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Review

Cancer stem cells and angiogenesis

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ABSTRACT

Most cancers contain tumor cells that display stem cell-like characteristics. How and when such cells appear in tumors are not clear, but may involve both stochastic as well as hierarchical events. Most likely, tumor cells that display stem cell-like characteristics can undergo asymmetric cell division giving rise to tumor cells that trigger angiogenic programs. As normal stem cells the cancer stem-like cells seem to adapt to hypoxic environments and will use metabolic pathways that involve increased conversion of glucose to pyruvate and lactate, and a concomitant decrease in mitochondrial metabolism and mitochondrial mass. The molecular pathways responsible for inducing glycolysis are now being explored. These pathways seem to mediate multiple metabolic functions in cancer stem-like cells, leading to a highly migratory and angiogenesis-independent phenotype. Future challenges will be to identify and validate molecular targets involved in anaerobic metabolic pathways active in cancer stem-like cells and to determine how these pathways differ from regulatory pathways involved in normal stem cell function.

1. The clonal evolution of cancer

It is well recognized that most tumors arise from a single cell clone that undergoes malignant transformation leading to the generation of multiple tumor cell populations forming a mass of heterogenous tumor cells. In 1976 Nowell introduced the idea that cancers are caused by multiple mutations into a general framework of tumor progression that through stochastic processes lead to the accumulation and selection of genetic changes [1]. However, during the last decade a very old concept of tumor progression has been revitalized based on the cancer stem cell theory originally proposed nearly 150 years ago [2,3]. This concept states that tumors are initiated and driven by an abnormal subtype of stem-like cells, capable to generate, through asymmetric cell division identical stem-like cells and heterogeneous tumor cell populations, resulting in a population of genetically identical tumor cells. Moreover, several pathways and genes required for normal stem cell function are activated in cancer stem-like cells and play essential roles in the development of tumors [4,5].

2. The stochastic model of tumor development

Variability in disease presentation and progression is a key feature of cancer. This is observed among similarly diagnosed cancers and in the same cancer at different time points. Tumor progression is believed to result from variability between tumor cell subpopulations partly initiated by conditions within the tumor micro-environment as well as by interactions between tumor cells and host cells. The development is characterized by a somatic evolution in which certain mutations give a cell a selective proliferation advantage [1,6]. Evidence strongly supports mutations as dominant factors in setting rate-limiting steps in tumor progression, resulting in variation in the timing of progression between tumors [7]. Tumorigenesis is thought to require four to six stochastic rate-limiting mutation events to occur in the lineage of one cell [8–10]. This model implies that there are several cell types within a tumor that have tumor initiating capabilities, based on the microenvironment in which they reside.

3. Cancer stem cells: the hierarchical model of tumor development

A cancer stem cell (CSC) is defined as a cancer cell, that possesses characteristics associated with normal stem cells, specifically the ability to give rise to all cell types found in a particular cancer. According to the hierarchical CSC hypothesis, CSCs represent the

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tumorigenic cells that generate tumors through the stem cell processes of self-renewal and differentiation. The CSCs are proposed to persist in tumors as a distinct population and cause relapse and metastasis by giving rise to new tumors. It has been argued that the long life span of a stem cell would allow the accumulation of mutations and epigenetic changes in the normally highly regulated pathways to promote increasing malignancy within these cells, which already have active self-renewal pathways [4,11]. Based on the fact that CSCs in different tumors are defined by multiple phenotypic markers and potentially may arise from multiple populations, makes a clear-cut definition of CSCs difficult (see below). Many authors have therefore chosen to use the term cancer stem-like cells instead of CSCs.

4. Which model is correct?

There is now a large body of evidence showing that cancer stem-like cells can be isolated from a variety of tumors. A common phenotype for the leukemia-initiating cells has been described [12–15]. In addition, cancer stem cells have also been described in breast [16] and brain [17,18] tumors, and possibly also in bone [19], lung [20] melanocytic [21] and prostate [22] neoplasms. Based on these studies putative CSC populations are heterogeneous between tumors. A problem is that in most cases CSCs are identified by cell surface markers that may not be connected to the functional properties of the stem cell and may vary over time. As previously mentioned, this makes the identification of CSCs very difficult. Although many tumors contain cells that display stem cell-like features, the identity of the normal cells that acquire the first genetic hits leading to the cancer-initiating cell still remains elusive [23,24]. Theoretically, CSCs may arise in different ways [23,25]. The cell of origin may either be a normal stem cell, the regulation of which is disrupted. Alternatively, it may be a differentiated cell which re-acquires stem cell properties, characterized by long-term self-renewal. In fact, several studies indicate that cells other than stem cells are capable of giving rise to cancer-initiating cells with stem cell characteristics, suggesting that their origin cannot be rigidly defined [26–30].

In xenotransplantation assays for melanomas, it has recently been shown that environmental factors are critical in determining the tumorigenicity of single tumor cell clones. These observations, at least for melanomas, raise doubts about the CSC hypothesis [31]. Moreover, it has recently been shown that a clonal initiation of cancer can occur also from tumor cells that do not display cancer stem cell markers [26,30]. Within the scientific community there is therefore also scepticism to the cancer stem cell concept [32,33].

Recent studies have shown that metastatic colon cancer contains clonally derived tumor cells that have all the critical properties expected of stem cells, including self-renewal and the ability to differentiate into phenotypes resembling mature colon cells. The cells showed however chromosomal instability suggesting that multiple phenotypic markers may define them [34]. This study indicates that tumor progression includes features of both the hierarchical model for CSCs and the stochastic model, driven by chromosomal instability [34].

A potential explanation to this controversy is that during tumor growth and progression, within a heterogeneous tumor cell population, there are cells that are highly selected to clonally expand within specific environmental niches. Such cells are clonogenic but are highly adapted to a particular environment. These cells can give rise to tumors, when they are present in a particular niche (Fig. 1). Other cells are capable of developing, based on clonal selection or adaptation, to display stem cell-like properties. These cancer stem-like cells show, as normal stem cells, high adaptability to new micro-environments and can thrive in different niches (Fig. 1). This

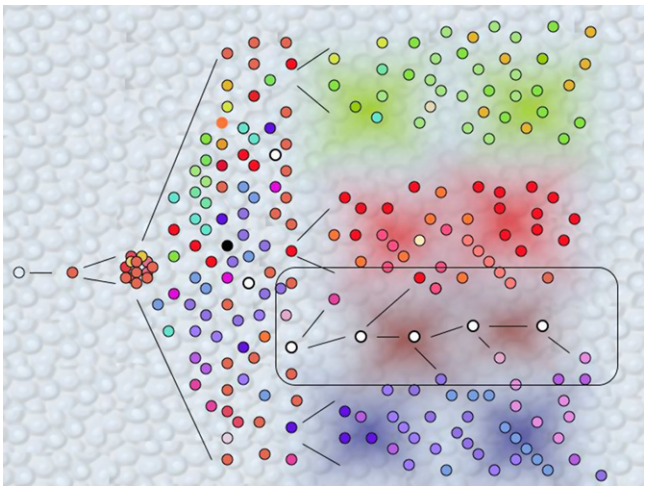

Fig. 1. Possible explanation of the controversy between the stochastic and the hierachical models of tumor development.
During a stochastic development of multiple tumor cell clones there may be a random formation of tumor cell clones that develop stem-cell-like properties including self-renewal and multipotency. Whereas most tumor clones (green, red or blue) will only survive in specific well adapted niches, the cancer stem-like cells (white clones) will thrive in all niches including unfavorable environmental niches (e.g. hypoxia) where they well may use a hierarchical program of development. Depending on the niche, these cells may or may not express stem cell markers.

implies that marker expression is dynamically regulated depending on the micro-environment. Thus these cells may or may not express stem cell markers at any given timepoint. A combination of both the stochastic and hierarchical model seems to be at play, depending on the interaction of the cells with their niche.

5. Glycolysis in cancer stem-like cells, unique cell survival programs

It has for a century been known that under low oxygen conditions mammalian cells can use anaerobic glycolysis for their growth and survival. Glycolysis implies the conversion of glucose to pyruvate and lactic acid for the generation of ATP. This process can, in normal cells, be inhibited by the presence of oxygen. More than 80 years ago, Otto Heinrich Warburg showed that tumor cells can use glycolytic activity even in the presence of oxygen [35]. This mechanism of aerobic glycolysis, defined as the Warburg effect, has been highly debated and its significance is poorly understood, since cancer cells also use oxidative respiration which, compared to glycolysis, is a much more efficient pathway of ATP production [36]. On the other hand, it is now well known that in cancer cells there is a shift from oxidative phosphorylation to anaerobic glycolysis in response to induced hypoxia [37].

Nevertheless, it is still an open question if there are subpopulations of tumor cells that selectively use glycolysis for their survival. It has been proposed that lactate production in pre-malignant lesions can lead to micro-environmental acidosis [37]. This in turn can select tumor cell clones that show up-regulated glycolysis and resistance to an acidic environment. Such cells may have a powerful growth advantage, characterized by unconstrained cell proliferation [37]. The acidic environment may induce cell migration and thus also explain increased invasive growth of these cells [38–40].

6. Do stem cells thrive in vascular or in hypoxic niches?

It has recently been shown that neural stem cells, haematopoietic stem cells, epithelial stem cells and early progenitor cells grow well under hypoxic conditions. In fact there is evidence that stem cells reside in hypoxic niches as for instance in the bone marrow

[41–43]. Under such conditions stem cells should have lower levels of reactive oxygen species (ROS) compared to more differentiated phenotypes that reside in well-vascularized organs [44]. This implies that stem cells may to a large extent use glycolysis for their survival. Indirect evidence for such survival mechanisms has been shown for neural stem cells that can be isolated and propagated from human autopsies even four days after death [45]. In this context we have been able to establish human mesenchymal stem cell cultures from bone marrow autopsies (unpublished data) which indicates that stem cells indeed use unique cell survival programs under reduced oxygen tensions.

Although advances have been made in understanding the role of hypoxia in the stem cell niche, little is known about a similar role of hypoxia in maintaining the tumor stem-like cells in specific niches. Recent evidence indicates, however, that stem-like cells, identified as the side population (SP), are localized in hypoxic zones of solid tumors in vivo [46]. The SP identified had a tendency to migrate to hypoxic areas similar to the migration of normal bone marrow stem cells in response to injury/hypoxia. The cancer stem-like population isolated from the SP fraction of tumors show a strong up-regulation of the phospho-inositide 3-kinase (PI3K)–Akt pathway, directly linking this pathway to stem cell-like tumor cells [47]. Moreover, Akt, but not its downstream target mammalian target of rapamycin (mTOR) can regulate the ATP-binding cassette transporter family member ABCG2 which is known to provide chemoresistance in stem cells [48]. It has recently also been shown that the PTEN/PI3K/Akt pathway and the Foxo3a downstream transcription factor is critical for self-renewal of hematopoietic stem cells [49].

These data collectively indicate that normal stem cells and tumor stem-like cells are predominantly located at the lowest end of an oxygen gradient scale where they well may use glycolysis as a cell survival program [50]. Within the hypoxic niche it has been suggested that self-renewal and differentiation activity is well balanced. However, with an increase in oxygen levels, proliferation may become a dominant feature mediated by an increase in the p38 MAPK and $p16^{ink4a}$ pathway leading to increased cell proliferation and exhaustion of the CSC pool [51].

7. The PI3K–Akt pathway in stem cells and CSCs

Glycolysis involves activation of the PI3K–Akt pathway. Akt is involved in multiple cellular functions including cell proliferation, cell survival, apoptosis resistance, enhanced glycolytic activity and a variety of metabolic functions [52] (Fig. 2). In particular, Akt is involved in many steps of both anaerobic and aerobic glycolysis regulation, as for instance localization of glucose transporters at the cell surface and maintenance of hexokinase function in the absence of extrinsic regulatory factors [52,53]. Akt is activated by specific growth factor receptors and oncogenes as for instance Her2/Neu, Ras, and Bcr-Abl and its activity is negatively regulated by the tumor suppressor PTEN. Activated Akt as well as cMyc can increase glucose uptake in the cancer cells by increasing the number of glucose transporters in the cell membrane which leads to elevated glucose concentrations in the cell. Under hypoxic conditions, increased Akt activity may also activate mTOR1 which can promote HIF1α accumulation. This in turn leads to increased glucose transporters, hexokinase 2, lactate dehydrogenase and lactate levels [54] (Fig. 2).

Activation of Akt may thus under hypoxic conditions lead to increased glucose levels and suppress oxidative phosphorylation. On the other hand, Akt is also known to increase oxidative phosphorylation through facilitating mitochondrial hexokinase association with voltage-dependent anion channels which leads to increased ATP levels and also to production of ROS. For instance it has recently been shown that Akt activation can induce premature senescence and sensitize cells to ROS by inhibiting the expression of ROS scavengers [55]. Thus, in contrast to its ability to inhibit apoptosis induced by multiple mechanisms, Akt may also promote ROS-mediated cell death.

8. Tumor angiogenesis-independent growth?

The common notion has been that solid tumors depend on angiogenesis for their growth [56,57] and the hypothesis that inhibition of neo-vascularisation is a promising treatment strategy for malignant disease is now more than three decades old [58]. The tumors can gain access to oxygen and nutrient supply by

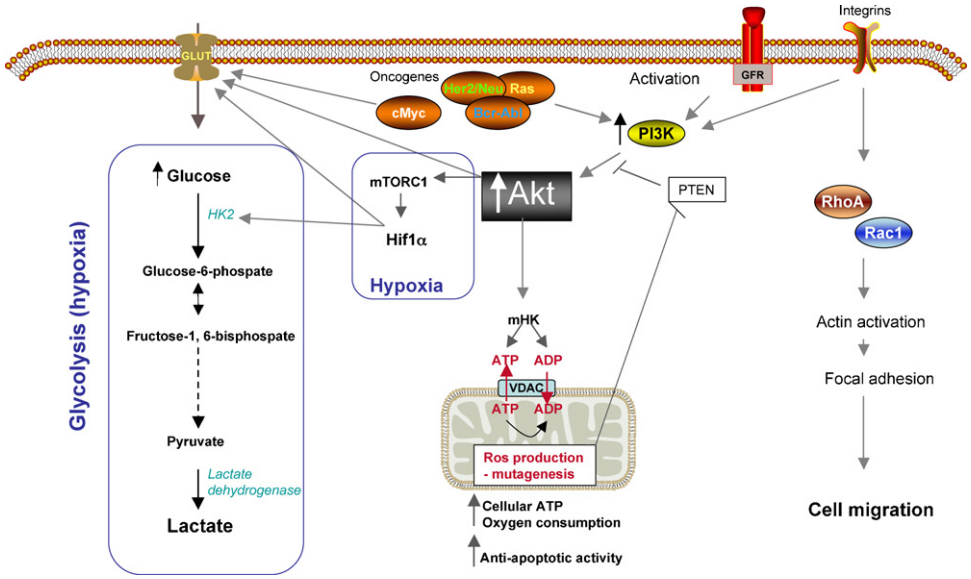

Fig. 2. Relationship between Akt activation and glycolysis in cancer stem-like cells.
The PI3K–Akt pathway can be activated by specific growth factor receptors (GFR) or by a number of oncogenes as for instance Her2/Neu, Ras, Bcr-Abl. Akt activation as well as cMyc can increase glucose uptake by increasing the number of glucose transporters (GLUT) in the cell membrane. Akt may also increase oxidative phosphorylation through facilitating mitochondrial hexokinase (mHK) association with voltage-dependent anion channels (VDAC) eventually leading to increased ATP levels and also to production of reactive oxygen species (ROS) which may cause mutations. Increased Akt activity may also activate mTOR1 which can promote HIF1α accumulation under hypoxic conditions. This can lead to an increase in GLUT, hexokinase 2 (HK2) and lactate dehydrogenase which are important in glycolysis. Finally, integrins may also activate PI3K, and have a central role in cell migration through among others, activation of the RhoA-Rac1 pathway.

using several strategies: vasculogenesis (*de novo* formation of vessels from pluripotent stem cells), angiogenesis (sprouting from pre-existing vessels), vascular co-option (tumor cell growth along pre-existing vessels), intussusception (cleavage of existing vessels by septal invagination) and or vascular mimicry (tumor cells mimicking endothelial cell functions) [59]. To what extent these events can be connected to specific cell clones within a growing tumor is not known. It is however highly likely that there are tumor cell clones in a heterogenous tumor that do not rely on angiogenic programs for their progression. Such cells may well use cooption mechanisms relying on normal vascular elements for their survival and may not respond to anti-angiogenic therapy. [60]. It is yet not known if such cells display stem-cell-like characteristics.

9. Potential consequences of anti-angiogenic treatment

Initially, anti-angiogenic treatments were believed to reduce vascular supply and increase tumor hypoxia and thereby drive tumor cells into hypoxic cell death. This has also been observed in the experimental situation where treatment with the vascular endothelial growth factor (VEGF) receptor tyrosine kinase inhibitor vandetanib decreased vascular density and increased tumor necrosis [61]. On the other hand, there are also some studies indicating that anti-VEGF treatments also may normalize the pathological tumor vasculature structurally as well as functionally leading to facilitated blood flow [62]. Angiogenesis inhibition, as opposed to vascular targeting strategies, therefore appears to have the dual effect changing the tumor micro-environment towards more hypoxic as well as less hypoxic states. These changes may be niche dependent and oxygenation may be increased in parts of the tumor due to vascular normalization, while in other parts of the tumor decreased perfusion and increased hypoxia may occur.

The changes in tumor micro-environment inflicted by anti-angiogenesis treatments may induce phenotypic changes in some niches due to clonal selection of tumor cells with the capacity to adapt to the new micro-environment. Today there is emerging evidence showing that the changes in tumor physiology induced by anti-angiogenic therapies may lead to a selection of tumor cell clones that show increased invasive and migratory potential [63]. At present it is not clear if these invasive cells result from a selection of specific cell clones or from an adaptation of tumor cells to a new micro-environment. For instance, in an assessment of safety patterns of response and recurrence in patients with recurrent high-grade gliomas treated with bevacizumab an anti-VEGF antibody, a high rate of distant tumor progression was observed, suggesting that the tumors may adapt to inhibition of angiogenesis by increased infiltration and vascular co-option [63]. Thus, cells not affected by the anti-angiogenic treatment may either use vessel co-option for survival and invasion or adapt to the hypoxic environment by using anaerobic glycolysis. A key question is whether these cells represent a distinct subpopulation within the tumors. Another open question is if these cells correspond to what is commonly called CSCs.

10. Do CSCs depend on angiogenesis?

Glioblastoma (GBM) represents the most aggressive and infiltrative brain tumor with a very poor prognosis. The tumors grow locally in the brain and are characterized by an extremely infiltrative growth within the brain. Moreover, the tumors are highly angiogenic and show evidence of central necrosis. By xenotransplanting human GBMs in the central nervous system of nude rats, we have shown that highly infiltrative brain tumors with a stem-like phenotype can be established [64]. These tumors co-opt the host vasculature and show an aggressive infiltrative pattern without signs of angiogenesis. The malignant cells express several markers of neural progenitor cells and reflect the migratory behavior of human neural stem cells. Serial passages in animals gradually transform the tumors into an angiogenesis-dependent phenotype accompanied by reduced invasion. The described

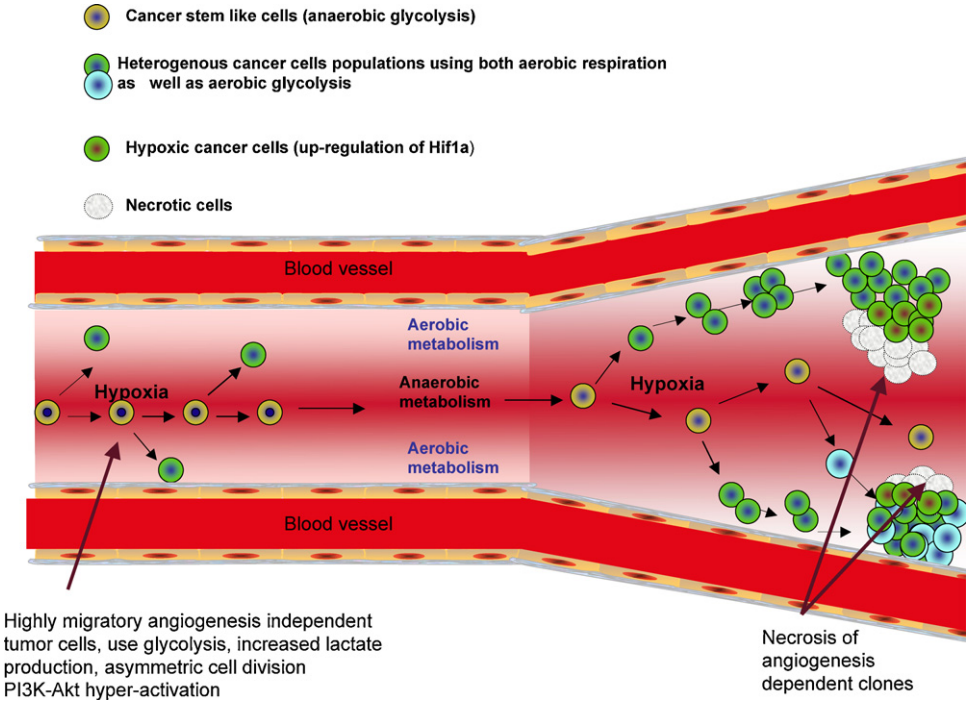

Fig. 3. Proposed model of cancer progression.
Cancer stem-like cells reside in hypoxic niches where they can undergo asymmetric cell division giving rise to tumor cell clones that depend on angiogenesis for their survival. The cancer stem-like cells show an up-regulation of Akt which is associated with increased glycolysis, anti-apoptotic activity and cell migration. These properties are shared with normal stem cells.

angiogenesis-independent tumor growth and the uncoupling of invasion and angiogenesis, represented by the stem-like cancer cells and the cells derived from them respectively, point at two completely independent mechanisms that drive tumor progression [64]. Interestingly, MRI spectroscopy has shown that the infiltrative tumor cells display a high lactate peak suggesting that these cells use a glycolytic pathway for energy generation [65]. In fact, proteomic analyses of the infiltrative cells have shown that these cells also display increased activity of the PI3K/Akt pathway linking these cells to cancer stem-like cells.

11. Cancer stem-like cells and the vascular niche

To what extent cancer stem-like cells are dependent on specific niche requirements is not clear based on the fact that there is not a clear-cut marker identifying the cancer stem cell. Yet it has been shown in experimental model systems that Nestin+/CD133+ cancer cells are located next to capillaries in brain tumors and that endothelial cells maintain these cells in a self-renewing and undifferentiated state. Moreover, anti-angiogenic therapies depleted tumor blood vessels and associated self-renewing Nestin+/CD133+ cancer cells leading to an arrest in tumor growth [66]. These findings somewhat contradict recent clinical studies showing tumor progression after anti-angiogenic therapy combined with chemotherapy [63,67,68]. Thus, the relevance of using Nestin+/CD133+ as a marker for the stem cell compartment at least for human brain tumors is questionable in particular since recent findings indicate that also CD133-cells can be tumorigenic and may show stem-like properties [26,30].

12. Epigenetic regulation

Recent studies have shown that hypoxia can keep normal cells in a hypomethylated state [69] and that hypoxia may enhance tumor stemness by increasing the invasive and tumorigenic ability of tumor side populations [46]. It is therefore possible that hypoxia may also keep cancer stem-like cells (that show an ability to utilize glycolysis) in a hypomethylated state and that these cells reside in hypoxic niches within a cancer. Through asymmetric cell division, these cells may well give rise to cells that depend more on oxygen and that will depend on angiogenesis for further growth (Fig. 3). Further studies should be aimed at isolating these potential subtypes within the tumors and also on determining to what extent epigenetic mechanisms regulate their adaptability and plasticity. Based on the fact that we have been able to generate both an angiogenesis-independent and -dependent phenotype *in vivo*, we should be able to determine to what extent epigenetic events contribute to cancer initiation and progression. We have previously performed array comparative genomic hybridization (array CGH) on the tumor phenotypes and shown that they have similar DNA profiles [64]. This indicates that a change from angiogenesis-independent to angiogenesis-dependent growth is transcriptionally regulated. In other words, the genetic machinery is the same but epigenetic events may determine to what extent adaptable cancer stem-like cells can give rise angiogenesis dependent cell clones.

13. Conclusion

It is still not clear if solid tumors contain CSCs that have followed a hierarchical pattern of growth and progression or if stochastic processes are involved in the selection of novel tumor cell clones during progressive tumor growth. There is, however, emerging evidence for the existence of tumor cells that utilize cell signaling pathways that are shared with normal stem cells. These signaling pathways, involve PI3K–Akt activation and seem to favor glycolysis, indicating that cancer cells that show stem-cell-like expression patterns may reside in hypoxic niches within solid tumors. Anti-angiogenic therapies may well affect oxygen dependent tumor cells that depend on oxidative respiration for their growth. However, tumor cells independent of oxygen levels can survive under these conditions and invade the surrounding tissue. It remains to be solved whether these cells represent a distinct highly adaptable multipotent stem-like cell population or whether they are niche-restricted tumor cells adapted to survive in the hypoxic environment (Figs. 1 and 3). Future studies should focus on finding molecular targets involved in anaerobic metabolic pathways utilized by invasive cancer stem-like cells that are unaffected by anti-angiogenic treatment.

Conflict of interest

None declared.

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