmann, G.N.; Cecchini, M.G.; et al. High aldehyde dehydrogenase activity identifies tumor-initiating and metastasis-initiating cells in human prostate cancer. *Cancer Res.* **2010**, *70*, 5163–5173. [CrossRef] [PubMed]

90. Ma, S.; Chan, K.W.; Lee, T.K.; Tang, K.H.; Wo, J.Y.; Zheng, B.J.; Guan, X.Y. Aldehyde dehydrogenase discriminates the CD133 liver cancer stem cell populations. *Mol. Cancer Res.* **2008**, *6*, 1146–1153. [CrossRef] [PubMed]

91. Sullivan, J.P.; Spinola, M.; Dodge, M.; Raso, M.G.; Behrens, C.; Gao, B.; Schuster, K.; Shao, C.; Larsen, J.E.; Sullivan, L.A.; et al. Aldehyde dehydrogenase activity selects for lung adenocarcinoma stem cells dependent on notch signaling. *Cancer Res.* **2010**, *70*, 9937–9948. [CrossRef] [PubMed]

92. Ueda, K.; Ogasawara, S.; Akiba, J.; Nakayama, M.; Todoroki, K.; Ueda, K.; Sanada, S.; Suekane, S.; Noguchi, M.; Matsuoka, K.; et al. Aldehyde dehydrogenase 1 identifies cells with cancer stem cell-like properties in a human renal cell carcinoma cell line. *PLoS ONE* **2013**, *8*, e75463. [CrossRef] [PubMed]

93. Eramo, A.; Lotti, F.; Sette, G.; Pilozzi, E.; Biffoni, M.; Di Virgilio, A.; Conticello, C.; Ruco, L.; Peschle, C.; de Maria, R. Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell Death Differ.* **2008**, *15*, 504–514. [CrossRef] [PubMed]

94. Justilien, V.; Regala, R.P.; Tseng, I.C.; Walsh, M.P.; Batra, J.; Radisky, E.S.; Murray, N.R.; Fields, A.P. Matrix metalloproteinase-10 is required for lung cancer stem cell maintenance, tumor initiation and metastatic potential. *PLoS ONE* **2012**, *7*, e35040. [CrossRef] [PubMed]

95. Ali, S.A.; Justilien, V.; Jamieson, L.; Murray, N.R.; Fields, A.P. Protein Kinase Cɩ Drives a NOTCH3-dependent Stem-like Phenotype in Mutant KRAS Lung Adenocarcinoma. *Cancer Cell* **2016**, *29*, 367–378. [CrossRef] [PubMed]

96. Song, W.; Li, H.; Tao, K.; Li, R.; Song, Z.; Zhao, Q.; Zhang, F.; Dou, K. Expression and clinical significance of the stem cell marker CD133 in hepatocellular carcinoma. *Int. J. Clin. Pract.* **2008**, *62*, 1212–1218. [CrossRef] [PubMed]

97. Collins, A.T.; Berry, P.A.; Hyde, C.; Stower, M.J.; Maitland, N.J. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res.* **2005**, *65*, 10946–10951. [CrossRef] [PubMed]

98. Haraguchi, N.; Ishii, H.; Mimori, K.; Tanaka, F.; Ohkuma, M.; Kim, H.M.; Akita, H.; Takiuchi, D.; Hatano, H.; Nagano, H.; Barnard, G.F.; Doki, Y.; Mori, M. CD13 is a therapeutic target in human liver cancer stem cells. *J. Clin. Invest.* **2010**, *120*, 3326–3339. [CrossRef] [PubMed]

99. Kim, H.M.; Haraguchi, N.; Ishii, H.; Ohkuma, M.; Okano, M.; Mimori, K.; Eguchi, H.; Yamamoto, H.; Nagano, H.; Sekimoto, M.; et al. Increased CD13 expression reduces reactive oxygen species, promoting survival of liver cancer stem cells via an epithelial-mesenchymal transition-like phenomenon. *Ann. Surg. Oncol.* **2012**, *19*, S539–S548. [CrossRef] [PubMed]

100. Yang, Z.F.; Ho, D.W.; Ng, M.N.; Lau, C.K.; Yu, W.C.; Ngai, P.; Chu, P.W.; Lam, C.T.; Poon, R.T.; Fan, S.T. Significance of CD90+ cancer stem cells in human liver cancer. *Cancer Cell* **2008**, *13*, 153–166. [CrossRef] [PubMed]

101. Cho, R.W.; Wang, X.; Diehn, M.; Shedden, K.; Chen, G.Y.; Sherlock, G.; Gurney, A.; Lewicki, J.; Clarke, M.F. Isolation and molecular characterization of cancer stem cells in MMTV-Wnt-1 murine breast tumors. *Stem Cells* **2008**, *26*, 364–371. [CrossRef] [PubMed]

102. He, J.; Liu, Y.; Zhu, T.; Zhu, J.; Dimeco, F.; Vescovi, A.L.; Heth, J.A.; Muraszko, K.M.; Fan, X.; Lubman, D.M. CD90 is identified as a candidate marker for cancer stem cells in primary high-grade gliomas using tissue microarrays. *Mol. Cell Proteomics* **2012**, *11*, M111.010744. [CrossRef] [PubMed]

103. Bussolati, B.; Bruno, S.; Grange, C.; Ferrando, U.; Camussi, G. Identification of a tumor-initiating stem cell population in human renal carcinomas. *FASEB J.* **2008**, *22*, 3696–3705. [CrossRef] [PubMed]

104. Addla, S.K.; Brown, M.D.; Hart, C.A.; Ramani, V.A.; Clarke, N.W. Characterization of the Hoechst 33342 side population from normal and malignant human renal epithelial cells. *Am. J. Physiol. Renal Physiol.* **2008**, *295*, F680–F687. [CrossRef] [PubMed]

105. Wang, X.; Liu, Y.; Zhou, K.; Zhang, G.; Wang, F.; Ren, J. Isolation and characterization of CD105+/CD90+ subpopulation in breast cancer MDA-MB-231 cell line. *Int. J. Clin. Exp. Pathol.* **2015**, *8*, 5105–5112. [PubMed]

106. Li, Y.; Zhai, Z.; Liu, D.; Zhong, X.; Meng, X.; Yang, Q.; Liu, J.; Li, H. CD105 promotes hepatocarcinoma cell invasion and metastasis through VEGF. *Tumour Biol.* **2015**, *36*, 737–745. [CrossRef] [PubMed]

107. Meacham, C.E.; Morrison, S.J. Tumour heterogeneity and cancer cell plasticity. *Nature* **2013**, *501*, 328–337. [CrossRef] [PubMed]

108. Blanco, S.; Bandiera, R.; Popis, M.; Hussain, S.; Lombard, P.; Aleksic, J.; Sajini, A.; Tanna, H.; Cortes-Garrido, R.; Gkatza, N.; et al. Stem cell function and stress response are controlled by protein synthesis. *Nature* **2016**, *534*, 335–340. [CrossRef] [PubMed]

109. Adams, J.M.; Strasser, A. Is tumor growth sustained by rare cancer stem cells or dominant clones? *Cancer Res.* **2008**, *68*, 4018–4021. [CrossRef] [PubMed]

110. Chen, J.; Li, Y.; Yu, T.S.; McKay, R.M.; Burns, D.K.; Kernie, S.G.; Parada, L.F. A restricted cell population propagates glioblastoma growth after chemotherapy. *Nature* **2012**, *488*, 522–526. [CrossRef] [PubMed]

111. Driessens, G.; Beck, B.; Caauwe, A.; Simons, B.D.; Blanpain, C. Defining the mode of tumour growth by clonal analysis. *Nature* **2012**, *488*, 527–530. [CrossRef] [PubMed]

112. Hermann, P.C.; Huber, S.L.; Herrler, T.; Aicher, A.; Ellwart, J.W.; Guba, M.; Bruns, C.J.; Heeschen, C. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell* **2007**, *1*, 313–323. [CrossRef] [PubMed]

113. Malanchi, I. Tumour cells coerce host tissue to cancer spread. *Bonekey Rep.* **2013**, *2*, 371. [CrossRef] [PubMed]

114. Baccelli, I.; Schneeweiss, A.; Riethdorf, S.; Stenzinger, A.; Schillert, A.; Vogel, V.; Klein, C.; Saini, M.; Bauerle, T.; Wallwiener, M.; et al. Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay. *Nat. Biotechnol.* **2013**, *31*, 539–544. [CrossRef] [PubMed]

115. Kim, R.J.; Nam, J.S. Oct4 expression enhances featuRes. of cancer stem cells in a mouse model of breast cancer. *Lab. Anim. Res.* **2011**, *27*, 147–152. [CrossRef] [PubMed]

116. Shan, J.; Shen, J.; Liu, L.; Xia, F.; Xu, C.; Duan, G.; Xu, Y.; Ma, Q.; Yang, Z.; Zhang, Q.; et al. Nanog regulates self-renewal of cancer stem cells through the insulin-like growth factor pathway in human hepatocellular carcinoma. *Hepatology* **2012**, *56*, 1004–1014. [CrossRef] [PubMed]

117. Boumahdi, S.; Driessens, G.; Lapouge, G.; Rorive, S.; Nassar, D.; Le Mercier, M.; Delatte, B.; Caauwe, A.; Lenglez, S.; Nkusi, E.; et al. Sox2 controls tumour initiation and cancer stem-cell functions in squamous-cell carcinoma. *Nature* **2014**, *511*, 246–250. [CrossRef] [PubMed]

118. Yu, F.; Li, J.; Chen, H.; Fu, J.; Ray, S.; Huang, S.; Zheng, H.; Ai, W. Kruppel-like factor 4 (Klf4) is required for maintenance of breast cancer stem cells and for cell migration and invasion. *Oncogene* **2011**, *30*, 2161–2172. [CrossRef] [PubMed]

119. Reynolds, B.A.; Weiss, S. Clonal and population analyses demonstrate that an egf-responsive mammalian embryonic cns precursor is a stem cell. *Dev. Biol.* **1996**, *175*, 1–13. [CrossRef] [PubMed]

120. Fillmore, C.M.; Gupta, P.B.; Rudnick, J.A.; Caballero, S.; Keller, P.J.; Lander, E.S.; Kuperwasser, C. Estrogen expands breast cancer stem-like cells through paracrine FGF/TBX3 signaling. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 21737–21742. [CrossRef] [PubMed]

121. Babaie, Y.; Herwig, R.; Greber, B.; Brink, T.C.; Wruck, W.; Groth, D.; Lehrach, H.; Burdon, T.; Adjaye, J. Analysis of Oct4-dependent transcriptional networks regulating self-renewal and pluripotency in human embryonic stem cells. *Stem Cells* **2007**, *25*, 500–510. [CrossRef] [PubMed]

122. Jerabek, S.; Merino, F.; Scholer, H.R.; Cojocaru, V. Oct4: Dynamic DNA binding pioneers stem cell pluripotency. *Biochim. Biophys. Acta* **2014**, *1839*, 138–154. [CrossRef] [PubMed]

123. Pan, G.; Li, J.; Zhou, Y.; Zheng, H.; Pei, D. A negative feedback loop of transcription factors that controls stem cell pluripotency and self-renewal. *FASEB J.* **2006**, *20*, 1730–1732. [CrossRef] [PubMed]

124. Lee, S.; Wottrich, S.; Bonavida, B. Crosstalks between Raf-kinase inhibitor protein and cancer stem cell transcription factors (Oct4, Klf4, Sox2, Nanog). *Tumour Biol.* **2017**, *39*, 1010428317692253. [CrossRef] [PubMed]

125. Nowell, P.C. The clonal evolution of tumor cell populations. *Science* **1976**, *194*, 23–28. [CrossRef] [PubMed]

126. Kreso, A.; Dick, J.E. Evolution of the cancer stem cell model. *Cell Stem Cell* **2014**, *14*, 275–291. [CrossRef] [PubMed]

127. Durrett, R.; Foo, J.; Leder, K.; Mayberry, J.; Michor, F. Intratumor heterogeneity in evolutionary models of tumor progression. *Genetics* **2011**, *188*, 461–477. [CrossRef] [PubMed]

128. Yachida, S.; Jones, S.; Bozic, I.; Antal, T.; Leary, R.; Fu, B.; Kamiyama, M.; Hruban, R.H.; Eshleman, J.R.; Nowak, M.A.; et al. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature* **2010**, *467*, 1114–1117. [CrossRef] [PubMed]

129. Campbell, P.J.; Yachida, S.; Mudie, L.J.; Stephens, P.J.; Pleasance, E.D.; Stebbings, L.A.; Morsberger, L.A.; Latimer, C.; McLaren, S.; Lin, M.L.; et al. The patterns and dynamics of genomic instability in metastatic pancreatic cancer. *Nature* **2010**, *467*, 1109–1113. [CrossRef] [PubMed]

130. Chaffer, C.L.; Brueckmann, I.; Scheel, C.; Kaestli, A.J.; Wiggins, P.A.; Rodrigues, L.O.; Brooks, M.; Reinhardt, F.; Su, Y.; Polyak, K.; et al. Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 7950–7955. [CrossRef] [PubMed]

131. Kong, D.; Banerjee, S.; Ahmad, A.; Li, Y.; Wang, Z.; Sethi, S.; Sarkar, F.H. Epithelial to mesenchymal transition is mechanistically linked with stem cell signatuRes. in prostate cancer cells. *PLoS ONE* **2010**, *5*, e12445. [CrossRef] [PubMed]

132. Wellner, U.; Schubert, J.; Burk, U.C.; Schmalhofer, O.; Zhu, F.; Sonntag, A.; Waldvogel, B.; Vannier, C.; Darling, D.; zur Hausen, A.; et al. The EMT-activator zeb1 promotes tumorigenicity by repressing stemness-inhibiting micrornas. *Nat. Cell Biol.* **2009**, *11*, 1487–1495. [CrossRef] [PubMed]

133. Mani, S.A.; Guo, W.; Liao, M.J.; Eaton, E.N.; Ayyanan, A.; Zhou, A.Y.; Brooks, M.; Reinhard, F.; Zhang, C.C.; Shipitsin, M.; et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* **2008**, *133*, 704–715. [CrossRef] [PubMed]

134. Rhim, A.D.; Mirek, E.T.; Aiello, N.M.; Maitra, A.; Bailey, J.M.; McAllister, F.; Reichert, M.; Beatty, G.L.; Rustgi, A.K.; Vonderheide, R.H.; et al. EMT and dissemination precede pancreatic tumor formation. *Cell* **2012**, *148*, 349–361. [CrossRef] [PubMed]

135. Weng, D.; Penzner, J.H.; Song, B.; Koido, S.; Calderwood, S.K.; Gong, J. Metastasis is an early event in mouse mammary carcinomas and is associated with cells bearing stem cell markers. *Breast Cancer Res.* **2012**, *14*, R18. [CrossRef] [PubMed]

136. Balic, M.; Lin, H.; Young, L.; Hawes, D.; Giuliano, A.; McNamara, G.; Datar, R.H.; Cote, R.J. Most early disseminated cancer cells detected in bone marrow of breast cancer patients have a putative breast cancer stem cell phenotype. *Clin. Cancer Res.* **2006**, *12*, 5615–5621. [CrossRef] [PubMed]

137. Aktas, B.; Tewes, M.; Fehm, T.; Hauch, S.; Kimmig, R.; Kasimir-Bauer, S. Stem cell and epithelial-mesenchymal transition markers are frequently overexpressed in circulating tumor cells of metastatic breast cancer patients. *Breast Cancer Res.* **2009**, *11*, R46. [CrossRef] [PubMed]

138. Van der Pluijm, G. Epithelial plasticity, cancer stem cells and bone metastasis formation. *Bone* **2011**, *48*, 37–43. [CrossRef] [PubMed]

139. Dunn, L.K.; Mohammad, K.S.; Fournier, P.G.; McKenna, C.R.; Davis, H.W.; Niewolna, M.; Peng, X.H.; Chirgwin, J.M.; Guise, T.A. Hypoxia and TGF-beta drive breast cancer bone metastases through parallel signaling pathways in tumor cells and the bone microenvironment. *PLoS ONE* **2009**, *4*, e6896. [CrossRef] [PubMed]

140. Buijs, J.T.; Henriquez, N.V.; van Overveld, P.G.; van der Horst, G.; ten Dijke, P.; van der Pluijm, G. Tgf-beta and bmp7 interactions in tumour progression and bone metastasis. *Clin. Exp. Metastasis* **2007**, *24*, 609–617. [CrossRef] [PubMed]

141. Buijs, J.T.; van der Pluijm, G. Osteotropic cancers: From primary tumor to bone. *Cancer Lett.* **2009**, *273*, 177–193. [CrossRef] [PubMed]

142. Ponta, H.; Sherman, L.; Herrlich, P.A. Cd44: From adhesion molecules to signalling regulators. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 33–45. [CrossRef] [PubMed]

143. Cao, J.J.; Singleton, P.A.; Majumdar, S.; Boudignon, B.; Burghardt, A.; Kurimoto, P.; Wronski, T.J.; Bourguignon, L.Y.; Halloran, B.P. Hyaluronan increases rankl expression in bone marrow stromal cells through cd44. *J. Bone Miner. Res.* **2005**, *20*, 30–40. [CrossRef] [PubMed]

144. Ariyoshi, W.; Takahashi, T.; Kanno, T.; Ichimiya, H.; Takano, H.; Koseki, T.; Nishihara, T. Mechanisms involved in enhancement of osteoclast formation and function by low molecular weight hyaluronic acid. *J. Biol. Chem.* **2005**, *280*, 18967–18972. [CrossRef] [PubMed]

145. Visvader, J.E.; Lindeman, G.J. Cancer stem cells in solid tumours: Accumulating evidence and unresolved questions. *Nat. Rev. Cancer* **2008**, *8*, 755–768. [CrossRef] [PubMed]

146. Zoller, M. Cd44: Can a cancer-initiating cell profit from an abundantly expressed molecule? *Nat. Rev. Cancer* **2011**, *11*, 254–267. [CrossRef] [PubMed]

147. Sheridan, C.; Kishimoto, H.; Fuchs, R.K.; Mehrotra, S.; Bhat-Nakshatri, P.; Turner, C.H.; Goulet, R., Jr.; Badve, S.; Nakshatri, H. Cd44+/cd24− breast cancer cells exhibit enhanced invasive properties: An early step necessary for metastasis. *Breast Cancer Res.* **2006**, *8*, R59. [CrossRef] [PubMed]

148. Reuben, J.M.; Lee, B.N.; Gao, H.; Cohen, E.N.; Mego, M.; Giordano, A.; Wang, X.; Lodhi, A.; Krishnamurthy, S.; Hortobagyi, G.N.; et al. Primary breast cancer patients with high risk clinicopathologic features have high percentages of bone marrow epithelial cells with ALDH activity and CD44(+)CD24lo cancer stem cell phenotype. *Eur. J. Cancer* **2011**, *47*, 1527–1536. [CrossRef] [PubMed]

149. Ponti, D.; Costa, A.; Zaffaroni, N.; Pratesi, G.; Petrangolini, G.; Coradini, D.; Pilotti, S.; Pierotti, M.A.; Daidone, M.G. Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. *Cancer Res.* **2005**, *65*, 5506–5511. [CrossRef] [PubMed]

150. Abraham, B.K.; Fritz, P.; McClellan, M.; Hauptvogel, P.; Athelogou, M.; Brauch, H. Prevalence of CD44+/CD24−/low cells in breast cancer may not be associated with clinical outcome but may favor distant metastasis. *Clin. Cancer Res.* **2005**, *11*, 1154–1159. [PubMed]

151. Hiraga, T.; Ito, S.; Nakamura, H. Cancer stem-like cell marker CD44 promotes bone metastases by enhancing tumorigenicity, cell motility, and hyaluronan production. *Cancer Res.* **2013**, *73*, 4112–4122. [CrossRef] [PubMed]

152. Wang, J.; Shiozawa, Y.; Wang, Y.; Jung, Y.; Pienta, K.J.; Mehra, R.; Loberg, R.; Taichman, R.S. The role of cxcr7/rdc1 as a chemokine receptor for cxcl12/sdf-1 in prostate cancer. *J. Biol. Chem.* **2008**, *283*, 4283–4294. [CrossRef] [PubMed]

153. Gassenmaier, M.; Chen, D.; Buchner, A.; Henkel, L.; Schiemann, M.; Mack, B.; Schendel, D.J.; Zimmermann, W.; Pohla, H. Cxc chemokine receptor 4 is essential for maintenance of renal cell carcinoma-initiating cells and predicts metastasis. *Stem Cells* **2013**, *31*, 1467–1476. [CrossRef] [PubMed]

154. Egeblad, M.; Nakasone, E.S.; Werb, Z. Tumors as organs: Complex tissues that interface with the entire organism. *Dev. Cell* **2010**, *18*, 884–901. [CrossRef] [PubMed]

155. Junttila, M.R.; de Sauvage, F.J. Influence of tumour micro-environment heterogeneity on therapeutic response. *Nature* **2013**, *501*, 346–354. [CrossRef] [PubMed]

156. Lloyd, M.C.; Cunningham, J.J.; Bui, M.M.; Gillies, R.J.; Brown, J.S.; Gatenby, R.A. Darwinian dynamics of intratumoral heterogeneity: Not solely random mutations but also variable environmental selection forces. *Cancer Res.* **2016**, *76*, 3136–3144. [CrossRef] [PubMed]

157. Zhang, X.H.; Jin, X.; Malladi, S.; Zou, Y.; Wen, Y.H.; Brogi, E.; Smid, M.; Foekens, J.A.; Massague, J. Selection of bone metastasis seeds by mesenchymal signals in the primary tumor stroma. *Cell* **2013**, *154*, 1060–1073. [CrossRef] [PubMed]

158. Zhang, X.H.; Wang, Q.; Gerald, W.; Hudis, C.A.; Norton, L.; Smid, M.; Foekens, J.A.; Massague, J. Latent bone metastasis in breast cancer tied to src-dependent survival signals. *Cancer Cell* **2009**, *16*, 67–78. [CrossRef] [PubMed]

159. Vermeulen, L.; de Sousa, E.M.F.; van der Heijden, M.; Cameron, K.; de Jong, J.H.; Borovski, T.; Tuynman, J.B.; Todaro, M.; Merz, C.; Rodermond, H.; et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat. Cell Biol.* **2010**, *12*, 468–476. [CrossRef] [PubMed]

160. Todaro, M.; Gaggianesi, M.; Catalano, V.; Benfante, A.; Iovino, F.; Biffoni, M.; Apuzzo, T.; Sperduti, I.; Volpe, S.; Cocorullo, G.; et al. Cd44v6 is a marker of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis. *Cell Stem Cell* **2014**, *14*, 342–356. [CrossRef] [PubMed]

161. Lotti, F.; Jarrar, A.M.; Pai, R.K.; Hitomi, M.; Lathia, J.; Mace, A.; Gantt, G.A., Jr.; Sukhdeo, K.; DeVecchio, J.; Vasanji, A.; et al. Chemotherapy activates cancer-associated fibroblasts to maintain colorectal cancer-initiating cells by IL-17a. *J. Exp. Med.* **2013**, *210*, 2851–2872. [CrossRef] [PubMed]

162. Zeuner, A.; Todaro, M.; Stassi, G.; de Maria, R. Colorectal cancer stem cells: From the crypt to the clinic. *Cell Stem Cell* **2014**, *15*, 692–705. [CrossRef] [PubMed]

163. Cheung, T.H.; Rando, T.A. Molecular regulation of stem cell quiescence. *Nat. Rev. Mol. Cell Biol.* **2013**, *14*, 329–340. [CrossRef] [PubMed]

164. Chaffer, C.L.; Weinberg, R.A. A perspective on cancer cell metastasis. *Science* **2011**, *331*, 1559–1564. [CrossRef] [PubMed]

165. Mohme, M.; Riethdorf, S.; Pantel, K. Circulating and disseminated tumour cells—Mechanisms of immune surveillance and escape. *Nat. Rev. Clin. Oncol.* **2017**, *14*, 155–167. [CrossRef] [PubMed]

166. Lu, X.; Mu, E.; Wei, Y.; Riethdorf, S.; Yang, Q.; Yuan, M.; Yan, J.; Hua, Y.; Tiede, B.J.; Haffty, B.G.; et al. VCAM-1 promotes osteolytic expansion of indolent bone micrometastasis of breast cancer by engaging alpha4beta1-positive osteoclast progenitors. *Cancer Cell* **2011**, *20*, 701–714. [CrossRef] [PubMed]

167. 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*, 225–238. [CrossRef] [PubMed]

168. Ema, H.; Suda, T. Two anatomically distinct niches regulate stem cell activity. *Blood* **2012**, *120*, 2174–2181. [CrossRef] [PubMed]

169. Wang, L.D.; Wagers, A.J. Dynamic niches in the origination and differentiation of haematopoietic stem cells. *Nat. Rev. Mol. Cell Biol.* **2011**, *12*, 643–655. [CrossRef] [PubMed]

170. Shiozawa, Y.; Pedersen, E.A.; Havens, A.M.; Jung, Y.; Mishra, A.; Joseph, J.; Kim, J.K.; Patel, L.R.; Ying, C.; Ziegler, A.M.; et al. Human prostate cancer metastases target the hematopoietic stem cell niche to establish footholds in mouse bone marrow. *J. Clin. Invest.* **2011**, *121*, 1298–1312. [CrossRef] [PubMed]

171. Lawson, M.A.; McDonald, M.M.; Kovacic, N.; Hua Khoo, W.; Terry, R.L.; Down, J.; Kaplan, W.; Paton-Hough, J.; Fellows, C.; Pettitt, J.A.; et al. Osteoclasts control reactivation of dormant myeloma cells by remodelling the endosteal niche. *Nat. Commun.* **2015**, *6*, 8983. [CrossRef] [PubMed]

172. Wang, H.; Yu, C.; Gao, X.; Welte, T.; Muscarella, A.M.; Tian, L.; Zhao, H.; Zhao, Z.; Du, S.; Tao, J.; et al. The osteogenic niche promotes early-stage bone colonization of disseminated breast cancer cells. *Cancer Cell* **2015**, *27*, 193–210. [CrossRef] [PubMed]

173. Calvi, L.M.; Adams, G.B.; Weibrecht, K.W.; Weber, J.M.; Olson, D.P.; Knight, M.C.; Martin, R.P.; Schipani, E.; Divieti, P.; Bringhurst, F.R.; et al. Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* **2003**, *425*, 841–846. [CrossRef] [PubMed]

174. Shiozawa, Y.; Havens, A.M.; Jung, Y.; Ziegler, A.M.; Pedersen, E.A.; Wang, J.; Lu, G.; Roodman, G.D.; Loberg, R.D.; Pienta, K.J.; et al. Annexin II/annexin II receptor axis regulates adhesion, migration, homing, and growth of prostate cancer. *J. Cell Biochem.* **2008**, *105*, 370–380. [CrossRef] [PubMed]

175. Shiozawa, Y.; Pedersen, E.A.; Patel, L.R.; Ziegler, A.M.; Havens, A.M.; Jung, Y.; Wang, J.; Zalucha, S.; Loberg, R.D.; Pienta, K.J.; et al. GAS6/AXL axis regulates prostate cancer invasion, proliferation, and survival in the bone marrow niche. *Neoplasia* **2010**, *12*, 116–127. [CrossRef] [PubMed]