Stem and Cancer Stem Cell_Markers

The conversion of healthy stem cells into cancer stem cells (CSCs) is believed to underlie tumor relapse after surgical removal and fuel tumor growth and invasiveness. CSCs often arise from the malignant transformation of resident multipotent stem cells, which are present in most human tissues. Some organs, such as the gut and the brain, can give rise to very aggressive types of cancers, contrary to the dental pulp, which is a tissue with a very remarkable resistance to oncogenesis.

1. Introduction

Adult multipotent stem cells are responsible for renewing cell populations in the different organs of the body. The physiology and proliferative activity of these very different populations of organ-specific stem cells are adapted to fulfil the different requirements of the host tissue. Tissues differ markedly in their rate of mature cell turnover: there exist some tissues with high cell turnover activity owing to very high stem cell activity, whereas others have very low rates of cell turnover owing to a relative scarcity and/or quiescence of their adult stem cell populations.

The intestinal epithelium is the tissue with the highest adult cell renewal rate in mammals [1]. Millions of enterocytes are shed from the gut every day, which have to be replaced with new cells. The adult intestinal epithelium is constantly renewed by a population of cells located in the base of the Lieberkühn crypts: the ISCs, or intestinal stem cells. These are adult multipotent stem cells that respond very quickly to regenerative niche signals, and divide every 24 h to generate a new population of transit-amplifying cells, which gradually migrate towards the top of the villi whilst differentiating into different cell lineages [2]. The highly proliferative activity of ISCs allows for a practically complete replacement of the intestinal villi and crypt epithelial cells in a period of a few days. This turnover rate may be even accelerated in the case of presence of gut parasites, where it contributes to parasite expulsion [3].

On the diametrically opposite scenario, we find the central nervous system (CNS) with a very low self-renewal rate. Most cells in the brain and spinal cord are postmitotic neurons and glial cells. Indeed, the very existence of neurogenesis in the adult human brain was widely questioned by the neuroscientific community until very recently [4]. However, nowadays it is accepted that new neurons are generated throughout the whole life of the human brain, by activation of endogenous neural stem cells, or NSCs [5]. This natural neuron renewal process takes places mainly in a region of the limbic system called the hippocampus, a brain structure involved in memory consolidation [6]. Gliogenesis is known to be more widespread than neurogenesis, and it can take place in both the gray and white matter parenchyma, to ensure the renewal of postmitotic oligodendrocytes and astrocytes [7]. Both neurogenesis and gliogenesis are known to be relatively quiescent processes in the healthy mammal CNS, but they increase sharply after CNS injury [8][9]. However, there is controversy about whether the exacerbated activation of NSCs and the consequent reactive gliosis following brain injury are harmful or beneficial events for the restoration of normal CNS function [10].

Despite the great cellular and physiological differences between the healthy gut and CNS, both organs can give rise to very aggressive types of cancers. Colorectal cancer (CRC) is one of the most abundant cancers in the world and develops from the epithelial cells lining the colon or rectum of the gastrointestinal tract. As in other tumors, colon cancer cells are morphologically heterogeneous, differing in markers expression, proliferation capacity, tumorigenicity and chemotherapy resistance [11]. Glioblastoma, on the other hand, is a type of stage IV human brain cancer with the poorest prognosis owing to its very high ability to spread and infiltrate into brain parenchyma, thus hampering its total eradication by conventional therapies [12][13][14]. Both CRC and glioblastoma have in common the ability to relapse after surgical removal, which is attributed to the presence of cancer stem cells (CSCs) within the tumor.

The Cancer Stem Cell theory states that tumor growth is fueled by small numbers of CSCs hidden within the bulk of the tumor mass [15]. Much as normal cell renewal in healthy adult tissues depends on activation and proliferation of their endogenous stem cells, cell renewal in malignant tumors would depend on the activation of CSCs [16]. This theory explains clinical observations, such as the recurrence of tumors after initially successful therapy, and the phenomena of tumor dormancy and metastasis [17]. These CSCs or tumor-initiating cells were first described in teratocarcinomas that contained highly tumorigenic cells that, as single cells, could differentiate into multiple non-tumorigenic cell types [18]. Accordingly, the most accepted view of the theory of CSCs and tumorigenesis is that CSCs arise from and/or are closely related to normal adult multipotent stem cells, which undergo a process of transformation owing to the combined effect of gene mutations and cellular niche perturbation. Thus, understanding how adult tissue-specific stem cells behave in the healthy adult body, and how this homeostasis is lost under specific circumstances, is of paramount importance for the study of the different types of cancer.

The dental pulp shows a cellular renewal rate in between the gut epithelium and the brain parenchyma. Dental pulp tissue is of relevance to carcinogenesis because no cases of malignancies primarily originating in that location have ever been reported, at least since 1937 [19]. Back in the late nineteenth century it was common to diagnose putative dental pulp neoplasms as "pulpitis chronica sarcomatosa", which was associated with bacterial infections and poor dental hygiene. However, closer examinations later on revealed that most, if not all, of those cases were not related to malignant neoplasms per se, but to a colonization of the exposed dental pulp space by the gingival epithelium [20].

It is unclear whether this dental pulp resistance to oncogenesis owes simply to the physical constraints of the reduced space of the dental pulp chamber, which would prevent a minimum tumor growth required for dissemination, or rather to other so far unknown factors [19]. The dental pulp contains its own stem cells: dental pulp stem cells (DPSCs), which have a particularly high self-renewal and multilineage differentiation capacity [21]. Interestingly, one surprising feature of DPSCs is that these cells are extremely resistant to anaerobiosis and lack of nutrients, as clearly demonstrated by some reported facts, such as their capability to survive and proliferate to confluency after travelling for more than one week in parafilm-sealed culture flasks under ambient temperature shipping conditions [22]. DPSCs in the healthy dental pulp are known to localize into neurovascular bundles containing nerves and blood vessels [23] and they are responsible for renewing populations of mature fibroblasts, odontoblasts, and myelinating Schwann cells [24]. DPSCs can generate complete dentin–pulp complexes in vitro and in vivo [21][25][26], and are also induced to activate and proliferate after dental injury [27].

Arguably, the tissues with the highest resemblance to the dental pulp would be embryonic mesenchymal tissues and also adult loose connective stromal tissues. Soft stromal connective tissues can be found distributed throughout the human body, and enriched in some particular locations including the skin, the bone marrow, the adipose tissue, among others [28] [29]. These loose connective tissues can also give rise to soft tissue sarcomas with a very low incidence in the human population [30][31]. Notably, all of these tissues also home their own resident multipotent mesenchymal stem cells (MSCs), which share a great deal of characteristics, but also present important differences, with DPSCs. The systematic comparison between MSCs and DPSCs has already been the main topic of many excellent reviews [28][32]. It is compelling that all those connective tissues with so many resemblances at the structural and cellular level present so little rates of malignancy, which again suggests that there might well be other factors apart from mere physical isolation that would explain the absence of human cancers originating in the dental pulp.

2. Cell Markers and Pluripotency Core Factors

2.1. Cell Surface or Membrane Markers

Searching for similarities between different types of normal and aberrant stem cells, numerous cell surface markers with different functions were found to be associated with the stemness characteristics of CCSCs (see Table 1).

Table 1. Expression of cell markers and core factors involved in cancer in ISCs, NSCs and their corresponding CSCs, and DPSCs. Green color represents confirmed expression as reported by the literature. Red color represents absent expression as reported by the literature. Yellow color implies that its expression is not well defined or needs to be further studied. "+" symbol implies the existence of expression-confirming reports, "-" symbol implies the existence of no-expression supporting reports and question mark "?" implies that the expression of that marker has not been yet thoroughly assessed for a particular cell type. "↑" or "↓" symbol refers to an upregulation/increase or downregulation/reduction in its activity/expression with respect to their normal cell counterparts. ISC: intestinal stem cells. CCSC: colorectal cancer stem cells. NSC: neural stem cells. GSC: glioblastoma stem cells. DPSC: dental pulp stem cells. CSC: cancer stem cells.

	Intestinal		Neural		Dental
	ISC	CSC/CCSC	NSC	CSC/GSC	DPSC
CD133	+[33]	+↑[34][35]	+[36][37][38]	+↑[37][39]	+/- [40][41]
CD15	?	+[42]	+[43][44]	+[45][46][47]	+[48]
LGR5	+[49]	+[50]	+[51][52]	+[53]	?
CD166	+[54][55]	+[55]	-[56]	+↑ [57]	+[58][59]
CD44	-[54]	+↑[60]	+[61]	+↑[62][63][64]	+[65]
CD90	-[66]	+↑[67]	-[56]	+↑ [68]	+[69][70]
CXCR4	+[71]	+↑[72]	+[73]	+↑[74]	+[75]
NESTIN	+[76]	+↑[77]	+[78]	+↑[79][77]	+[48][69]
MUSASHI	+[80]	+↑[81]	+[82]	+↑[83]	+[84]
SOX2	?	+↑[85]	+[46][86]	+↑[46][87][88]	+[48][69][89][90]
OCT4	?	+↑[91]	+[92]	+↑[87][88][93]	+[48][94][89][90]
NANOG	?	+↑[95]	+[92][96][97]	+↑[87][88]	+[48][94][89][90]
PTEN	+[98]	+↓[99]	+[100]	+↓[100]	+[101]

The first identified marker of stem cells and early progenitors in the mouse small intestine was CD133, a transmembrane glycoprotein, also known as Prominin-1 [33]. More recent studies found that CD133 was also expressed in a subpopulation of CCSCs [34][68][102] and these cells were present in metastasis and angiogenesis of colorectal adenocarcinoma [102][103]. Moreover, other reports using subcutaneous injection into immunodeficient mice, after comparison of CD133+ versus CD133- populations showed that CD133- ones were unable to form tumors or grow as undifferentiated tumorspheres [104]. Similarly, CD133 was one of the first reported markers of brain CSCs [39]. This marker was identified in brain stem cells of fetal [105] and premature infants [79] but initially reported as absent in adult human NSCs, contrary to murine NSCs [35][77] [106]. Interestingly, CD133 has been postulated as an embryonic stem cell marker [107] and its presence or absence may suggest a different cancer origin accordingly to the cell transcriptional profiles. CD133+ brain CSCs behave similarly to fetal neural stem cells forming tumorspheres whereas CD133- brain CSCs display a semi adherent growth and its transcriptional profile is similar to adult NSCs [108].

CD133 expression has not yet been thoroughly evaluated for DPSCs. One study reporting a positive staining of DPSCs to CD133 relied exclusively on immunocytochemical characterization, which showed no clear membrane staining [109]. Other studies reported an absence of expression, as assessed by flow cytometry [110]. All these observations make CD133 an interesting potential marker for tumorigenic susceptibility across different tissues, and it would be interesting to address unequivocally whether CD133 is expressed or not in DPSC cultures, and whether this expression changes or not with different experimental conditions. Another comprehensive flow cytometry assessment also revealed the absence of CD133 in the more DPSC-related human bone marrow MSCs [111]. However, CD133 expression could be detected in CSC-like cells derived from malignant fibrosarcomas [112].

All this evidence combined points to CD133 as an interesting marker to identify CSCs in a large variety of tissues. However, some controversy remains with regard to the adoption of CD133 as a genuine universal CSC marker [113]. CD133 expression in CSCs has been related to particularly aggressive phenotypes but, as shown by different reports, this CD133 marker is also expressed by some healthy stem cell types such as ISCs and NSCs of the gut and the brain. However, it is precisely those organs, which give rise to very aggressive types of cancers, are also the ones that apparently show a higher basal CD133 expression in their resident stem cells, contrary to loose connective tissues, and the dental pulp in particular. This brings another issue: may eventually a positive relationship be established between the basal expression of CD133 in healthy stem cells and the malignity of neoplasms arising from CSCs in different tissues? This may deserve closer investigation. For instance, if CD133 expression was more consistently and comparatively assessed between different human stem cell types to fill in the existing information gaps, this could decisively help to clarify this question.

Another interesting surface marker of NSCs and neural CSCs is CD15, also known as SSEA-1 or Lewis X (Lex). This embryonic stem cell marker is expressed by some multipotent murine and human stem cells, and also by human CSCs [36][37][38][114][40][41][115][58][116]. Interestingly, CD133 and CD15 coexpression in a same cell (NSC or CSC) is very rare [40][117][118] with the exception of primitive neuroectodermal tumors which show higher clonogenicity for CD133+/CD15+ than CD133-/CD15+ cells [119]. CD133+ and CD15+ cells are sensitive to killing by oncolytic herpes simplex viruses [120]. In the intestine, CD15 expression has been found in Paneth cells in the vicinity of stem cell niches [94] but it is yet not clear whether CD15 is expressed or not by ISCs. CD15 expression is progressively increased during colon cancer development [36]. CD15/SSEA-1 has also been found to be expressed by both MSCs and DPSCs, as assessed by flow cytometry and RT-PCR. The expression of CD15/SSEA-1 was also found to be higher in DPSCs than in bone marrow MSCs [80]. Moreover, this embryonic marker was clearly upregulated in DPSCs after experimental conditions that increase the stemness of these cells, such as Wnt/ß-catenin activation [41][110]. The expression of CD15 has also been identified in fibrosarcoma-derived cells [121]. It would also be very interesting to compare the relative levels of CD15 expression in CSCs vs. MSCs and/or DPSCs to further validate the utility of CD15 as a CSC marker.

Another surface receptor that is present in human NSCs and CSCs is the Leucine-rich repeat-containing G-protein coupled receptor 5, or LGR5 [45][122]. LGR5, also known as GPR49, works as an important regulator of canonical Wnt/ß-catenin signaling and it physically interacts with Wnt Frizzled-5/LRP6 receptors [71]. LGR5 binds to R-spondins, which are secreted Wnt activator protein ligands. The LGR5 receptor has been postulated as a widespread CSC marker [123]. Interestingly, LGR5 is required for the tumorigenicity of glioblastoma cells [43]. LGR5 is also expressed by healthy colorectal ISCs [124] and its expression is maintained in CCSCs [125]. LGR5 is considered an ISC cell cycle marker and is related to survival, proliferation, and differentiation [126]. LGR5 can also mediate integrin signaling through MyoX and integrins present at LGR5 cytonemes [84][127]. Some subsets of MSCs have been shown to express LGR5, although the expression of this marker seems to be highly dependent on the mesenchymal cell source [128]. The expression of LGR5 in DPSCs also remains to be fully elucidated, although one report showed a strong expression of LRG5 in the dental pulp and the odontoblastic cell layer of permanent teeth [100]. Notably, the loss of expression of some specific LGR5 splicing variants has been associated with a poor prognosis in soft tissue sarcomas [129]. It is yet unclear how this differential expression of LRG5 isoforms may promote the development of human cancers, particularly with regard to the regulation of Wnt/ß-catenin activity.

The CD184 receptor, also known as Fusin or CXCR4, is a G-protein coupled receptor expressed by both brain NSCs and CSCs [49][50]. CXCR4 expression increases in vivo glioma perivascular invasion capacity [96]. In the gut, this receptor has been used as a marker to identify the CCSC population, together with LGR5 [97]. Furthermore, colon cancer cells double positive for CD133+ and CXCR4+ exhibit metastatic potential and their presence is linked to poor prognosis [53]. CXCR4 activation induces several cellular responses ranging from gene transcription and chemotaxis to cell survival and proliferation [130]. CXCR4 is amply expressed by human MSCs and DPSCs, where it contributes to stimulate their migration and chemotaxis, through activation of PI3K/AKT and Wnt/ß-catenin pathways [98][131]. In all cases, the activation of CXCR4 in stem and/or cancer stem cells is linked to the acquisition of a migratory cell phenotype and/or metastatic ability.

The transmembrane glycoprotein, CD166 also known as ALCAM (activated leukocyte cell adhesion molecule) is an adhesion protein binding to the ECM and is expressed in both ISCs and CCSCs [46][47]. CD166 is also expressed by plastic-adherent human MSCs and DPSCs [58][115]. CD166 is not present in NSCs, nor in neural stem-like cells induced from conversion of bone marrow stromal cells (CD166+) [101]. The association between CD166 expression and poor prognosis of colorectal cancer is not yet sufficiently elucidated, with studies reporting different results [86]. However, in CD133+ GSCs, CD166 has been shown to increase cellular invasion [48]. The acquired expression of CD166 in GSCs

could be associated with a higher migration and dissemination capacity, by the conversion to a more mesenchymal-like migratory phenotype. Under this viewpoint, an increased expression of CD166 in CCSCs could also be regarded as a contributing factor to explain the high tendency of CRC to form secondary metastases.

There are other mesenchymal stem cell markers associated with a higher tumor invasiveness. CD44 is a hyaluronic acid-binding surface receptor expressed in DPSCs, GSCs and CCSCs, but not in their corresponding healthy ISCs. Thus, CD44 constitutes a marker for prediction of hepatic metastases and poor prognosis in CRC [59][132][133][134][135]. CD90, also known as THY-1, is a GPI-anchored adhesion protein of the immunoglobulin superfamily, which is another widespread mesenchymal marker related to poor prognosis in many cancers [85]. As expected for a mesenchymal cell marker, CD90 is also expressed by DPSCs [52][136]. CD90 is a candidate marker for GSCs, but its expression is completely absent from healthy brain tissues [91]. Interestingly, CD90 has also been involved in the proliferation, migration and adhesion of human glioma-associated mesenchymal stem cells [51]. The cases of CD184, CD44, CD90 and CD166 constitute typical examples of mesenchymal surface proteins involved in cell chemotaxis and adhesion, whose expression is associated with the emergence of CSC phenotypes and particularly poor prognosis in many human cancers. However, because of the prominent expression of these markers in several types of healthy stem cells, such as MSCs and DPSCs, they also could not be considered to be genuine markers of CSCs.

2.2. Cytoplasmic Markers

One of the most prominent proteins required for self-renewal of NSCs is the intermediate filament Nestin [95]. Nestin is also expressed in several types of cancers [92], and also by CD133+ brain CSCs [137]. Nestin abundance is significantly correlated with prognosis, clinicopathological features and the histological grade of the glioma in patients [93][138]. Nestin+ tumor cells have been observed to be the origin of tumor regrowth after chemotherapeutic treatment with the alkylating agent temozolomide [87]. With respect to CRC, Nestin expression is upregulated in stromal cells and its knockdown inhibits migration and cell cycle arrest at S phase, thus halting cell proliferation [88]. GFP-tagged Nestin protein of several cancer cell lines including CRC revealed a high GFP expression in proliferating endothelial cells and nascent blood vessels in the growing tumors [139]. DPSCs in vitro are also practically 100% Nestin+ [52]. The widespread expression of neural markers by DPSCs is associated with the neural crest origin of these cells. In fact, contrary to other mesoderm-derived mesenchymal stem cells, such as those obtained by the bone marrow or the adipose tissue, DPSCs have been reported to show a much better capacity to differentiate to neuronal and glial cells [52][89][90] and these reasons partly account for the expectation raised by DPSCs as a non-conventional source of stem cells for neural regeneration [44][56]. Another important feature of DPSCs related to their use for neural cell therapy is their ability to secrete neurotrophic factors [52][66] and differentiate to endoteliocytes and pericytes to generate new blood vessels within the CNS [60].

The intracellular RNA binding-protein MUSASHI is a marker of poor prognosis in many human cancers and regarded as a putative marker for CSCs. It is known to be expressed by ISCs [140], and it is overexpressed in CRC, where its levels correlate with other stem cell marker of the intestinal epithelium such as ß1-integrin and LRG5, suggesting its involvement in CCSC generation [67][141]. MUSASHI is involved in the maintenance of adult stem cell fate, and also expressed by NSCs and GSCs [34][70][76] where it participates in enhancing tumoral cell migration [78]. MUSASHI has also been shown to be expressed by DPSCs and other stem cells of the oral cavity, and its expression was reported to increase in response to osteogenic differentiation [142]. There exist yet no reports of MUSASHI expression in soft tissue sarcomas.

One marker whose loss is related to poor prognosis in human cancers is the Phosphatase and Tensin Homolog (PTEN). This enzyme is critical for stem cell maintenance and PTEN deficiencies can cause the development of CSCs [81]. It has been observed that PTEN loss reprograms healthy stem cells to adopt a glioblastoma stem cell-like phenotype [69]. PTEN is also involved in the migration of precursor cells [82] and it is expressed in adult NSCs and progenitors [62][63]. Its deletion or loss of function has been reported to alter neurogenesis and provokes cellular alterations in adult hippocampal neural progenitor and stem cells [83]. PTEN has also been involved in the control of the proliferation rate and number of ISCs, and similar to what is observed in other regions, the absence or dysfunction of PTEN provokes an intestinal polyposis due to an excessive cellular proliferation becoming a precancerous neoplasia [64].

Recent investigations show that DPSCs present unusually high levels of PTEN expression [65]. This accounts for critical differences between DPSCs and other related multipotent stem cells, such as mesodermal MSCs. In a comparative study between DPSCs and bone marrow MSCs, the high levels of PTEN expression naturally present in DPSCs were shown to downregulate the oncogenic PI3K/AKT pathway, thus contributing to an increased osteo/odontogenic capacity, at the expense of a diminished tumorigenic capacity. Remarkably, when both bone marrow MSCs and DPSCs were transfected in parallel with pRB and CMYC oncogenes, MSCs readily acquired a tumor phenotype whereas DPSCs could only be induced to transform when PTEN was also simultaneously inhibited [65]. Due to its role as a tumor suppressor gene, it

might be postulated that at least some of the resistance of DPSCs to oncogenesis may be attributed to a high expression of PTEN. Another implication of this hypothesis would be that stem cells from other body locations could also be more vulnerable to transformation because of an insufficient PTEN expression.

2.3. Nuclear Proteins

One of the principal characteristics of CSCs that distinguish them from the rest of tumor cells is their overexpression of nuclear transcription factors traditionally associated to stemness and pluripotency. SOX2 is one of the principal core factors related to cell pluripotency [143] and encodes a transcription factor member of the SRY-related HMG-box (SOX) family. In CRC, SOX2 positive cells were found to display several characteristics of CSCs, together with a decreased expression of the intestinal epithelial marker CDX2, contributing to a poor prognosis [72]. OCT4A is another core factor which has been linked to chemoresistance of colon CSCs [144] and NANOG has been recently related to colony formation and growth of CRC cells [61]. Notably, SOX2 is expressed by both healthy NSCs and GSCs [114]. Interestingly neural progenitor cells have also been reported to express mRNA for NANOG and OCT4 [145]. It should be taken into account that the expression of these stemness factors, together with others such as KLF4, leads to the development of induced pluripotent stem cells (iPSCs) with tumorigenic capacity [57] and SOX2, OCT4 and NANOG are all pluripotency markers [75] that are found in circulating tumor cells present in the blood of patients with glioblastoma [146].

Nevertheless, it should be noted that the mere coexpression of these three markers per se does not necessarily induce CSC-related cell phenotypes. Healthy non-tumorigenic DPSC cultures, for instance, also show coexpression of SOX2, OCT4A and NANOG [41][99]. Moreover, the expression of these pluripotency core factors rises in DPSCs subjected to activation of Wnt/ßcatenin signaling to enhance their stemness potential, but without leading to cell transformation [41][54][55]. Wnt/ßcatenin signaling was also shown to promote the maintenance of pluripotency in embryonic stem cells [116].

3. Oncogenic Signaling

3.1. Wingless (Wg)-Related Integration Site (Wnt)

Wnt genes are extensively conserved between invertebrates and mammals, thus highlighting the importance of this signaling pathway to regulate cell development and gene expression [147]. Once secreted, Wnt proteins bind to specific membrane Frizzled/LRP5-6 coreceptors on the target cell. These events lead to the membrane recruitment of an intracellular multiprotein complex containing (among others) AXIN2, APC and Glycogen Synthase Kinase-3ß, which causes the inactivation of the latter enzyme, and the dephosphorylation and eventual accumulation of ß-catenin protein, a fundamental transcriptional coactivator of Wnt target genes. Wnt/ß-catenin signaling can be potentiated by simultaneous activation of LRG5 by R-spondins [71], or alternatively weakened by other LRP5-6 ligands such as DKK-1 [148]. Many malignant cancer cells show a pathological hyperactivation of canonical Wnt/ß-catenin signaling [149] and mutations that promote a constitutive activation of the Wnt pathway, such as inactivation of APC or DKK-1, very often lead to colorectal cancer [150][151]. ISCs are positive for LGR5 [124] and AXIN2 [152], which underscores the importance of canonical Wnt signaling in controlling the homeostasis of these cells [124][152][153]. On the contrary, the loss of Wnt function is associated with defects in epithelial cell renewal in many organs, including the gut [154][155].

Wnt activity in NSCs regulates their homeostasis and adult hippocampal neurogenesis [156]. In human glioma cells, it has been described as an important regulator of cell proliferation [157][158][159]. Thus, both healthy ISCs and NSCs are sensitive to Wnt signaling, and an excessive Wnt/ß-catenin hyperactivation is believed to promote the transformation of stem cells to CSCs in both cases [150]. This parallelism shows a common ground between ISCs, NSCs, CCSCs and GSCs [155][160][161][162]. Strikingly, the "need" for the Wnt pathway and the struggle this creates between cancer cells and neural cells reaches to levels of "vampirization" in which neurons end dying from the subsequent enwrapping and Wnt receptor depletion taken by the squeezing and invading glioma cells. Using this strategy, cancer cells manage to increase their available space and their own proliferation and infiltration capabilities within the brain [163]. Furthermore, Wnt activity is required for self-renewal of GSCs [164]. Taking everything into account, it is not surprising that the targeting of the Wnt pathway has been recently regarded as a high priority for therapeutic advances [149].

Another cell type with a high sensitivity to Wnt signaling is the DPSC. It was recently shown that even very short-term applications of Wnt-3a in DPSCs are associated with an increased self-renewal and an enhancement of their stemness properties [41]. Moreover, the increase in multilineage differentiation potential in DPSCs is associated with a deep remodeling of DPSC physiology at both the metabolic and epigenetic level [54][55]. However, Wnt/ß-catenin activation caused only a modest increase in the self-renewal capacity of DPSCs [55]. It is currently unknown whether the non-tumorigenic phenotype of DPSCs could have any relationship with a tighter regulation of Wnt signaling, in comparison with CCSCs or glioma CSCs [160]. It should also be taken into account that not only the tumor cells themselves, but also

stromal cells around the tumor may also secrete additional factors activating the Wnt/ß-catenin signaling pathway, to promote tumor cell invasion and metastasis. The contribution of stromal cells, especially fibroblasts, endothelial cells and pericytes, appears to be very relevant for the progression of both malignant CRC and glioblastoma [165][166].

3.2. Transforming Growth Factor Beta (TGF-ß) Signaling

TGF-β superfamily signaling plays key roles in cell differentiation and proliferation [167], and comprises over 30 different members including activins, nodals, bone morphogenetic proteins (BMPs), and growth and differentiation factors (GDFs) (see review [168]). TGF-β pathway activity is able to lengthen the progression of the cell cycle in aged NSCs [169]. This signaling also increases in the neurogenic niches during aging or after a high dose of radiation inducing the quiescence of NSCs [170]. However, it may also induce epithelial–mesenchymal transition (EMT) of normal cells to acquire migratory and stem cell properties [171]. TGF-ß1 protein is known to be up-regulated during ageing, brain lesions or during neurodegeneration [172] but is also involved in the development and progression of high-grade gliomas [173][174]. TGF-β also promotes tissue invasion, angiogenesis and evasion from immune attack [175][176].

TGF-β signaling also regulates stemness of normal stem cells and CSCs [177]. Indeed, during development Activin and Nodal proteins regulate NANOG expression maintaining cellular pluripotency in human and mouse embryonic stem cells [178]. The importance of TGF-ß signaling in the maintenance of stemness of DPSCs needs further clarification. One report showed significant expression changes of expression in several TGF-ß-related genes after induction of DPSC differentiation to osteo/odontoblasts by standard pharmacological protocols. Specifically, Activin A, TGF-ß1 and TGF-ß2 expression were shown to be downregulated, but TFG-ß receptors II and III upregulated, after DPSC osteoblastic differentiation [179].

In the large intestine, it is assumed that the TGFβ/BMP signal gradually increases along the intestinal axis of the villi of the crypt, while the gradient towards the base of the crypt decreases, thus inhibiting the regeneration of stem cells and supporting the differentiation of epithelial cells, thereby playing a vital role in balancing the effect of Wnt signaling on intestinal homeostasis [180]. However, dysregulation of TGF-β signaling is involved in cell proliferation, differentiation, migration and apoptosis, and it could lead to the development of CCSCs [181]. Interestingly, a cross-talk between TGF-β signaling and the R-spondin/LGR5 axis was reported in CRC cells, where LGR5-induced TGF-ß activity in tumor cells was associated with a decreased tumor invasion and metastasis [182]. However, the activity of the TGF-β pathway in stromal cells is associated with a higher risk of metastasis in CRC, and pharmacological inhibition of TGF-ß receptor I impairs tumor metastasis in CRC [183]. Once again, these results show the involvement of TGF-ß signaling to promote an oncogenic tissue microenvironment and highlight the importance of the crosstalk between tumor and stromal cells to sustain cancer malignancy.

4. Telomerase Activity

The regulation of cellular telomerase activity depends on the transcriptional control of its two essential components, hTERC (RNA component) and hTERT (reverse transcriptase component) [184]. Wnt/β-catenin signaling was shown to have a positive regulatory effect on the expression of telomerase reverse transcriptase (hTERT) and CSC-related proteins [185]. In turn, telomerase directly modulates Wnt/β-catenin signaling, by activating quiescent stem cells [186]. Importantly, the emergence of CSCs is promoted by the overexpression of hTERT [187][188][189]. Interestingly, it has been reported that a mutated TERT fragment is able to induce brain cancer stemness independently of its telomerase activity [190]. Furthermore, telomere dysfunction promotes tumorigenesis by inducing chromosomal instability in tumor initiating cells (see review [191]). Chromosomal instability is a source of genetic variation, favoring tumor adaptations to stressful environments and cytotoxic anticancer drugs, contributing to the progression at multiple stages of tumor evolution [192][193]. Normally, telomerase activity is downregulated after human brain embryonic development even in adult multipotent stem cells [194][195]. However, multipotent stem cells such as ISCs, MSCs, DPSCs and NSCs all present a basal telomerase activity and hTERT expression [196][197][198][199].

Brain telomerase activity in adult mice has been found to be restricted to the subventricular zone and olfactory bulb [200]. It plays an important role in cell proliferation in the adult but not in embryonic NSCs [201]. Telomere length has also been demonstrated to be important for neuronal differentiation and neuritogenesis [202] (see also review [203]). Its deficiency leads to a compromised olfactory bulb neurogenesis [201] although NSCs lose telomerase activity upon differentiation into astrocytes [204]. DPSCs also lose progressively their telomerase activity upon their spontaneous in vitro differentiation to osteoblastic/odontoblastic cells in conditions of high culture passages [198].

It should be emphasized that telomerase is reactivated in some malignancies such as CRC and most of brain cancers [205]. However, the mere absence of telomerase activity does not guarantee cellular resistance to oncogenic transformation, because CSCs may also use a mechanism of alternative lengthening of telomeres (ALT) [206][207]. ALT preserves telomeres by homologous recombination machinery independently of hTERT and hTERC [208]. ALT has been reported to be present in 10–15% of human cancers, including GSCs [114][209], but also in colon cancer cells with BRCA2 deletion [210] or hereditary and sporadic colon cancer [211]. ALT has not been reported to date in NSCs nor ISCs, suggesting that the origin of ALT in CSCs of brain and colon cancer could also be related to a dedifferentiation process from somatic cells [211]. ALT has not been yet reported for DPSCs. However, this mechanism is very active in malignant tumors of mesenchymal origin [212][213]. It remains to be studied whether the ability to activate ALT could constitute another important difference between DPSCs and MSCs.

References

1. Williams, J.M.; Duckworth, C.A.; Burkitt, M.D.; Watson, A.J.M.; Campbell, B.J.; Pritchard, D.M. Epithelial cell shedding and barrier function: A matter of life and death at the small intestinal villus tip. Vet. Pathol. 2015, 52, 445–455.

2. Umar, S. Intestinal Stem Cells. Curr. Gastroenterol. Rep. 2010, 12, 340–348.

3. Cliffe, L.J.; Humphreys, N.E.; Lane, T.E.; Potten, C.S.; Booth, C.; Grencis, R.K. Accelerated intestinal epithelial cell turnover: A new mechanism of parasite expulsion. Science 2005, 308, 1463–1465.

4. Paredes, M.F.; Sorrells, S.F.; Cebrian-Silla, A.; Sandoval, K.; Qi, D.; Kelley, K.W.; James, D.; Mayer, S.; Chang, J.; Auguste, K.I.; et al. Does Adult Neurogenesis Persist in the Human Hippocampus? Cell Stem Cell 2018, 23, 780–781.

5. Moreno-Jiménez, E.P.; Flor-García, M.; Terreros-Roncal, J.; Rábano, A.; Cafini, F.; Pallas-Bazarra, N.; Ávila, J.; Llorens-Martín, M. Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. Nat. Med. 2019, 25, 554–560.

6. Scoville, W.B.; Milner, B. Loss of recent memory after bilateral hippocampal lesions. J. Neurol. Neurosurg. Psychiatry 1957, 20, 11–21.

7. Rusznák, Z.; Henskens, W.; Schofield, E.; Kim, W.S.; Fu, Y. Adult Neurogenesis and Gliogenesis: Possible Mechanisms for Neurorestoration. Exp. Neurobiol. 2016, 25, 103–112.

8. Arvidsson, A.; Collin, T.; Kirik, D.; Kokaia, Z.; Lindvall, O. Neuronal replacement from endogenous precursors in the adult brain after stroke. Nat. Med. 2002, 8, 963–970.

9. Robel, S.; Berninger, B.; Götz, M. The stem cell potential of glia: Lessons from reactive gliosis. Nat. Rev. Neurosci. 2011, 12, 88–104.

10. Sierra, A.; Martin-Suarez, S.; Valcarcel-Martin, R.; Pascual-Brazo, J.; Aelvoet, S.A.; Abiega, O.; Deudero, J.J.; Brewster, A.L.; Bernales, I.; Anderson, A.E.; et al. Neuronal hyperactivity accelerates depletion of neural stem cells and impairs hippocampal neurogenesis. Cell Stem Cell 2015, 16, 488–503.

11. Brenner, H.; Kloor, M.; Pox, C.P. Colorectal cancer. Lancet 2014, 383, 1490–1502.

12. Seo, Y.-S.; Ko, I.O.; Park, H.; Jeong, Y.J.; Park, J.-A.; Kim, K.S.; Park, M.-J.; Lee, H.-J. Radiation-Induced Changes in Tumor Vessels and Microenvironment Contribute to Therapeutic Resistance in Glioblastoma. Front. Oncol. 2019, 9, 1259.

13. Wang, D.; Wang, C.; Wang, L.; Chen, Y. A comprehensive review in improving delivery of small-molecule chemotherapeutic agents overcoming the blood-brain/brain tumor barriers for glioblastoma treatment. Drug Deliv. 2019, 26, 551–565.

14. Stupp, R.; Hegi, M.E.; Mason, W.P.; van den Bent, M.J.; Taphoorn, M.J.B.; Janzer, R.C.; Ludwin, S.K.; Allgeier, A.; Fisher, B.; Belanger, K.; et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol. 2009, 10, 459–466.

15. Visvader, J.E.; Lindeman, G.J. Cancer stem cells in solid tumours: Accumulating evidence and unresolved questions. Nat. Rev. Cancer 2008, 8, 755–768.

16. Sell, S. Stem cell origin of cancer and differentiation therapy. Crit. Rev. Oncol. Hematol. 2004, 51, 1–28.

17. Wang, K.; Wu, X.; Wang, J.; Huang, J. Cancer stem cell theory: Therapeutic implications for nanomedicine. Int. J. Nanomed. 2013, 8, 899–908.

18. Kleinsmith, L.J.; Pierce, G.B. MULTIPOTENTIALITY OF SINGLE EMBRYONAL CARCINOMA CELLS. Cancer Res. 1964, 24, 1544–1551.

19. Neuhaus, K.W. Teeth: Malignant neoplasms in the dental pulp? Lancet Oncol. 2007, 8, 75–78.

20. Neuhaus, K.W. Dental Pulp Neoplasms. In Encyclopedia of Cancer; Schwab, M., Ed.; Springer: Berlin/Heidelberg, Germany, 2011; pp. 1084–1086. ISBN 978-3-642-16483-5.

21. Gronthos, S.; Mankani, M.; Brahim, J.; Robey, P.G.; Shi, S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc. Natl. Acad. Sci. USA 2000, 97, 13625–13630.

22. Mitsiadis, T.A.; Woloszyk, A. Odyssey of human dental pulp stem cells and their remarkable ability to survive in extremely adverse conditions. Front. Physiol. 2015, 6.

23. Shi, S.; Gronthos, S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. J. Bone Miner. Res. 2003, 18, 696–704.

24. Kaukua, N.; Shahidi, M.K.; Konstantinidou, C.; Dyachuk, V.; Kaucka, M.; Furlan, A.; An, Z.; Wang, L.; Hultman, I.; Ahrlund-Richter, L.; et al. Glial origin of mesenchymal stem cells in a tooth model system. Nature 2014, 513, 551–554.

25. Yoshida, S.; Wada, N.; Hasegawa, D.; Miyaji, H.; Mitarai, H.; Tomokiyo, A.; Hamano, S.; Maeda, H. Semaphorin 3A Induces Odontoblastic Phenotype in Dental Pulp Stem Cells. J. Dent. Res. 2016, 95, 1282–1290.

26. Yang, X.; Zhang, S.; Fan, M.; Li, X.; Liu, T.; Yao, Y. Effects of interleukin-1β on mineralization potential of dental pulp stem cells. Zhonghua Kou Qiang Yi Xue Za Zhi 2011, 46, 406–411.

27. Rombouts, C.; Jeanneau, C.; Bakopoulou, A.; About, I. Dental Pulp Stem Cell Recruitment Signals within Injured Dental Pulp Tissue. Dent. J. 2016, 4, 8.

28. Ullah, I.; Subbarao, R.B.; Rho, G.J. Human mesenchymal stem cells—Current trends and future prospective. Biosci. Rep. 2015, 35.

29. Huang, G.T.-J.; Gronthos, S.; Shi, S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: Their biology and role in regenerative medicine. J. Dent. Res. 2009, 88, 792–806.

30. Ducimetière, F.; Lurkin, A.; Ranchère-Vince, D.; Decouvelaere, A.-V.; Péoc'h, M.; Istier, L.; Chalabreysse, P.; Muller, C.; Alberti, L.; Bringuier, P.-P.; et al. Incidence of Sarcoma Histotypes and Molecular Subtypes in a Prospective Epidemiological Study with Central Pathology Review and Molecular Testing. PLoS ONE 2011, 6.

31. Burningham, Z.; Hashibe, M.; Spector, L.; Schiffman, J.D. The Epidemiology of Sarcoma. Clin. Sarcoma Res. 2012, 2, 14.

32. Lv, F.-J.; Tuan, R.S.; Cheung, K.M.C.; Leung, V.Y.L. Concise review: The surface markers and identity of human mesenchymal stem cells. Stem Cells 2014, 32, 1408–1419.

33. Snippert, H.J.; van Es, J.H.; van den Born, M.; Begthel, H.; Stange, D.E.; Barker, N.; Clevers, H. Prominin-1/CD133 marks stem cells and early progenitors in mouse small intestine. Gastroenterology 2009, 136, 2187–2194.e1.

34. Todaro, M.; Perez Alea, M.; Scopelliti, A.; Medema, J.P.; Stassi, G. IL-4-mediated drug resistance in colon cancer stem cells. Cell Cycle 2008, 7, 309–313.

35. Pfenninger, C.V.; Roschupkina, T.; Hertwig, F.; Kottwitz, D.; Englund, E.; Bengzon, J.; Jacobsen, S.E.; Nuber, U.A. CD133 is not present on neurogenic astrocytes in the adult subventricular zone, but on embryonic neural stem cells, ependymal cells, and glioblastoma cells. Cancer Res. 2007, 67, 5727–5736.

36. Jang, T.J.; Park, J.B.; Lee, J.I. The Expression of CD10 and CD15 Is Progressively Increased during Colorectal Cancer Development. Korean J. Pathol. 2013, 47, 340–347.

37. Daynac, M.; Tirou, L.; Faure, H.; Mouthon, M.-A.; Gauthier, L.R.; Hahn, H.; Boussin, F.D.; Ruat, M. Hedgehog Controls Quiescence and Activation of Neural Stem Cells in the Adult Ventricular-Subventricular Zone. Stem Cell Rep. 2016, 7, 735–748.

38. Son, M.J.; Woolard, K.; Nam, D.-H.; Lee, J.; Fine, H.A. SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. Cell Stem Cell 2009, 4, 440–452.

39. Singh, S.K.; Clarke, I.D.; Terasaki, M.; Bonn, V.E.; Hawkins, C.; Squire, J.; Dirks, P.B. Identification of a cancer stem cell in human brain tumors. Cancer Res. 2003, 63, 5821–5828.

40. Mao, X.-G.; Zhang, X.; Xue, X.-Y.; Guo, G.; Wang, P.; Zhang, W.; Fei, Z.; Zhen, H.-N.; You, S.-W.; Yang, H. Brain Tumor Stem-Like Cells Identified by Neural Stem Cell Marker CD15. Transl. Oncol. 2009, 2, 247–257.

41. Uribe-Etxebarria, V.; Luzuriaga, J.; Garcia-Gallastegui, P.; Agliano, A.; Unda, F.; Ibarretxe, G. Notch/Wnt cross-signalling regulates stemness of dental pulp stem cells through expression of neural crest and core pluripotency factors. Eur. Cell Mater. 2017, 34, 249–270.

42. Fu, L.; Zhu, L.; Huang, Y.; Lee, T.D.; Forman, S.J.; Shih, C.-C. Derivation of Neural Stem Cells from Mesenchymal Stem Cells: Evidence for a Bipotential Stem Cell Population. Stem Cells Dev. 2008, 17, 1109–1121.

43. Hiraoka, K.; Hayashi, T.; Kaneko, R.; Nasu-Nishimura, Y.; Koyama-Nasu, R.; Kawasaki, Y.; Akiyama, T. SOX9-mediated upregulation of LGR5 is important for glioblastoma tumorigenicity. Biochem. Biophys. Res. Commun. 2015, 460, 216–221.

44. Ibarretxe, G.; Crende, O.; Aurrekoetxea, M.; García-Murga, V.; Etxaniz, J.; Unda, F. Neural crest stem cells from dental tissues: A new hope for dental and neural regeneration. Stem Cells Int. 2012, 2012, 103503.

45. Yu, Y.; Moberly, A.H.; Bhattarai, J.P.; Duan, C.; Zheng, Q.; Li, F.; Huang, H.; Olson, W.; Luo, W.; Wen, T.; et al. The Stem Cell Marker Lgr5 Defines a Subset of Postmitotic Neurons in the Olfactory Bulb. J. Neurosci. 2017, 37, 9403–9414.

46. Wang, F.; Scoville, D.; He, X.C.; Mahe, M.M.; Box, A.; Perry, J.M.; Smith, N.R.; Lei, N.Y.; Davies, P.S.; Fuller, M.K.; et al. Isolation and characterization of intestinal stem cells based on surface marker combinations and colony-formation assay. Gastroenterology 2013, 145, 383–395.e1–21.

47. Levin, T.G.; Powell, A.E.; Davies, P.S.; Silk, A.D.; Dismuke, A.D.; Anderson, E.C.; Swain, J.R.; Wong, M.H. Characterization of the intestinal cancer stem cell marker CD166 in the human and mouse gastrointestinal tract. Gastroenterology 2010, 139, 2072–2082.e5.

48. Kijima, N.; Hosen, N.; Kagawa, N.; Hashimoto, N.; Nakano, A.; Fujimoto, Y.; Kinoshita, M.; Sugiyama, H.; Yoshimine, T. CD166/activated leukocyte cell adhesion molecule is expressed on glioblastoma progenitor cells and involved in the regulation of tumor cell invasion. Neuro-Oncology 2012, 14, 1254–1264.

49. Ho, S.-Y.; Ling, T.-Y.; Lin, H.-Y.; Liou, J.T.-J.; Liu, F.-C.; Chen, I.-C.; Lee, S.-W.; Hsu, Y.; Lai, D.-M.; Liou, H.-H. SDF-1/CXCR4 Signaling Maintains Stemness Signature in Mouse Neural Stem/Progenitor Cells. Stem Cells Int. 2017, 2017, 2493752.

50. Yi, L.; Zhou, X.; Li, T.; Liu, P.; Hai, L.; Tong, L.; Ma, H.; Tao, Z.; Xie, Y.; Zhang, C.; et al. Notch1 signaling pathway promotes invasion, self-renewal and growth of glioma initiating cells via modulating chemokine system CXCL12/CXCR4. J. Exp. Clin. Cancer Res. 2019, 38, 339.

51. Zhang, Q.; Yi, D.-Y.; Xue, B.-Z.; Wen, W.-W.; Lu, Y.-P.; Abdelmaksou, A.; Sun, M.-X.; Yuan, D.-T.; Zhao, H.-Y.; Xiong, N.-X.; et al. CD90 determined two subpopulations of glioma-associated mesenchymal stem cells with different roles in tumour progression. Cell Death Dis. 2018, 9, 1101.

52. Luzuriaga, J.; Pineda, J.R.; Irastorza, I.; Uribe-Etxebarria, V.; García-Gallastegui, P.; Encinas, J.M.; Chamero, P.; Unda, F.; Ibarretxe, G. BDNF and NT3 Reprogram Human Ectomesenchymal Dental Pulp Stem Cells to Neurogenic and Gliogenic Neural Crest Progenitors Cultured in Serum-Free Medium. Cell. Physiol. Biochem. 2019, 52, 1361–1380.

53. Zhang, S.; Han, Z.; Jing, Y.; Tao, S.; Li, T.; Wang, H.; Wang, Y.; Li, R.; Yang, Y.; Zhao, X.; et al. CD133(+)CXCR4(+) colon cancer cells exhibit metastatic potential and predict poor prognosis of patients. BMC Med. 2012, 10, 85.

54. Uribe-Etxebarria, V.; Agliano, A.; Unda, F.; Ibarretxe, G. Wnt signaling reprograms metabolism in dental pulp stem cells. J. Cell. Physiol. 2019, 234, 13068–13082.

55. Uribe-Etxebarria, V.; García-Gallastegui, P.; Pérez-Garrastachu, M.; Casado-Andrés, M.; Irastorza, I.; Unda, F.; Ibarretxe, G.; Subirán, N. Wnt-3a Induces Epigenetic Remodeling in Human Dental Pulp Stem Cells. Cells 2020, 9, 652.

56. Aurrekoetxea, M.; Garcia-Gallastegui, P.; Irastorza, I.; Luzuriaga, J.; Uribe-Etxebarria, V.; Unda, F.; Ibarretxe, G. Dental pulp stem cells as a multifaceted tool for bioengineering and the regeneration of craniomaxillofacial tissues. Front. Physiol. 2015, 6.

57. Asadi, M.H.; Khalifeh, K.; Mowla, S.J. OCT4 spliced variants are highly expressed in brain cancer tissues and inhibition of OCT4B1 causes G2/M arrest in brain cancer cells. J. Neurooncol. 2016, 130, 455–463.

58. D'Alimonte, I.; Nargi, E.; Mastrangelo, F.; Falco, G.; Lanuti, P.; Marchisio, M.; Miscia, S.; Robuffo, I.; Capogreco, M.; Buccella, S.; et al. Vascular endothelial growth factor enhances in vitro proliferation and osteogenic differentiation of human dental pulp stem cells. J. Biol. Regul. Homeost. Agents 2011, 25, 57–69.

59. Jing, F.; Kim, H.J.; Kim, C.H.; Kim, Y.J.; Lee, J.H.; Kim, H.R. Colon cancer stem cell markers CD44 and CD133 in patients with colorectal cancer and synchronous hepatic metastases. Int. J. Oncol. 2015, 46, 1582–1588.

60. Luzuriaga, J.; Pastor-Alonso, O.; Encinas, J.M.; Unda, F.; Ibarretxe, G.; Pineda, J.R. Human Dental Pulp Stem Cells Grown in Neurogenic Media Differentiate Into Endothelial Cells and Promote Neovasculogenesis in the Mouse Brain. Front. Physiol. 2019, 10, 347.

61. Zhang, M.; Xu, C.; Wang, H.-Z.; Peng, Y.-N.; Li, H.-O.; Zhou, Y.-J.; Liu, S.; Wang, F.; Liu, L.; Chang, Y.; et al. Soft fibrin matrix downregulates DAB2IP to promote Nanog-dependent growth of colon tumor-repopulating cells. Cell Death Dis. 2019, 10, 151.

62. Gregorian, C.; Nakashima, J.; Le Belle, J.; Ohab, J.; Kim, R.; Liu, A.; Smith, K.B.; Groszer, M.; Garcia, A.D.; Sofroniew, M.V.; et al. Pten deletion in adult neural stem/progenitor cells enhances constitutive neurogenesis. J. Neurosci. 2009, 29, 1874–1886.

63. Groszer, M.; Erickson, R.; Scripture-Adams, D.D.; Lesche, R.; Trumpp, A.; Zack, J.A.; Kornblum, H.I.; Liu, X.; Wu, H. Negative regulation of neural stem/progenitor cell proliferation by the Pten tumor suppressor gene in vivo. Science 2001, 294, 2186–2189.

64. He, X.C.; Yin, T.; Grindley, J.C.; Tian, Q.; Sato, T.; Tao, W.A.; Dirisina, R.; Porter-Westpfahl, K.S.; Hembree, M.; Johnson, T.; et al. PTEN-deficient intestinal stem cells initiate intestinal polyposis. Nat. Genet. 2007, 39, 189–198.

65. Shen, W.-C.; Lai, Y.-C.; Li, L.-H.; Liao, K.; Lai, H.-C.; Kao, S.-Y.; Wang, J.; Chuong, C.-M.; Hung, S.-C. Methylation and PTEN activation in dental pulp mesenchymal stem cells promotes osteogenesis and reduces oncogenesis. Nat. Commun. 2019, 10, 2226.

66. Pagella, P.; Miran, S.; Neto, E.; Martin, I.; Lamghari, M.; Mitsiadis, T.A. Human dental pulp stem cells exhibit enhanced properties in comparison to human bone marrow stem cells on neurites outgrowth. FASEB J. 2020.

67. Fan, L.-F.; Dong, W.-G.; Jiang, C.-Q.; Xia, D.; Liao, F.; Yu, Q.-F. Expression of putative stem cell genes Musashi-1 and beta1-integrin in human colorectal adenomas and adenocarcinomas. Int. J. Colorectal Dis. 2010, 25, 17–23.

68. Paschall, A.V.; Yang, D.; Lu, C.; Redd, P.S.; Choi, J.-H.; Heaton, C.M.; Lee, J.R.; Nayak-Kapoor, A.; Liu, K. CD133+CD24lo defines a 5-Fluorouracil-resistant colon cancer stem cell-like phenotype. Oncotarget 2016, 7, 78698–78712.

69. Duan, S.; Yuan, G.; Liu, X.; Ren, R.; Li, J.; Zhang, W.; Wu, J.; Xu, X.; Fu, L.; Li, Y.; et al. PTEN deficiency reprogrammes human neural stem cells towards a glioblastoma stem cell-like phenotype. Nat. Commun. 2015, 6, 10068.

70. Kaneko, Y.; Sakakibara, S.; Imai, T.; Suzuki, A.; Nakamura, Y.; Sawamoto, K.; Ogawa, Y.; Toyama, Y.; Miyata, T.; Okano, H. Musashi1: An evolutionarily conserved marker for CNS progenitor cells including neural stem cells. Dev. Neurosci. 2000, 22, 139–153.

71. Carmon, K.S.; Lin, Q.; Gong, X.; Thomas, A.; Liu, Q. LGR5 interacts and cointernalizes with Wnt receptors to modulate Wnt/β-catenin signaling. Mol. Cell. Biol. 2012, 32, 2054–2064.

72. Lundberg, I.V.; Edin, S.; Eklöf, V.; Öberg, Å.; Palmqvist, R.; Wikberg, M.L. SOX2 expression is associated with a cancer stem cell state and down-regulation of CDX2 in colorectal cancer. BMC Cancer 2016, 16, 471.

73. Chiou, G.-Y.; Yang, T.-W.; Huang, C.-C.; Tang, C.-Y.; Yen, J.-Y.; Tsai, M.-C.; Chen, H.-Y.; Fadhilah, N.; Lin, C.-C.; Jong, Y.-J. Musashi-1 promotes a cancer stem cell lineage and chemoresistance in colorectal cancer cells. Sci. Rep. 2017, 7, 2172.

74. Sakakibara, S.; Imai, T.; Hamaguchi, K.; Okabe, M.; Aruga, J.; Nakajima, K.; Yasutomi, D.; Nagata, T.; Kurihara, Y.; Uesugi, S.; et al. Mouse-Musashi-1, a neural RNA-binding protein highly enriched in the mammalian CNS stem cell. Dev. Biol. 1996, 176, 230–242.

75. Hattermann, K.; Flüh, C.; Engel, D.; Mehdorn, H.M.; Synowitz, M.; Mentlein, R.; Held-Feindt, J. Stem cell markers in glioma progression and recurrence. Int. J. Oncol. 2016, 49, 1899–1910.

76. Chen, H.-Y.; Lin, L.-T.; Wang, M.-L.; Laurent, B.; Hsu, C.-H.; Pan, C.-M.; Jiang, W.-R.; Chen, P.-Y.; Ma, H.-I.; Chen, Y.-W.; et al. Musashi-1 Enhances Glioblastoma Cell Migration and Cytoskeletal Dynamics through Translational Inhibition of Tensin3. Sci. Rep. 2017, 7, 8710.

77. Beckervordersandforth, R.; Tripathi, P.; Ninkovic, J.; Bayam, E.; Lepier, A.; Stempfhuber, B.; Kirchhoff, F.; Hirrlinger, J.; Haslinger, A.; Lie, D.C.; et al. In vivo fate mapping and expression analysis reveals molecular hallmarks of prospectively isolated adult neural stem cells. Cell Stem Cell 2010, 7, 744–758.

78. Lin, J.-C.; Tsai, J.-T.; Chao, T.-Y.; Ma, H.-I.; Liu, W.-H. Musashi-1 Enhances Glioblastoma Migration by Promoting ICAM1 Translation. Neoplasia 2019, 21, 459–468.

79. Schwartz, P.H.; Bryant, P.J.; Fuja, T.J.; Su, H.; O'Dowd, D.K.; Klassen, H. Isolation and characterization of neural progenitor cells from post-mortem human cortex. J. Neurosci. Res. 2003, 74, 838–851.

80. Bakopoulou, A.; Apatzidou, D.; Aggelidou, E.; Gousopoulou, E.; Leyhausen, G.; Volk, J.; Kritis, A.; Koidis, P.; Geurtsen, W. Isolation and prolonged expansion of oral mesenchymal stem cells under clinical-grade, GMP-compliant conditions differentially affects "stemness" properties. Stem Cell Res. Ther. 2017, 8, 247.

81. Hill, R.; Wu, H. PTEN, Stem Cells, and Cancer Stem Cells. J. Biol. Chem. 2009, 284, 11755–11759.

82. Li, L.; Liu, F.; Ross, A.H. PTEN regulation of neural development and CNS stem cells. J. Cell Biochem. 2003, 88, 24–28.

83. Amiri, A.; Cho, W.; Zhou, J.; Birnbaum, S.G.; Sinton, C.M.; McKay, R.M.; Parada, L.F. Pten Deletion in Adult Hippocampal Neural Stem/Progenitor Cells Causes Cellular Abnormalities and Alters Neurogenesis. J. Neurosci. 2012, 32, 5880–5890.

84. Schoumacher, M.; Goldman, R.D.; Louvard, D.; Vignjevic, D.M. Actin, microtubules, and vimentin intermediate filaments cooperate for elongation of invadopodia. J. Cell Biol. 2010, 189, 541–556.

85. Kumar, A.; Bhanja, A.; Bhattacharyya, J.; Jaganathan, B.G. Multiple roles of CD90 in cancer. Tumour Biol. 2016, 37, 11611–11622.

86. Shafaei, S.; Sharbatdaran, M.; Kamrani, G.; Khafri, S. The association between CD166 detection rate and clinicopathologic parameters of patients with colorectal cancer. Caspian J. Intern. Med. 2013, 4, 768–772.

87. 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.

88. Li, J.; Wang, R.; Yang, L.; Wu, Q.; Wang, Q.; Nie, Z.; Yu, Y.; Ma, J.; Pan, Q. Knockdown of Nestin inhibits proliferation and migration of colorectal cancer cells. Int. J. Clin. Exp. Pathol. 2015, 8, 6377–6386.

89. Gervois, P.; Struys, T.; Hilkens, P.; Bronckaers, A.; Ratajczak, J.; Politis, C.; Brone, B.; Lambrichts, I.; Martens, W. Neurogenic maturation of human dental pulp stem cells following neurosphere generation induces morphological and electrophysiological characteristics of functional neurons. Stem Cells Dev. 2015, 24, 296–311.

90. Martens, W.; Sanen, K.; Georgiou, M.; Struys, T.; Bronckaers, A.; Ameloot, M.; Phillips, J.; Lambrichts, I. Human dental pulp stem cells can differentiate into Schwann cells and promote and guide neurite outgrowth in an aligned tissue-engineered collagen construct in vitro. FASEB J. 2014, 28, 1634–1643.

91. Parry, P.V.; Engh, J.A. CD90 is identified as a marker for cancer stem cells in high-grade gliomas using tissue microarrays. Neurosurgery 2012, 70, N23–N24.

92. Yang, X.H.; Wu, Q.L.; Yu, X.B.; Xu, C.X.; Ma, B.F.; Zhang, X.M.; Li, S.N.; Lahn, B.T.; Xiang, A.P. Nestin expression in different tumours and its relevance to malignant grade. J. Clin. Pathol. 2008, 61, 467–473.

93. Lv, D.; Lu, L.; Hu, Z.; Fei, Z.; Liu, M.; Wei, L.; Xu, J. Nestin Expression Is Associated with Poor Clinicopathological Features and Prognosis in Glioma Patients: An Association Study and Meta-analysis. Mol. Neurobiol. 2017, 54, 727–735.

94. Ariza, A.; López, D.; Castellà, E.M.; Muñoz, C.; Zújar, M.J.; Mate, J.L. Expression of CD15 in normal and metaplastic Paneth cells of the digestive tract. J. Clin. Pathol. 1996, 49, 474–477.

95. Park, D.; Xiang, A.P.; Mao, F.F.; Zhang, L.; Di, C.-G.; Liu, X.-M.; Shao, Y.; Ma, B.-F.; Lee, J.-H.; Ha, K.-S.; et al. Nestin is required for the proper self-renewal of neural stem cells. Stem Cells 2010, 28, 2162–2171.

96. Yadav, V.N.; Zamler, D.; Baker, G.J.; Kadiyala, P.; Erdreich-Epstein, A.; DeCarvalho, A.C.; Mikkelsen, T.; Castro, M.G.; Lowenstein, P.R. CXCR4 increases in-vivo glioma perivascular invasion, and reduces radiation induced apoptosis: A genetic knockdown study. Oncotarget 2016, 7, 83701–83719.

97. Wu, W.; Cao, J.; Ji, Z.; Wang, J.; Jiang, T.; Ding, H. Co-expression of Lgr5 and CXCR4 characterizes cancer stem-like cells of colorectal cancer. Oncotarget 2016, 7, 81144–81155.

98. Xiu, G.; Li, X.; Yin, Y.; Li, J.; Li, B.; Chen, X.; Liu, P.; Sun, J.; Ling, B. SDF-1/CXCR4 Augments the Therapeutic Effect of Bone Marrow Mesenchymal Stem Cells in the Treatment of Lipopolysaccharide-Induced Liver Injury by Promoting Their Migration Through PI3K/Akt Signaling Pathway. Cell Transplant. 2020, 29, 963689720929992.

99. Potdar, P.D.; Jethmalani, Y.D. Human dental pulp stem cells: Applications in future regenerative medicine. World J. Stem Cells 2015, 7, 839–851.

100. Kim, J.-H.; Jeon, M.; Song, J.-S.; Lee, J.-H.; Choi, B.-J.; Jung, H.-S.; Moon, S.J.; DenBesten, P.K.; Kim, S.-O. Distinctive genetic activity pattern of the human dental pulp between deciduous and permanent teeth. PLoS ONE 2014, 9, e102893.

101. Hermann, A.; Gastl, R.; Liebau, S.; Popa, M.O.; Fiedler, J.; Boehm, B.O.; Maisel, M.; Lerche, H.; Schwarz, J.; Brenner, R.; et al. Efficient generation of neural stem cell-like cells from adult human bone marrow stromal cells. J. Cell. Sci. 2004, 117, 4411–4422.

102. Shmelkov, S.V.; Butler, J.M.; Hooper, A.T.; Hormigo, A.; Kushner, J.; Milde, T.; St Clair, R.; Baljevic, M.; White, I.; Jin, D.K.; et al. CD133 expression is not restricted to stem cells, and both CD133+ and CD133- metastatic colon cancer cells initiate tumors. J. Clin. Investig. 2008, 118, 2111–2120.

103. Choi, D.; Lee, H.-W.; Hur, K.-Y.; Kim, J.-J.; Park, G.-S.; Jang, S.-H.; Song, Y.-S.; Jang, K.-S.; Paik, S.-S. Cancer stem cell markers CD133 and CD24 correlate with invasiveness and differentiation in colorectal adenocarcinoma. World J. Gastroenterol. 2009, 15, 2258–2264.

104. Glumac, P.M.; LeBeau, A.M. The role of CD133 in cancer: A concise review. Clin. Transl. Med. 2018, 7, 18.

105. Uchida, N.; Buck, D.W.; He, D.; Reitsma, M.J.; Masek, M.; Phan, T.V.; Tsukamoto, A.S.; Gage, F.H.; Weissman, I.L. Direct isolation of human central nervous system stem cells. Proc. Natl. Acad. Sci. USA 2000, 97, 14720–14725.

106. Florek, M.; Haase, M.; Marzesco, A.-M.; Freund, D.; Ehninger, G.; Huttner, W.B.; Corbeil, D. Prominin-1/CD133, a neural and hematopoietic stem cell marker, is expressed in adult human differentiated cells and certain types of kidney cancer. Cell Tissue Res. 2005, 319, 15–26.

107. Virant-Klun, I.; Skerl, P.; Novakovic, S.; Vrtacnik-Bokal, E.; Smrkolj, S. Similar Population of CD133+ and DDX4+ VSEL-Like Stem Cells Sorted from Human Embryonic Stem Cell, Ovarian, and Ovarian Cancer Ascites Cell Cultures: The Real Embryonic Stem Cells? Cells 2019, 8, 706.

108. Lottaz, C.; Beier, D.; Meyer, K.; Kumar, P.; Hermann, A.; Schwarz, J.; Junker, M.; Oefner, P.J.; Bogdahn, U.; Wischhusen, J.; et al. Transcriptional profiles of CD133+ and CD133- glioblastoma-derived cancer stem cell lines suggest different cells of origin. Cancer Res. 2010, 70, 2030–2040.

109. Viña-Almunia, J.; Mas-Bargues, C.; Borras, C.; Gambini, J.; El Alami, M.; Sanz-Ros, J.; Peñarrocha, M.; Vina, J. Influence of Partial O_2 Pressure on the Adhesion, Proliferation, and Osteogenic Differentiation of Human Dental Pulp Stem Cells on β-Tricalcium Phosphate Scaffold. Int. J. Oral. Maxillofac. Implants 2017, 32, 1251–1256.

110. Bonnamain, V.; Thinard, R.; Sergent-Tanguy, S.; Huet, P.; Bienvenu, G.; Naveilhan, P.; Farges, J.-C.; Alliot-Licht, B. Human dental pulp stem cells cultured in serum-free supplemented medium. Front. Physiol 2013, 4.

111. Lee, T.-C.; Lee, T.-H.; Huang, Y.-H.; Chang, N.-K.; Lin, Y.-J.; Chien, P.-W.C.; Yang, W.-H.; Lin, M.H.-C. Comparison of surface markers between human and rabbit mesenchymal stem cells. PLoS ONE 2014, 9, e111390.

112. Feng, B.-H.; Liu, A.-G.; Gu, W.-G.; Deng, L.; Cheng, X.-G.; Tong, T.-J.; Zhang, H.-Z. CD133+ subpopulation of the HT1080 human fibrosarcoma cell line exhibits cancer stem-like characteristics. Oncol. Rep. 2013, 30, 815–823.

113. Irollo, E.; Pirozzi, G. CD133: To be or not to be, is this the real question? Am. J. Transl Res. 2013, 5, 563–581.

114. Silvestre, D.C.; Pineda, J.R.; Hoffschir, F.; Studler, J.M.; Mouthon, M.A.; Pflumio, F.; Junier, M.P.; Chneiweiss, H.; Boussin, F.D. Alternative lengthening of telomeres in human glioma stem cells. Stem Cells 2011, 29, 440–451.

115. Hadaegh, Y.; Niknam, M.; Attar, A.; Maharlooei, M.K.; Tavangar, M.S.; Aarabi, A.M.; Monabati, A. Characterization of stem cells from the pulp of unerupted third molar tooth. Indian J. Dent. Res. 2014, 25, 14–21.

116. Sokol, S.Y. Maintaining embryonic stem cell pluripotency with Wnt signaling. Development 2011, 138, 4341–4350.

117. Barraud, P.; Stott, S.; Møllgård, K.; Parmar, M.; Björklund, A. In vitro characterization of a human neural progenitor cell coexpressing SSEA4 and CD133. J. Neurosci. Res. 2007, 85, 250–259.

118. Patru, C.; Romao, L.; Varlet, P.; Coulombel, L.; Raponi, E.; Cadusseau, J.; Renault-Mihara, F.; Thirant, C.; Leonard, N.; Berhneim, A.; et al. CD133, CD15/SSEA-1, CD34 or side populations do not resume tumor-initiating properties of long-term cultured cancer stem cells from human malignant glio-neuronal tumors. BMC Cancer 2010, 10, 66.

119. Kahlert, U.D.; Bender, N.O.; Maciaczyk, D.; Bogiel, T.; Bar, E.E.; Eberhart, C.G.; Nikkhah, G.; Maciaczyk, J. CD133/CD15 defines distinct cell subpopulations with differential in vitro clonogenic activity and stem cell-related gene expression profile in in vitro propagated glioblastoma multiforme-derived cell line with a PNET-like component. Folia Neuropathol. 2012, 50, 357–368.

120. Friedman, G.K.; Moore, B.P.; Nan, L.; Kelly, V.M.; Etminan, T.; Langford, C.P.; Xu, H.; Han, X.; Markert, J.M.; Beierle, E.A.; et al. Pediatric medulloblastoma xenografts including molecular subgroup 3 and CD133+ and CD15+ cells are sensitive to killing by oncolytic herpes simplex viruses. Neuro-Oncology 2016, 18, 227–235.

121. Yudoh, K.; Matsui, H.; Tsuji, H. Nitric oxide induced by tumor cells activates tumor cell adhesion to endothelial cells and permeability of the endothelium in vitro. Clin. Exp. Metastasis 1997, 15, 557–567.

122. Satoh, J.; Obayashi, S.; Tabunoki, H.; Wakana, T.; Kim, S.U. Stable expression of neurogenin 1 induces LGR5, a novel stem cell marker, in an immortalized human neural stem cell line HB1.F3. Cell. Mol. Neurobiol. 2010, 30, 415–426.

123. Xu, L.; Lin, W.; Wen, L.; Li, G. Lgr5 in cancer biology: Functional identification of Lgr5 in cancer progression and potential opportunities for novel therapy. Stem Cell Res. Ther. 2019, 10, 219.

124. Barker, N.; Clevers, H. Tracking down the stem cells of the intestine: Strategies to identify adult stem cells. Gastroenterology 2007, 133, 1755–1760.

125. de Sousa e Melo, F.; Kurtova, A.V.; Harnoss, J.M.; Kljavin, N.; Hoeck, J.D.; Hung, J.; Anderson, J.E.; Storm, E.E.; Modrusan, Z.; Koeppen, H.; et al. A distinct role for Lgr5+ stem cells in primary and metastatic colon cancer. Nature 2017, 543, 676–680.

126. Barker, N.; van Es, J.H.; Kuipers, J.; Kujala, P.; van den Born, M.; Cozijnsen, M.; Haegebarth, A.; Korving, J.; Begthel, H.; Peters, P.J.; et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 2007, 449,

1003–1007.

127. Snyder, J.C.; Rochelle, L.K.; Marion, S.; Lyerly, H.K.; Barak, L.S.; Caron, M.G. Lgr4 and Lgr5 drive the formation of long actin-rich cytoneme-like membrane protrusions. J. Cell Sci. 2015, 128, 1230–1240.

128. Lin, W.; Xu, L.; Lin, S.; Shi, L.; Wang, B.; Pan, Q.; Lee, W.Y.W.; Li, G. Characterisation of multipotent stem cells from human peripheral blood using an improved protocol. J. Orthop. Translat. 2019, 19, 18–28.

129. Rot, S.; Taubert, H.; Bache, M.; Greither, T.; Würl, P.; Eckert, A.W.; Schubert, J.; Vordermark, D.; Kappler, M. A novel splice variant of the stem cell marker LGR5/GPR49 is correlated with the risk of tumor-related death in soft-tissue sarcoma patients. BMC Cancer 2011, 11, 429.

130. Ganju, R.K.; Brubaker, S.A.; Meyer, J.; Dutt, P.; Yang, Y.; Qin, S.; Newman, W.; Groopman, J.E. The alpha-chemokine, stromal cell-derived factor-1alpha, binds to the transmembrane G-protein-coupled CXCR-4 receptor and activates multiple signal transduction pathways. J. Biol. Chem. 1998, 273, 23169–23175.

131. Li, M.; Sun, X.; Ma, L.; Jin, L.; Zhang, W.; Xiao, M.; Yu, Q. SDF-1/CXCR4 axis induces human dental pulp stem cell migration through FAK/PI3K/Akt and GSK3β/β-catenin pathways. Sci. Rep. 2017, 7, 40161.

132. Su, W.; Foster, S.C.; Xing, R.; Feistel, K.; Olsen, R.H.J.; Acevedo, S.F.; Raber, J.; Sherman, L.S. CD44 Transmembrane Receptor and Hyaluronan Regulate Adult Hippocampal Neural Stem Cell Quiescence and Differentiation. J. Biol. Chem. 2017, 292, 4434–4445.

133. Nishikawa, M.; Inoue, A.; Ohnishi, T.; Kohno, S.; Ohue, S.; Matsumoto, S.; Suehiro, S.; Yamashita, D.; Ozaki, S.; Watanabe, H.; et al. Significance of Glioma Stem-Like Cells in the Tumor Periphery That Express High Levels of CD44 in Tumor Invasion, Early Progression, and Poor Prognosis in Glioblastoma. Stem Cells Int. 2018, 2018, 5387041.

134. Rimkus, T.K.; Carpenter, R.L.; Sirkisoon, S.; Zhu, D.; Pasche, B.C.; Chan, M.D.; Lesser, G.J.; Tatter, S.B.; Watabe, K.; Debinski, W.; et al. Truncated Glioma-Associated Oncogene Homolog 1 (tGLI1) Mediates Mesenchymal Glioblastoma via Transcriptional Activation of CD44. Cancer Res. 2018, 78, 2589–2600.

135. Wang, H.-H.; Liao, C.-C.; Chow, N.-H.; Huang, L.L.-H.; Chuang, J.-I.; Wei, K.-C.; Shin, J.-W. Whether CD44 is an applicable marker for glioma stem cells. Am. J. Transl Res. 2017, 9, 4785–4806.

136. Macrin, D.; Alghadeer, A.; Zhao, Y.T.; Miklas, J.W.; Hussein, A.M.; Detraux, D.; Robitaille, A.M.; Madan, A.; Moon, R.T.; Wang, Y.; et al. Metabolism as an early predictor of DPSCs aging. Sci. Rep. 2019, 9, 2195.

137. Singh, S.K.; Hawkins, C.; Clarke, I.D.; Squire, J.A.; Bayani, J.; Hide, T.; Henkelman, R.M.; Cusimano, M.D.; Dirks, P.B. Identification of human brain tumour initiating cells. Nature 2004, 432, 396–401.

138. Ehrmann, J.; Kolár, Z.; Mokry, J. Nestin as a diagnostic and prognostic marker: Immunohistochemical analysis of its expression in different tumours. J. Clin. Pathol. 2005, 58, 222–223.

139. Amoh, Y.; Yang, M.; Li, L.; Reynoso, J.; Bouvet, M.; Moossa, A.R.; Katsuoka, K.; Hoffman, R.M. Nestin-linked green fluorescent protein transgenic nude mouse for imaging human tumor angiogenesis. Cancer Res. 2005, 65, 5352–5357.

140. Potten, C.S.; Booth, C.; Tudor, G.L.; Booth, D.; Brady, G.; Hurley, P.; Ashton, G.; Clarke, R.; Sakakibara, S.; Okano, H. Identification of a putative intestinal stem cell and early lineage marker; musashi-1. Differentiation 2003, 71, 28–41.

141. Wang, Y.; Jiang, C.-Q.; Fan, L.-F. Correlation of Musashi-1, Lgr5, and pEGFR expressions in human small intestinal adenocarcinomas. Tumour Biol. 2015, 36, 6075–6082.

142. Padial-Molina, M.; de Buitrago, J.G.; Sainz-Urruela, R.; Abril-Garcia, D.; Anderson, P.; O'Valle, F.; Galindo-Moreno, P. Expression of Musashi-1 During Osteogenic Differentiation of Oral MSC: An In Vitro Study. Int. J. Mol. Sci. 2019, 20, 2171.

143. Zhang, S.; Cui, W. Sox2, a key factor in the regulation of pluripotency and neural differentiation. World J. Stem Cells 2014, 6, 305–311.

144. Wen, K.; Fu, Z.; Wu, X.; Feng, J.; Chen, W.; Qian, J. Oct-4 is required for an antiapoptotic behavior of chemoresistant colorectal cancer cells enriched for cancer stem cells: Effects associated with STAT3/Survivin. Cancer Lett. 2013, 333, 56–65.

145. Vincent, P.H.; Benedikz, E.; Uhlén, P.; Hovatta, O.; Sundström, E. Expression of Pluripotency Markers in Nonpluripotent Human Neural Stem and Progenitor Cells. Stem Cells Dev. 2017, 26, 876–887.

146. Liu, T.; Xu, H.; Huang, M.; Ma, W.; Saxena, D.; Lustig, R.A.; Alonso-Basanta, M.; Zhang, Z.; O'Rourke, D.M.; Zhang, L.; et al. Circulating Glioma Cells Exhibit Stem Cell-like Properties. Cancer Res. 2018, 78, 6632–6642.

147. Schubert, M.; Holland, L.Z. The Wnt Gene Family and the Evolutionary Conservation of Wnt Expression; Landes Bioscience: Austin, TX, USA, 2013.

148. González-Sancho, J.M.; Aguilera, O.; García, J.M.; Pendás-Franco, N.; Peña, C.; Cal, S.; García de Herreros, A.; Bonilla, F.; Muñoz, A. The Wnt antagonist DICKKOPF-1 gene is a downstream target of beta-catenin/TCF and is

downregulated in human colon cancer. Oncogene 2005, 24, 1098–1103.

149. Jung, Y.-S.; Park, J.-I. Wnt signaling in cancer: Therapeutic targeting of Wnt signaling beyond β-catenin and the destruction complex. Exp. Mol. Med. 2020, 52, 183–191.

150. Duchartre, Y.; Kim, Y.-M.; Kahn, M. The Wnt signaling pathway in cancer. Crit. Rev. Oncol. Hematol. 2016, 99, 141–149.

151. Zhang, L.; Shay, J.W. Multiple Roles of APC and its Therapeutic Implications in Colorectal Cancer. J. Natl. Cancer Inst. 2017, 109.

152. de Roo, J.J.D.; Breukel, C.; Chhatta, A.R.; Linssen, M.M.; Vloemans, S.A.; Salvatori, D.; Mikkers, H.M.M.; Verbeek, S.J.; Staal, F.J.T. Axin2-mTurquoise2: A novel reporter mouse model for the detection of canonical Wnt signalling. Genesis 2017, 55.

153. Kim, B.-M.; Mao, J.; Taketo, M.M.; Shivdasani, R.A. Phases of canonical Wnt signaling during the development of mouse intestinal epithelium. Gastroenterology 2007, 133, 529–538.

154. Haegebarth, A.; Clevers, H. Wnt signaling, lgr5, and stem cells in the intestine and skin. Am. J. Pathol. 2009, 174, 715–721.

155. Kahlert, U.D.; Mooney, S.M.; Natsumeda, M.; Steiger, H.-J.; Maciaczyk, J. Targeting cancer stem-like cells in glioblastoma and colorectal cancer through metabolic pathways. Int. J. Cancer 2017, 140, 10–22.

156. Lie, D.-C.; Colamarino, S.A.; Song, H.-J.; Désiré, L.; Mira, H.; Consiglio, A.; Lein, E.S.; Jessberger, S.; Lansford, H.; Dearie, A.R.; et al. Wnt signalling regulates adult hippocampal neurogenesis. Nature 2005, 437, 1370–1375.

157. Zhou, F.; Cao, W.; Xu, R.; Zhang, J.; Yu, T.; Xu, X.; Zhi, T.; Yin, J.; Cao, S.; Liu, N.; et al. MicroRNA-206 attenuates glioma cell proliferation, migration, and invasion by blocking the WNT/β-catenin pathway via direct targeting of Frizzled 7 mRNA. Am. J. Transl Res. 2019, 11, 4584–4601.

158. Augustin, I.; Goidts, V.; Bongers, A.; Kerr, G.; Vollert, G.; Radlwimmer, B.; Hartmann, C.; Herold-Mende, C.; Reifenberger, G.; von Deimling, A.; et al. The Wnt secretion protein Evi/Gpr177 promotes glioma tumourigenesis. EMBO Mol. Med. 2012, 4, 38–51.

159. Yu, J.M.; Jun, E.S.; Jung, J.S.; Suh, S.Y.; Han, J.Y.; Kim, J.Y.; Kim, K.W.; Jung, J.S. Role of Wnt5a in the proliferation of human glioblastoma cells. Cancer Lett. 2007, 257, 172–181.

160. Zuccarini, M.; Giuliani, P.; Ziberi, S.; Carluccio, M.; Iorio, P.D.; Caciagli, F.; Ciccarelli, R. The Role of Wnt Signal in Glioblastoma Development and Progression: A Possible New Pharmacological Target for the Therapy of This Tumor. Genes 2018, 9, 105.

161. Shevchenko, V.; Arnotskaya, N.; Korneyko, M.; Zaytsev, S.; Khotimchenko, Y.; Sharma, H.; Bryukhovetskiy, I. Proteins of the Wnt signaling pathway as targets for the regulation of CD133+ cancer stem cells in glioblastoma. Oncol. Rep. 2019, 41, 3080–3088.

162. Gonçalves, C.S.; Vieira de Castro, J.; Pojo, M.; Martins, E.P.; Queirós, S.; Chautard, E.; Taipa, R.; Pires, M.M.; Pinto, A.A.; Pardal, F.; et al. WNT6 is a novel oncogenic prognostic biomarker in human glioblastoma. Theranostics 2018, 8, 4805–4823.

163. Portela, M.; Venkataramani, V.; Fahey-Lozano, N.; Seco, E.; Losada-Perez, M.; Winkler, F.; Casas-Tintó, S. Glioblastoma cells vampirize WNT from neurons and trigger a JNK/MMP signaling loop that enhances glioblastoma progression and neurodegeneration. PLoS Biol. 2019, 17, e3000545.

164. Rajakulendran, N.; Rowland, K.J.; Selvadurai, H.J.; Ahmadi, M.; Park, N.I.; Naumenko, S.; Dolma, S.; Ward, R.J.; So, M.; Lee, L.; et al. Wnt and Notch signaling govern self-renewal and differentiation in a subset of human glioblastoma stem cells. Genes Dev. 2019, 33, 498–510.

165. Oskarsson, T.; Batlle, E.; Massagué, J. Metastatic stem cells: Sources, niches, and vital pathways. Cell Stem Cell 2014, 14, 306–321.

166. Valdor, R.; García-Bernal, D.; Riquelme, D.; Martinez, C.M.; Moraleda, J.M.; Cuervo, A.M.; Macian, F.; Martinez, S. Glioblastoma ablates pericytes antitumor immune function through aberrant up-regulation of chaperone-mediated autophagy. Proc. Natl. Acad. Sci. USA 2019, 116, 20655–20665.

167. Sakaki-Yumoto, M.; Katsuno, Y.; Derynck, R. TGF-β family signaling in stem cells. Biochim. Biophys. Acta 2013, 1830, 2280–2296.

168. Weiss, A.; Attisano, L. The TGFbeta superfamily signaling pathway. Wiley Interdiscip Rev. Dev. Biol. 2013, 2, 47–63.

169. Daynac, M.; Pineda, J.R.; Chicheportiche, A.; Gauthier, L.R.; Morizur, L.; Boussin, F.D.; Mouthon, M.-A. TGFβ lengthens the G1 phase of stem cells in aged mouse brain. Stem Cells 2014, 32, 3257–3265.

170. Pineda, J.R.; Daynac, M.; Chicheportiche, A.; Cebrian-Silla, A.; Sii Felice, K.; Garcia-Verdugo, J.M.; Boussin, F.D.; Mouthon, M.-A. Vascular-derived TGF-β increases in the stem cell niche and perturbs neurogenesis during aging and following irradiation in the adult mouse brain. EMBO Mol. Med. 2013, 5, 548–562.

171. 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.

172. Flanders, K.C.; Ren, R.F.; Lippa, C.F. Transforming growth factor-betas in neurodegenerative disease. Prog. Neurobiol. 1998, 54, 71–85.

173. Derynck, R.; Goeddel, D.V.; Ullrich, A.; Gutterman, J.U.; Williams, R.D.; Bringman, T.S.; Berger, W.H. Synthesis of messenger RNAs for transforming growth factors alpha and beta and the epidermal growth factor receptor by human tumors. Cancer Res. 1987, 47, 707–712.

174. TGFβ Promotes Immune Evasion to Limit the Efficacy of Anti-PD-1/PD-L1. Cancer Discov 2018, 8, OF10.

175. Platten, M.; Wick, W.; Weller, M. Malignant glioma biology: Role for TGF-beta in growth, motility, angiogenesis, and immune escape. Microsc. Res. Tech. 2001, 52, 401–410.

176. Derynck, R.; Akhurst, R.J.; Balmain, A. TGF-beta signaling in tumor suppression and cancer progression. Nat. Genet. 2001, 29, 117–129.

177. Bellomo, C.; Caja, L.; Moustakas, A. Transforming growth factor β as regulator of cancer stemness and metastasis. Br. J. Cancer 2016, 115, 761–769.

178. Vallier, L.; Mendjan, S.; Brown, S.; Chng, Z.; Teo, A.; Smithers, L.E.; Trotter, M.W.B.; Cho, C.H.-H.; Martinez, A.; Rugg-Gunn, P.; et al. Activin/Nodal signalling maintains pluripotency by controlling Nanog expression. Development 2009, 136, 1339–1349.

179. Liu, J.; Jin, T.; Chang, S.; Ritchie, H.H.; Smith, A.J.; Clarkson, B.H. Matrix and TGF-beta-related gene expression during human dental pulp stem cell (DPSC) mineralization. In Vitro Cell. Dev. Biol. Anim. 2007, 43, 120–128.

180. Qi, Z.; Li, Y.; Zhao, B.; Xu, C.; Liu, Y.; Li, H.; Zhang, B.; Wang, X.; Yang, X.; Xie, W.; et al. BMP restricts stemness of intestinal Lgr5+ stem cells by directly suppressing their signature genes. Nat. Commun. 2017, 8, 13824.

181. Ahmadi, A.; Najafi, M.; Farhood, B.; Mortezaee, K. Transforming growth factor-β signaling: Tumorigenesis and targeting for cancer therapy. J. Cell. Physiol. 2019, 234, 12173–12187.

182. Zhou, X.; Geng, L.; Wang, D.; Yi, H.; Talmon, G.; Wang, J. R-Spondin1/LGR5 Activates TGFβ Signaling and Suppresses Colon Cancer Metastasis. Cancer Res. 2017, 77, 6589–6602.

183. Calon, A.; Espinet, E.; Palomo-Ponce, S.; Tauriello, D.V.F.; Iglesias, M.; Céspedes, M.V.; Sevillano, M.; Nadal, C.; Jung, P.; Zhang, X.H.-F.; et al. Dependency of colorectal cancer on a TGF-β-driven program in stromal cells for metastasis initiation. Cancer Cell 2012, 22, 571–584.

184. Ulaner, G.A.; Hu, J.F.; Vu, T.H.; Giudice, L.C.; Hoffman, A.R. Telomerase activity in human development is regulated by human telomerase reverse transcriptase (hTERT) transcription and by alternate splicing of hTERT transcripts. Cancer Res. 1998, 58, 4168–4172.

185. Chen, K.; Chen, L.; Li, L.; Qu, S.; Yu, B.; Sun, Y.; Wan, F.; Chen, X.; Liang, R.; Zhu, X. A positive feedback loop between Wnt/β-catenin signaling and hTERT regulates the cancer stem cell-like traits in radioresistant nasopharyngeal carcinoma cells. J. Cell. Biochem. 2020.

186. Park, J.-I.; Venteicher, A.S.; Hong, J.Y.; Choi, J.; Jun, S.; Shkreli, M.; Chang, W.; Meng, Z.; Cheung, P.; Ji, H.; et al. Telomerase modulates Wnt signalling by association with target gene chromatin. Nature 2009, 460, 66–72.

187. Saha, A.; Shree Padhi, S.; Roy, S.; Banerjee, B. HCT116 colonospheres shows elevated expression of hTERT and β-catenin protein—A short report. J. Stem Cells 2014, 9, 243–251.

188. Kerem Terali, K. On the Far Side of Telomeres: The Many Roles of Telomerase in the Acquisition and Retention of Cancer Stemness. In Telomere—A Complex End of a Chromosome; Intechopen: London, UK, 2016; pp. 1–28. ISBN 978-953-51-2753-6.

189. Rhyu, M.S. Telomeres, telomerase, and immortality. J. Natl. Cancer Inst. 1995, 87, 884–894.

190. Beck, S.; Jin, X.; Sohn, Y.-W.; Kim, J.-K.; Kim, S.-H.; Yin, J.; Pian, X.; Kim, S.-C.; Nam, D.-H.; Choi, Y.-J.; et al. Telomerase Activity-Independent Function of TERT Allows Glioma Cells to Attain Cancer Stem Cell Characteristics by Inducing EGFR Expression. Mol. Cells 2011, 31, 9–15.

191. Gunes, C.; Avila, A.I.; Rudolph, K.L. Telomeres in cancer. Differentiation 2018, 99, 41–50.

192. Tanaka, K.; Hirota, T. Chromosomal instability: A common feature and a therapeutic target of cancer. Biochim. Biophys. Acta 2016, 1866, 64–75.

193. McClelland, S.E. Role of chromosomal instability in cancer progression. Endocr. Relat. Cancer 2017, 24, T23–T31.

194. Ishaq, A.; Hanson, P.S.; Morris, C.M.; Saretzki, G. Telomerase Activity is Downregulated Early During Human Brain Development. Genes 2016, 7, 27.

195. Hiyama, E.; Hiyama, K. Telomere and telomerase in stem cells. Br. J. Cancer 2007, 96, 1020–1024.

196. Schepers, A.G.; Vries, R.; van den Born, M.; van de Wetering, M.; Clevers, H. Lgr5 intestinal stem cells have high telomerase activity and randomly segregate their chromosomes. EMBO J. 2011, 30, 1104–1109.

197. Ninagawa, N.; Murakami, R.; Isobe, E.; Tanaka, Y.; Nakagawa, H.; Torihashi, S. Mesenchymal stem cells originating from ES cells show high telomerase activity and therapeutic benefits. Differentiation 2011, 82, 153–164.

198. Horibe, H.; Murakami, M.; Iohara, K.; Hayashi, Y.; Takeuchi, N.; Takei, Y.; Kurita, K.; Nakashima, M. Isolation of a stable subpopulation of mobilized dental pulp stem cells (MDPSCs) with high proliferation, migration, and regeneration potential is independent of age. PLoS ONE 2014, 9, e98553.

199. Jeon, B.-G.; Kang, E.-J.; Kumar, B.M.; Maeng, G.-H.; Ock, S.-A.; Kwack, D.-O.; Park, B.-W.; Rho, G.-J. Comparative analysis of telomere length, telomerase and reverse transcriptase activity in human dental stem cells. Cell Transplant. 2011, 20, 1693–1705.

200. Caporaso, G.L.; Lim, D.A.; Alvarez-Buylla, A.; Chao, M.V. Telomerase activity in the subventricular zone of adult mice. Mol. Cell Neurosci. 2003, 23, 693–702.

201. Ferrón, S.; Mira, H.; Franco, S.; Cano-Jaimez, M.; Bellmunt, E.; Ramírez, C.; Fariñas, I.; Blasco, M.A. Telomere shortening and chromosomal instability abrogates proliferation of adult but not embryonic neural stem cells. Development 2004, 131, 4059–4070.

202. Ferrón, S.R.; Marqués-Torrejón, M.A.; Mira, H.; Flores, I.; Taylor, K.; Blasco, M.A.; Fariñas, I. Telomere shortening in neural stem cells disrupts neuronal differentiation and neuritogenesis. J. Neurosci. 2009, 29, 14394–14407.

203. Liu, M.-Y.; Nemes, A.; Zhou, Q.-G. The Emerging Roles for Telomerase in the Central Nervous System. Front. Mol. Neurosci. 2018, 11, 160.

204. Miura, T.; Katakura, Y.; Yamamoto, K.; Uehara, N.; Tsuchiya, T.; Kim, E.H.; Shirahata, S. Neural stem cells lose telomerase activity upon differentiating into astrocytes. Cytotechnology 2001, 36, 137–144.

205. Kim, N.W.; Piatyszek, M.A.; Prowse, K.R.; Harley, C.B.; West, M.D.; Ho, P.L.; Coviello, G.M.; Wright, W.E.; Weinrich, S.L.; Shay, J.W. Specific association of human telomerase activity with immortal cells and cancer. Science 1994, 266, 2011–2015.

206. Zhao, S.; Wang, F.; Liu, L. Alternative Lengthening of Telomeres (ALT) in Tumors and Pluripotent Stem Cells. Genes 2019, 10, 1030.

207. Arnoult, N.; Karlseder, J. ALT telomeres borrow from meiosis to get moving. Cell 2014, 159, 11–12.

208. Farooqi, A.; Yang, J.; Sharin, V.; Ezhilarasan, R.; Danussi, C.; Alvarez, C.; Dharmaiah, S.; Irvin, D.; Huse, J.; Sulman, E.P. Identification of patient-derived glioblastoma stem cell (GSC) lines with the alternative lengthening of telomeres phenotype. Acta Neuropathol. Commun 2019, 7, 76.

209. Lafferty-Whyte, K.; Cairney, C.J.; Will, M.B.; Serakinci, N.; Daidone, M.-G.; Zaffaroni, N.; Bilsland, A.; Keith, W.N. A gene expression signature classifying telomerase and ALT immortalization reveals an hTERT regulatory network and suggests a mesenchymal stem cell origin for ALT. Oncogene 2009, 28, 3765–3774.

210. Pompili, L.; Maresca, C.; Dello Stritto, A.; Biroccio, A.; Salvati, E. BRCA2 Deletion Induces Alternative Lengthening of Telomeres in Telomerase Positive Colon Cancer Cells. Genes 2019, 10, 697.

211. Heaphy, C.M.; Subhawong, A.P.; Hong, S.-M.; Goggins, M.G.; Montgomery, E.A.; Gabrielson, E.; Netto, G.J.; Epstein, J.I.; Lotan, T.L.; Westra, W.H.; et al. Prevalence of the Alternative Lengthening of Telomeres Telomere Maintenance Mechanism in Human Cancer Subtypes. Am. J. Pathol. 2011, 179, 1608–1615.

212. Lawlor, R.T.; Veronese, N.; Pea, A.; Nottegar, A.; Smith, L.; Pilati, C.; Demurtas, J.; Fassan, M.; Cheng, L.; Luchini, C. Alternative lengthening of telomeres (ALT) influences survival in soft tissue sarcomas: A systematic review with meta-analysis. BMC Cancer 2019, 19, 232.

213. Venturini, L.; Motta, R.; Gronchi, A.; Daidone, M.; Zaffaroni, N. Prognostic relevance of ALT-associated markers in liposarcoma: A comparative analysis. BMC Cancer 2010, 10, 254.