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Altered gene products involved in the malignant reprogramming of cancer stem/progenitor cells and multitargeted therapies

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Abstract

Recent studies in the field of cancer stem cells have revealed that the alterations in key gene products involved in the epithelial-mesenchymal transition (EMT) program, altered metabolic pathways such as enhanced glycolysis, lipogenesis and/or autophagy and treatment resistance may occur in cancer stem/progenitor cells and their progenies during cancer progression. Particularly, the sustained activation of diverse developmental cascades such as hedgehog, epidermal growth factor receptor (EGFR), Wnt/ β -catenin, Notch, transforming growth factor- β (TGF- β)/TGF- β R receptors and/or stromal cell-derived factor-1 (SDF-1)/CXC chemokine receptor 4 (CXCR4) can play critical functions for high self-renewal potential, survival, invasion and metastases of cancer stem/progenitor cells and their progenies. It has also been observed that cancer cells may be reprogrammed to re-express different pluripotency-associated stem cell-like markers such as Myc, Oct-3/4, Nanog and Sox-2 along the EMT process and under stressful and hypoxic conditions. Moreover, the enhanced expression and/or activities of some drug resistance-associated molecules such as Bcl-2, Akt/molecular target of rapamycin (mTOR), nuclear factor-kappaB (NF- κ B), hypoxia-inducible factors (HIFs), macrophage inhibitory cytokine-1 (MIC-1) and ATP-binding cassette (ABC) multidrug transporters frequently occur in cancer cells during cancer progression and metastases. These molecular events may cooperate for the survival and acquisition of a more aggressive and migratory behavior by cancer stem/progenitor cells and their progenies during cancer transition to metastatic and recurrent disease states. Of therapeutic interest, these altered gene products may also be exploited as molecular biomarkers and therapeutic targets to develop novel multitargeted strategies for improving current cancer therapies and preventing disease relapse.

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Keywords

Biomarkers; Therapeutic targets; Cancer stem/progenitor cells; Cancer-initiating cells; Epithelial-mesenchymal transition; Hypoxia; Metabolic pathways; Metastases; Metastasis-initiating cells; Personalized medicine; Multitargeted therapies

1. Introduction

Recent advances in cancer research have led to the identification of distinct gene products that are often deregulated in cancer cells during primary cancer progression and metastases at surrounding and distant tissues and associated with a poor prognosis of cancer patients (Bismar et al., 2006; Glinsky et al., 2005; Manjili et al., 2012; Marchini et al., 2010; Markert et al., 2011; Perez-Villamil et al., 2012; Sergeant et al., 2012; Van Laar, 2012; Varambally et al., 2005). Although this important advance, the heterogeneity of the most cancers and progressive changes of the expression and/or activity of numerous candidate biomarkers and therapeutic targets during disease progression and between patients have limited their applications in clinical settings. It is become evident that the validation of novel gene signatures combining different biomarkers and/or molecular targets in cancer cells and their local microenvironment will be necessary for overcoming the intervariability between cancer subtypes and improving the therapeutic management and survival of cancer patients.

Importantly, a growing body of experimental evidence has revealed that cancer stem/progenitor cells endowed with stem cell-like properties, also designated as cancer-, tumor- and metastasis-initiating cells, can play critical functions for the leukemia development or tumor growth, metastases, treatment resistance and disease relapse. It has been shown that the most cancers may originate from the malignant transformation of immature tissue-resident adult stem/progenitor cells or their early progenies endowed with a high self-renewal ability and aberrant differentiation potential (Mimeault and Batra, 2010b; Mimeault and Batra, 2010c; Mimeault and Batra, 2010d; Mimeault and Batra, 2013). In support with this, cancer stem/progenitor cells expressing specific stem cell-like markers such as CD133, CD44^{high}, aldehyde dehydrogenase (ALDH^{high}), CD90, CXC chemokine receptor 4 (CXCR4^{high}) and high levels of ATP-binding cassette (ABC) multidrug transporters have been identified and isolated from primary and metastatic tissues of cancer patients and established cancer cell lines (Al-Hajj et al., 2003; Bapat et al., 2005; Chiba et al., 2006; Chiba et al., 2007; Chiba et al., 2008; Eramo et al., 2008; Fillmore and Kuperwasser, 2008; Friel et al., 2008; Galli et al., 2004; Hemmati et al., 2003; Hermann et al., 2007; Huang et al., 2009; Kim et al., 2005; Ma et al., 2007; Ma et al., 2008a; Ma et al., 2008b; Maitland et al., 2006; Marsden et al., 2012; Mimeault et al., 2007b; Mimeault et al., 2012; Mimeault and Batra, 2011; Mimeault and Batra, 2013; Mitsutake et al., 2007; Ponti et al., 2005; Prince et al., 2007; Qin et al., 2012; Ricci-Vitiani et al., 2007; She et al., 2008; Shi et al., 2008; Singh et al., 2004; Sung et al., 2008; Wright et al., 2008; Yang et al., 2008; Yu et al., 2008; Yuan et al., 2004; Zhang et al., 2008a). The cancer types harboring a cancer stem/progenitor cell subpopulation with stem cell-like properties include leukemias, lymphomas, sarcomas, melanomas, brain tumors and diverse epithelial cancers such as skin, head and neck, cervical, lung, liver, thyroid, renal, esophageal, gastrointestinal, colon, bladder, pancreatic, prostate, ovarian and breast cancers (Al-Hajj et al., 2003; Bapat et al., 2005; Chiba et al.,

2008; Eramo et al., 2008; Fillmore and Kuperwasser, 2008; Frank et al., 2005; Friel et al., 2008; Galli et al., 2004; Hemmati et al., 2003; Hermann et al., 2007; Huang et al., 2009; Kim et al., 2005; Maitland et al., 2006; Marsden et al., 2012; Ponti et al., 2005; Prince et al., 2007; Qin et al., 2012; Ricci-Vitiani et al., 2007; She et al., 2008; Shi et al., 2008; Singh et al., 2004; Sung et al., 2008; Wright et al., 2008; Yang et al., 2008; Yu et al., 2008; Yuan et al., 2004; Zhang et al., 2008a). It has been observed that cancer stem/progenitor cells displayed higher clonogenic and sphere-forming abilities than their differentiated progenies *in vitro* and were able to give rise to the total cancer cell mass that reconstituted the histological architecture and molecular characteristics closely resembling to original patient's cancer subtypes *in vivo* (Al-Hajj et al., 2003; Bapat et al., 2005; Chiba et al., 2008; Eramo et al., 2008; Fillmore and Kuperwasser, 2008; Frank et al., 2005; Friel et al., 2008; Galli et al., 2004; Hemmati et al., 2003; Hermann et al., 2007; Huang et al., 2009; Kim et al., 2005; Maitland et al., 2006; Marsden et al., 2012; Ponti et al., 2005; Prince et al., 2007; Qin et al., 2012; Ricci-Vitiani et al., 2007; She et al., 2008; Shi et al., 2008; Singh et al., 2004; Sung et al., 2008; Wright et al., 2008; Yang et al., 2008; Yu et al., 2008; Yuan et al., 2004; Zhang et al., 2008a). It has also been noted that different cancer subtypes may contain distinct subsets and/or a different number of cancer stem/progenitor cells during primary cancer progression and metastasis formation at distant sites as well as before or after therapy initiation and disease recurrence (Bao et al., 2006; Das et al., 2008; Dylla et al., 2008; Griffiero et al., 2009; Huang et al., 2009; Kelly et al., 2007; Liu et al., 2006a; Mimeault and Batra, 2013; Quintana et al., 2008; Schmidt, 2008; Shmelkov et al., 2008).

In addition, accumulating lines of evidence have also indicated that cancer- and metastasis-initiating cells with stem cell-like features may express some drug resistance-associated molecules and be more resistant than their differentiated progenies to current anti-hormonal, radiation and chemotherapeutic treatments (Alvero et al., 2009; Bao et al., 2006; Chiba et al., 2008; Fillmore and Kuperwasser, 2008; Frank et al., 2005; Friel et al., 2008; Hamada et al., 2012; Haraguchi et al., 2006; Hermann et al., 2007; Hirschmann-Jax et al., 2004; Huang et al., 2009; Kurrey et al., 2009; Lee et al., 2012; Liu et al., 2006a; Loebinger et al., 2008; Ma et al., 2008b; Maitland et al., 2006; Mimeault et al., 2007b; Mimeault et al., 2010a; Mimeault et al., 2010b; Mimeault et al., 2012; Mimeault and Batra, 2011; Mimeault and Batra, 2013; Qin et al., 2012; Salmaggi et al., 2006; She et al., 2008; Shi et al., 2008; Steg et al., 2012; Sung et al., 2008; Todaro et al., 2007; Wang et al., 2007a; Wang et al., 2012b; Wright et al., 2008; Zhang et al., 2008a; Zhang et al., 2008b; Zhou et al., 2008).

Consequently, the persistence of highly leukemic or tumorigenic cancer stem/progenitor cells in primary cancer, peripheral circulation and/or distant metastatic sites after treatment initiation may contribute to the leukemic or tumor re-growth, metastases and disease recurrence. These observations underline great clinical interest to identify and validate novel biomarkers in cancer-initiating cells detected in leukemias or primary tumor specimens, circulating tumor cells and/or metastasis-initiating cells with stem cell-like properties that could be used, either alone or in combination, to predict the risk of disease progression, metastases and disease relapse. These new biomarkers could also be exploited as potential therapeutic targets to optimize the choice of therapeutic treatments of cancer patients and prevent leukemic or tumor re-growth and disease recurrence after treatment initiation. In this matter, we review recent advances on the characterization of oncogenic events that

frequently occur in cancer stem/progenitor cells and their progenies during cancer initiation and progression to locally invasive and metastatic disease states. The emphasis is on the gene products that are often altered during the epithelial-mesenchymal transition (EMT) process and metabolic reprogramming and which are involved in the acquisition of more malignant phenotypes, survival and treatment resistance of cancer stem/progenitor cells and their progenies under normoxic and hypoxic conditions. Of therapeutic interest, we also discussed the potential therapeutic drug targets in cancer stem/progenitor cells and their differentiated progenies as well as their local microenvironment. The information provided should help to design new multitargeted strategies for eradicating the total mass of cancer cells and improving current treatments against aggressive, metastatic, recurrent and lethal cancers.

2. Molecular transforming events in cancer stem/progenitor cells and their local microenvironment associated with cancer progression and metastases

Cancers generally consist a heterogeneous population of cancer cells organized under a hierarchy in which only a subset of cancer cells with different tumorigenic potential and aberrant differentiation ability can drive cancer development and progression to metastatic disease state. In this regard, a growing body of evidence suggests that the most cancers may arise from the accumulation of different genetic and/or epigenetic alterations in multipotent tissue-resident adult stem/progenitor cells or their early progenies endowed with a high self-renewal capacity concomitant with the changes in their local microenvironment, niches (Hanahan and Weinberg, 2011; Kim et al., 2005; Mimeault et al., 2007b; Mimeault et al., 2012; Mimeault and Batra, 2010b; Mimeault and Batra, 2011; Mimeault and Batra, 2013; Nijhof et al., 2007; Shiras et al., 2007; Sugiarto et al., 2011; Vaish, 2007). Particularly, cancer initiation and development is often accompanied by a down-regulation of specific tumor suppressor gene products such as p16^{INK4A}, phosphatase and tensin homolog deleted on chromosome 10 (PTEN), p53 and/or retinoblastoma (pRb) combined with the overexpression of different oncogenic products, including chromosomal translocations generating oncogenic fusion proteins in cancer-initiating cells (Chen et al., 2007; Clement et al., 2007; Mimeault and Batra, 2009; Mimeault and Batra, 2010b; Mimeault and Batra, 2011; Mimeault and Batra, 2013; Motohara et al., 2011; Sengupta et al., 2007; Wang et al., 2007a). Complex signaling networks involving the interplay and cross-talks between different growth factor signaling cascades in cancer stem/progenitor cells and their progenies usually contribute to their gain of more malignant phenotypes during primary cancer progression and transition to metastatic disease states (Bao et al., 2006; Haraguchi et al., 2006; Liu et al., 2006a; Mimeault et al., 2007b; Mimeault and Batra, 2010b; Mimeault and Batra, 2011; Mimeault and Batra, 2013; Sengupta et al., 2007; Shiozawa et al., 2011b; Tso et al., 2006). The growth factor cascades that are often altered in highly leukemic and tumorigenic cancer cells comprise hedgehog, epidermal growth factor receptor (EGFR), Wnt/ β -catenin, Notch, stem cell factor (SCF)/KIT receptor, hyaluronan/CD44, transforming growth factor- β (TGF- β)/TGF- β R receptors and/or stromal cell-derived factor-1 (SDF-1)/CXCR4 33 (Bao et al., 2011; Bourguignon et al., 2008; Bourguignon et al., 2009; Chen et al., 2006; Cheng et al., 2012a; Clement et al., 2007; Eichhorn et al., 2012; Haraguchi et al.,

2006; Huynh et al., 2011; Kucia et al., 2005; Liu et al., 2006a; Liu et al., 2006b; Mimeault et al., 2007b; Mimeault et al., 2012; Mimeault and Batra, 2010b; Mimeault and Batra, 2011; Mimeault and Batra, 2013; Moriyama et al., 2010; Sengupta et al., 2007; Tso et al., 2006; Wang et al., 2012b; Wang et al., 2012c). These tumorigenic pathways can cooperate to activate different downstream signaling effectors, including phosphatidylinositol 3'-kinase (PI3K)/Akt/molecular target of rapamycin (mTOR), nuclear factor-kappaB (NF- κ B), mitogen-activated protein kinases (MAPKs), Myc and polycomb group (PcG) proteins such as B lymphoma Mo-MLV insertion region 1 homolog (BMI-1) (Birnie et al., 2008; Chiba et al., 2008; Ma et al., 2008b; Mimeault et al., 2007b; Mimeault et al., 2012; Shiras et al., 2007; Vaish, 2007; Venugopal et al., 2012; Wang et al., 2012d). The cooperation between these signal transduction elements, in turn, may play critical roles for the high self-renewal ability, sustained proliferation, survival, invasion and metastasis of cancer stem/progenitor cells and their progenies and treatment resistance (Birnie et al., 2008; Chiba et al., 2008; Ma et al., 2008b; Mimeault et al., 2007b; Mimeault et al., 2012; Shiras et al., 2007; Vaish, 2007; Venugopal et al., 2012; Wang et al., 2012d). Furthermore, the acquisition of stem cell-like features and a more malignant behavior by cancer stem/progenitor cells and their progenies as well as the alterations in their metabolic pathways may occur during the cancer progression, and more particularly through the EMT program, and contribute to their adaptation and survival under oxidative stress, hypoxia and nutrient deprivation (Bapat et al., 2005; Brabletz et al., 2005; Das et al., 2008; Hermann et al., 2007; Mimeault et al., 2007b; Mimeault and Batra, 2007b; Mimeault and Batra, 2008b; Mimeault and Batra, 2013; Tso et al., 2006).

2.1. Implications of the EMT process in the malignant transformation and survival of cancer stem/progenitor cells and their progenies

The EMT phenomenon, which occurs through the tissue and organ morphogenesis and patterning during embryonic development and tissue regeneration and wound healing in adult, is also re-activated during the progression of numerous epithelial cancers such as skin, lung, hepatic, gastrointestinal, colorectal, pancreatic, prostate, ovarian and breast cancers (Alvero et al., 2009; Bao et al., 2011; Cao et al., 2012; Chen et al., 2007; Du et al., 2011; Feldmann et al., 2007; Giarnieri et al., 2013; Hanahan and Weinberg, 2011; Kabashima et al., 2009; Kikuta et al., 2010; Mani et al., 2008; Mimeault and Batra, 2007b; Mimeault and Batra, 2008b; Mimeault and Batra, 2010c; Pühr et al., 2012; Rhim et al., 2012; Shah et al., 2007; Wang et al., 2012b; Wang et al., 2009; Zhang et al., 2012b). Moreover, malignant cells involved in the development of non-epithelial cancers, such as melanomas and brain cancers, can acquire variable degrees of mesenchymal markers and a more invasive ability through an EMT-like process resembling embryonic neural crest invasion program (Bailey et al., 2012; Cheng et al., 2012b; De Bacco F. et al., 2012; Galavotti et al., 2012). The EMT process is generally associated with a disruption of cell-cell junctions, decreased contact inhibition and extensive reorganization of the actin cytoskeleton and remodeling of extracellular matrix (ECM) components that results in the loss of epithelial phenotypes concomitant with the gain of mesenchymal markers by cancer cells (Alvero et al., 2009; Bao et al., 2011; Beachy et al., 2004; Cao et al., 2012; Chen et al., 2007; Du et al., 2011; Feldmann et al., 2007; Giarnieri et al., 2013; Kabashima et al., 2009; Mani et al., 2008; Mimeault et al., 2007a; Mimeault et al., 2008a; Mimeault et al., 2008b; Mimeault and Batra,

2006; Mimeault and Batra, 2007b; Mimeault and Batra, 2008b; Mimeault and Batra, 2010c; Puhr et al., 2012; Rhim et al., 2012; Shah et al., 2007; Wang et al., 2012b; Wang et al., 2007b; Wang et al., 2009; Zhang et al., 2012b). The growth factor cascades that may cooperate to induce the EMT program in cancer stem/progenitor cells and their progenies include TGF- β /TGF- β R, EGFR and its ligands, including epidermal growth factor (EGF) and TGF- α , hedgehog, Notch, platelet-derived growth factor (PDGF)/PDGFR receptors and hepatocyte growth factor (HGF)/MET receptor (Fig. 1) (Bao et al., 2011; Cao et al., 2012; De Bacco F. et al., 2012; Galavotti et al., 2012; Kabashima et al., 2009; Kurrey et al., 2009; Mimeault and Batra, 2007b; Mimeault and Batra, 2010b; Shah et al., 2007; Wang et al., 2012b; Wang et al., 2009). The gene products modulated through the activation of these tumorigenic pathways in cancer stem/progenitor cells and their progenies during the EMT program comprise a decreased expression of E-cadherin localized at the plasma membrane concomitant with an up-regulation of N-cadherin, vimentin, snail, slug, twist, Cripto-1, tenascin C, β -catenin, CXCR4 and/or different anti-apoptotic factors (Fig. 1) (Bao et al., 2011; Cao et al., 2012; Du et al., 2011; Giarnieri et al., 2013; Kabashima et al., 2009; Kurrey et al., 2009; Mimeault and Batra, 2007b; Mimeault and Batra, 2010b; Puhr et al., 2012; Shah et al., 2007; Wang et al., 2012b; Wang et al., 2009). The enhanced expression levels and activities of these signaling effectors may contribute to the sustained growth, enhanced survival, invasion, metastases and/or drug resistance of cancer cells (Fig. 1) (Bao et al., 2011; Cao et al., 2012; Du et al., 2011; Kabashima et al., 2009; Kurrey et al., 2009; Mimeault and Batra, 2007b; Mimeault and Batra, 2010b; Puhr et al., 2012; Shah et al., 2007; Wang et al., 2012b; Wang et al., 2009). More specifically, the activation of TGF- β /TGF- β R pathway can play critical roles for the induction of the EMT program and cooperate with other growth factors such as the EGF/EGFR system to the malignant transformation of cancer stem/progenitor cells and their progenies as well as the activation of cancer-associated stromal fibroblasts (Gulhati et al., 2011; Kabashima et al., 2009; Lamouille et al., 2012; Mimeault and Batra, 2007b; Mimeault and Batra, 2010b; van den Hoogen et al., 2011b; Wang et al., 2012a; Wang et al., 2012b). The stimulation of TGF- β /TGF- β R axis may activate multiple intracellular signaling elements, such as MAPKs, PI3K/Akt/mTOR complex 1 and 2 (mTORC1 and mTORC2), Ras homolog gene family member A (RhoA), small GTP binding protein Rac-1, tissue transglutaminase (TG2) and ALDH and up-regulate expression of the EMT process-associated molecules (Cao et al., 2012; Gulhati et al., 2011; Lamouille et al., 2012; Mimeault and Batra, 2007b; van den Hoogen et al., 2011b). These altered gene products, in turn, may contribute to the acquisition of more migratory and invasive abilities by cancer cells.

Importantly, it has also been shown that cancer cells with stem cell-like features can undergo the EMT process and acquire more aggressive, migratory and invasive potentials than their differentiated progenies during cancer progression and more particularly in hypoxic zones of tumors (Das et al., 2008; Giarnieri et al., 2013; Lin et al., 2012; Lock et al., 2012; Mimeault and Batra, 2013; Salnikov et al., 2012; Wang et al., 2012a). Moreover, despite differentiated cancer cells can undergo mesenchymal-epithelial transition upon withdrawal of hypoxia, poorly differentiated cancer stem/progenitor cells appear only to partially undergo mesenchymal-epithelial transition in normoxic zones after tumor re-oxygenation, and thereby conserve their migratory potential (Mimeault and Batra, 2013; Salnikov et al.,

2012). Hence, the gain of a more invasive phenotype and selective survival advantages by cancer stem/progenitor cells as compared to their differentiated progenies under stressful conditions, including intermittent hypoxia and nutrient deprivation, prevalent in primary tumors may confer them with important functions in cancer progression to metastatic disease states and enhanced treatment resistance (Bao et al., 2011; Brabletz et al., 2005; Cao et al., 2012; Gotzmann et al., 2004; Gulhati et al., 2011; Kabashima et al., 2009; Kuphal et al., 2005; Kurrey et al., 2009; Lamouille et al., 2012; Lin et al., 2012; Mani et al., 2008; Mimeault et al., 2007b; Mimeault and Batra, 2007b; Mimeault and Batra, 2010b; Mimeault and Batra, 2011; Mimeault and Batra, 2013; Onoue et al., 2006; Rhim et al., 2012; Tso et al., 2006; Wang et al., 2012b; Zhang et al., 2012b). For instance, it has been reported that CD133⁺ colorectal cancer-initiating cells exhibiting high expression levels of stemness- and EMT program-associated molecules were enriched and survived under hypoxia and serum depletion conditions while CD133⁻ colorectal cancer cells undergo apoptosis (Lin et al., 2012). It has also been noted that the activation of p38 MAPK-heat shock protein (Hsp27) pathway may inhibit the cleavage of caspases-9 and -3 in CD133⁺ colorectal cancer-initiating cells and contribute to their survival under hypoxia and serum depletion conditions (Lin et al., 2012). In addition, the up-regulation of synthesis and stability of hypoxia-inducible factors (HIFs) under normoxic and hypoxic conditions may also lead to the enhanced transcriptional expression of stem cell-like markers, EMT process-associated molecules, glycolytic enzymes and angiogenic factors such as VEGF in cancer cells (Mimeault and Batra, 2013). HIF-induced altered signaling elements may cooperate for the acquisition of a more aggressive behavior and survival advantages by cancer stem/progenitor cells and their differentiated progenies as well as tumor angiogenic switch (Lock et al., 2012; Mimeault and Batra, 2013). More specifically, hypoxia and HIFs may up-regulate the expression of carbonic anhydrase IX (CAIX) that plays critical functions in the regulation of intracellular pH homeostasis, expression of EMT and stemness markers, such as Notch1 and Jagged1, survival and invasion of cancer cells in hypoxic microenvironments (Lock et al., 2012). Moreover, the alterations in the metabolic pathways frequently occur in cancer cells during cancer progression and may play critical functions for their survival under stressful conditions.

2.2. Implications of the altered metabolic pathways in the malignant transformation and survival of cancer stem/progenitor cells and their progenies under normoxic and hypoxic conditions

The cancer progression is often accompanied by the alterations in the metabolic pathways, including enhanced glycolysis, lipogenesis and/or autophagy in highly proliferative cancer cells, including cancer stem/progenitor cells as well as activated fibroblasts in the stromal compartment (Fig. 1) (Das et al., 2008; Guido et al., 2012b; Hanahan and Weinberg, 2011; Kondoh, 2008; Mimeault and Batra, 2013). The altered metabolic pathways may supply the energy and nutrients necessary for their sustained growth and survival under normoxic and hypoxic conditions (Fig. 1) (Das et al., 2008; Guido et al., 2012b; Hanahan and Weinberg, 2011; Kondoh, 2008; Mimeault and Batra, 2013).

2.2.1. Implications of the enhanced glycolysis in the metabolic reprogramming and survival of cancer stem/progenitor cells and their

progenies under normoxic and hypoxic conditions—Unlike normal cells that use oxygen to generate energy from sugars, including glucose, *via* the aerobic cellular respiration, cancer cells and tumor-associated stromal myofibroblasts can exhibit alterations in mitochondrial functions and enhanced glycolytic metabolism (Bhatia et al., 2012; Mimeault and Batra, 2013). The enhanced glycolysis in cancer cells even in the presence of oxygen, also known as the Warburg effect, is generally associated with an increased expression and/or activities of different growth factor signaling cascades (Bhatia et al., 2012; Mimeault and Batra, 2013). More specifically, the stimulation of different receptor tyrosine kinases, such as EGFR, MET, and PDGFRs as well as hedgehog and TGF- β /TGF- β R pathways may lead to the activation of PI3K/Akt/mTOR, MAPKs and/or HIF-1 α and HIF-2 α (Bhatia et al., 2012; Mimeault and Batra, 2013). These signaling elements, in turn, can cooperate to up-regulate the glycolytic pathway by inducing the enhanced expression of glucose transporters (GLUTs) and glycolytic enzymes in cancer cells and stromal fibroblasts under stressful conditions (Fig. 1)(Bhatia et al., 2012; Mimeault and Batra, 2013). For instance, the stimulation of sonic hedgehog (SHH) cascade in primary cerebellar granule neural precursors (CGNPs), which have been proposed as the cells involved in the medulloblastoma development, may up-regulate the expression of fatty acid-activated peroxisome proliferator-activated receptor- γ (PPAR- γ). PPAR- γ can act as a nutrient sensor and metabolic transcriptional regulator by up-regulating the expression of GLUT-4 and glycolytic enzymes such as hexokinase II (HK II) and pyruvate kinase M2 (PKM2) that contribute to glucose uptake and enhanced glycolysis (Bhatia et al., 2012). On the other hand, it has also been shown that the overexpression of metabolic enzymes, including glycine decarboxylase, in CD166⁺ non-small cell lung cancer (NSCLC)-initiating cells may lead to an accumulation of glycolytic intermediates and their conversion into glycine and serine biosynthesis that plays critical roles for their growth and tumorigenicity (Zhang et al., 2012a).

In addition, a substantial up-regulation of anaerobic glycolysis frequently occurs in cancer cells and stromal myofibroblasts localized within intratumoral hypoxic zones during cancer progression (Mimeault and Batra, 2013). The enhanced anaerobic glycolysis can allow cancer cells to survive and grow in a hostile microenvironment associated with a poor tumor vascularization and decreased blood flow perfusion and adapt to oxygen and nutrient deprivation (Mimeault and Batra, 2013). Particularly, an enhanced expression and/or activity of NF- κ B, HIF-1 α and HIF-2 α , vascular endothelial growth factor (VEGF)-VEGFR receptors, MMPs, urokinase plasminogen activator (uPA) system and COX-2 often occurs in cancer cells and stromal cells under stressful and hypoxic conditions (Das et al., 2008; Frank et al., 2011; Guido et al., 2012b; Hashimoto et al., 2011; Kondoh, 2008; Kuphal et al., 2010; Ma et al., 2011; Mathieu et al., 2011; Olivotto and Dello, 2008; Wang et al., 2012a). These signaling elements can drive inflammation- and hypoxia-induced oxidative stress and promote tumor growth, angiogenic switch and cancer progression to locally invasive and metastatic states (Das et al., 2008; Frank et al., 2011; Guido et al., 2012b; Hashimoto et al., 2011; Kondoh, 2008; Kuphal et al., 2010; Ma et al., 2011; Mathieu et al., 2011; Mimeault and Batra, 2013; Olivotto and Dello, 2008). Moreover, cancer stem/progenitor cells and their progenies may also be reprogrammed to re-express high levels of several stem cell-like markers (CD44, ALDH and/or CXCR4), pluripotency-associated transcription factors, such

as V-myc myelocytomatosis viral oncogene homolog (Myc), octamer-binding transcription factor 4 (Oct-3/4), Nanog and/or sex determining region Y (SRY)-box 2 (Sox-2), as well as EMT program-associated molecules (N-cadherin, vimentin, twist and/or slug) under hypoxic conditions (Bao et al., 2012a; Das et al., 2008; Hashimoto et al., 2011; Kuphal et al., 2010; Ma et al., 2011; Mathieu et al., 2011; Mimeault et al., 2007b; Mimeault et al., 2012; Mimeault and Batra, 2007b; Mimeault and Batra, 2010b; Mimeault and Batra, 2011; Olivotto and Dello, 2008; Shiozawa et al., 2011a; Shiozawa et al., 2011b). These deregulated gene products, in turn, can contribute to their high self-renewal potential, invasion, metastases and treatment resistance (Bao et al., 2012a; Box et al., 2010; Das et al., 2008; Hashimoto et al., 2011; Kuphal et al., 2010; Li and Neaves, 2006; Lyden et al., 2001; Ma et al., 2011; Mathieu et al., 2011; Mimeault et al., 2007b; Mimeault et al., 2012; Mimeault and Batra, 2007b; Mimeault and Batra, 2010b; Mimeault and Batra, 2011; Neiva et al., 2005; Olivotto and Dello, 2008; Shiozawa et al., 2011a; Shiozawa et al., 2011b). More specifically, it has been observed that hypoxia may lead to an increased expression of stem cell-like signature genes (Nanog, Oct-4 and enhancer of zeste homolog 2 and EZH2), angiogenic factors (VEGF and IL-6) and microRNAs (miR-21 and miR-210) in human pancreatic cancer cell lines, AsPC1 and MiaPaCa-2, concomitant with their gain of pancreatosphere-forming ability (Bao et al., 2012a).

2.2.2. Implications of the enhanced cholesterol synthesis and lipogenesis in the metabolic reprogramming and survival of cancer stem/progenitor cells and their differentiated progenies—

The alterations in the signaling elements of *de novo* lipid biosynthetic pathways and lipolysis, also may occur in cancer stem/progenitor cells and their differentiated progenies during the tumor development of some highly proliferative and aggressive cancers, including brain, lung, pancreatic, colorectal, breast and prostate cancers (Baron et al., 2004; Bhatia et al., 2011; Ettinger et al., 2004; Guido et al., 2012b; Guo et al., 2009a; Huang et al., 2012b; McGhee et al., 2011; Menendez et al., 2005; Menendez and Lupu, 2007; Moon et al., 2011; Nomura et al., 2011; Pandey et al., 2012; Scott et al., 2012; Walter et al., 2009; Witkiewicz et al., 2008; Yang et al., 2011a). The cholesterol and fatty acid signaling network can contribute in conjunction with enhanced glycolysis to the gain of a more aggressive behavior, survival and treatment resistance of cancer cells under stressful conditions (Fig. 1) (Bhatia et al., 2011; Ettinger et al., 2004; Guido et al., 2012b; Guo et al., 2009a; Huang et al., 2012b; McGhee et al., 2011; Menendez et al., 2005; Menendez and Lupu, 2007; Nomura et al., 2011; Pandey et al., 2012; Scott et al., 2012; Yang et al., 2011a). For instance, it has been reported that the enhanced expression and activation of sterol regulatory element-binding proteins (SREBPs), SREBP-1 and SREBP-2, which act as master regulators of lipid synthesis by up-regulating transcriptional expression of key lipogenic enzymes, often occur in cancer cells during disease progression (Bhatia et al., 2011; Ettinger et al., 2004; Guo et al., 2009a; Huang et al., 2012b; Menendez et al., 2005; Menendez and Lupu, 2007; Pandey et al., 2012). The stimulation of SREBP-1 and SREBP-2 transcription factors may be mediated through the activation of PI3K/Akt/mTOR and MAPKs induced by different hormone and growth factor cascades, such as androgen receptor, EGFR, erbB2 (HER-2) and hedgehog, or in response to extracellular acidosis (Bhatia et al., 2011; Ettinger et al., 2004; Guo et al., 2009a; Huang et al., 2012b; Menendez et al., 2005). In fact, the activation of SREBP precursors, which occurs through

their proteolytic cleavage that generate the mature SREBPs forms followed by their nuclear import can result in the up-regulation of expression levels of some enzymes, including acetyl coenzyme A carboxylase (ACC), 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and fatty acid synthase (FASN), in cancer cells (Fig. 1) (Bhatia et al., 2011; Ettinger et al., 2004; Guo et al., 2009a; Huang et al., 2012b; Menendez et al., 2005). These enzymes, in turn, can catalyze *de novo* lipid biosynthetic pathway that generates novel molecules of cholesterol and fatty acids that contribute to the maintenance of membrane integrity and nutrient supply that is necessary for the high proliferative rate and survival of cancer cells (Fig. 1). For instance, it has been observed that the lipogenic genes were significantly up-regulated in ductal carcinoma *in situ* (DCIS) specimens of breast cancer patients (Pandey et al., 2012). Importantly, it has also been noted that CS24⁻/CD44⁺/ESA⁺ breast cancer stem-like cells (BCSCs) isolated from DCIS.com cell line endowed with a high tumor-initiating ability exhibited higher expression levels of all lipogenic genes than the bulk mass of cancer cells (Pandey et al., 2012). Moreover, the ectopic expression of SREBP-1 in non-tumorigenic breast cancer cell line, MCF10A resulted in a significant increase of lipogenesis in the BCSC fraction and promoted their growth and mammosphere-forming ability while the SREBP-1 expression in tumorigenic MCF10AT breast cell line improved the survival of BCSCs (Pandey et al., 2012). In the same way, it has also been observed that the activation of Sonic hedgehog (SHH) cascade in CGNPs led to the inactivation of the retinoblastoma tumor suppressor protein (pRb) concomitant with the induction of E2F1 transcription factor and enhanced SREBP-1 protein expression (Bhatia et al., 2011). These molecular events, in turn, promoted enhanced expression of lipogenic enzymes, ACC and FASN, and *de novo* neutral lipid synthesis in the proliferative CGNPs and SHH-driven medulloblastoma development (Bhatia et al., 2011). In addition, it has also been reported that a lipolytic enzyme, monoacylglycerol lipase (MAGL), which is also overexpressed in melanoma, ovarian, breast and prostate cancer cell lines, can catalyze the hydrolysis of monoacylglycerols (MAGs) into free fatty acids, including lysophosphatidic acid and prostaglandin E₂ (Nomura et al., 2010; Nomura et al., 2011). These free fatty acids, in turn, can activate oncogenic signaling elements in cancer cells that are involved in their migration, survival and *in vivo* tumor growth (Nomura et al., 2010; Nomura et al., 2011).

2.2.3. Implications of the enhanced autophagy in the metabolic reprogramming and survival of cancer stem/progenitor cells and their progenies under normoxic and hypoxic conditions—

The activation of cellular autophagy also may play essential roles for the maintenance of high self-renewal and tumorigenic potentials of cancer stem/progenitor cells as well as in the regulation of their differentiation and selective adaptation to growth factor and nutrient starvation and hypoxia (Galavotti et al., 2012; Gong et al., 2013; Rausch et al., 2012; Singh et al., 2012a; Yang et al., 2011b). In fact, the autophagy is a lysosome-dependent cellular catabolic mechanism that mediates the turnover of intracellular damaged organelles, including mitochondria, proteins, lipids, carbohydrates and nucleic acids, and in which membrane structures first engulf cytoplasmic components forming double-membraned vesicles called autophagosomes (Fig. 1) (Yang et al., 2011b). In the late stage of autophagy, the autophagosomes can fuse with lysosomes to form single-membraned autolysosomes in which the contents are digested by hydrolases. Hence, the breakdown products such as amino acids, fatty acids, nucleosides

and sugars generated during the autophagic process may be recycled through the synthetic and metabolic pathways for the biosynthesis of new proteins, lipids and nucleic acids and energy supply to cancer cells (Fig. 1) (Galavotti et al., 2012; Gong et al., 2013; Rausch et al., 2012; Yang et al., 2011b). Despite the tumor suppressor proteins, including nuclear p53, usually up-regulate the autophagy while oncogenic products, such as Akt/mTOR and Bcl-2, rather inhibit the autophagic process, it has been reported that the dysfunctions in key signaling elements involved in the stringent regulation of autophagy may be prevalent in cancer cells (Yang et al., 2011b). For instance, it has been observed that pancreatic cancer stem cell-like markers (CD44 and CD24) and markers of hypoxia (CAIX) and autophagy (beclin-1 and microtubule-associated protein 1A/1B-light chain 3, LC3) were co-expressed in pancreatic ductal adenocarcinoma tissues from patients (Rausch et al., 2012). Moreover, human MiaPaCa-2 pancreatic cancer cells expressing higher levels of stem cell-like markers than human BxPc-3 pancreatic cancer cells, also exhibited higher levels of autophagy-related gene products and autophagic and acidic vesicles, enhanced colony-forming and migratory capacity, fibroblastoid-like morphology and survived under hypoxia and nutrient starvation (Rausch et al., 2012). In contrast, BxPC-3 pancreatic cancer cells did not survive in these stressful conditions (Rausch et al., 2012). In the same way, it has also been noted that the expression of the autophagy-related protein beclin-1 was higher in mammospheres derived from human breast tumors from patients with invasive ductal breast cancer or human MCF-7 and BT-474 breast cancer cell lines than in parental adherent breast cancer cells (Gong et al., 2013). The basal and starvation-induced autophagic flux was also superior in ALDH1⁺ breast cancer cells detected in mammospheres than in the bulk population of ALDH1⁻ breast cancer cells (Gong et al., 2013). Moreover, different autophagy-associated factors such as DNA-damage regulated autophagy modulator protein 1 (DRAM1) or p62 encoded by sequestosome 1 (*SQSTM1*) have also been observed to be highly expressed in glioblastomas (GBMs) exhibiting a mesenchymal gene signature and aggressive phenotypes and associated with a poor overall survival of GBM patients (Galavotti et al., 2012). The enhanced expression of DRAM1 and p62 in GBMs also correlated with an up-regulated expression of the mesenchymal marker MET, activation of MAPKs and enhanced motility and invasion of glioma stem cells (Galavotti et al., 2012).

2.2.4. Implications of the mutual interactions between cancer cells and cancer-associated stromal fibroblasts in their metabolic reprogramming—

Recent studies have also indicated that multiple interactions between cancer cells and stromal cells can contribute to their mutual metabolic reprogramming (Fiaschi et al., 2012; Giannoni et al., 2010; Giannoni et al., 2011; Guido et al., 2012a; Guido et al., 2012b; Martinez-Outschoorn et al., 2012). For instance, it has been reported that cancer cells can activate and induce the oxidative stress and enhanced catabolic metabolism in adjacent normal fibroblasts through the secretion of reactive oxygen species (ROS), TGF- β and/or interleukin-6 (IL-6) (Fiaschi et al., 2012; Giannoni et al., 2010; Giannoni et al., 2011; Guido et al., 2012a; Guido et al., 2012b; Martinez-Outschoorn et al., 2012). These molecular events may lead to the metabolic reprogramming of tumor-associated fibroblasts through the activation of NF- κ B and HIFs and induction of mitochondrial dysfunctions, enhanced autophagy/mitophagy and aerobic glycolysis (Fiaschi et al., 2012; Giannoni et al., 2010; Giannoni et al., 2011; Guido et al., 2012a; Guido et al., 2012b; Martinez-Outschoorn et al.,

2012). Reciprocally, the activated fibroblasts, in turn, can produce L-lactate, ketone bodies and glutamine in the extracellular medium which may be used by cancer cells to generate the nutrients and energy that are necessary for their survival (Fiaschi et al., 2012; Guido et al., 2012a; Guido et al., 2012b; Martinez-Outschoorn et al., 2012). Furthermore, cancer-associated myofibroblasts may also secrete matrix metalloproteinases (MMPs) that promote the EMT program and acquisition of stem cell-like features and migratory and aggressive phenotypes by cancer cells *via* the stimulation of cyclooxygenase-2 (COX-2)/NF- κ B/HIF-1 α signaling elements (Giannoni et al., 2010; Giannoni et al., 2011).

Altogether, these recent investigations have indicated that the acquisition of a more aggressive behavior and migratory property by cancer stem/progenitor cells and their progenies during the EMT program and alterations in their metabolic pathways, and more particularly at intratumoral hypoxic zones of primary neoplasms, may result in their survival, invasion and metastases at distant tissues and organs. Therefore, the targeting of oncogenic signaling elements, metabolic pathways and drug resistance-associated molecules that are altered in cancer- and metastasis-initiating cells and their progenies and their local microenvironment under normoxic and hypoxic conditions during cancer progression may constitute promising therapeutic strategies to overcome treatment resistance and prevent disease recurrence.

3. Novel multitargeted strategies for improving current cancer therapies

Significant advances in the basic and clinical oncology in the last years have led to earlier diagnosis and more effective therapeutic management of patients with leukemias and organ-confined tumors in the clinics (Mimeault and Batra, 2010b; Mimeault and Batra, 2012; Siegel et al., 2012). Although the surgical tumor resection may result in some cases to a complete remission, the rapid cancer progression of aggressive cancers to locally invasive and metastatic disease states is generally associated with the development of resistance mechanisms by cancer cells to current anti-hormonal, radiation, chemotherapies and/or stem cell-based therapies and disease relapse (Mimeault and Batra, 2010b; Mimeault and Batra, 2012; Siegel et al., 2012). At the present time, metastatic cancers remain the leading cause of the death of cancer patients (Mimeault and Batra, 2010b; Mimeault and Batra, 2012; Siegel et al., 2012). Consequently, many efforts have been made to validate novel therapeutic targets in cancer stem/progenitor cells and their differentiated progenies for optimizing the individualized treatment of cancer patients and preventing cancer progression and metastases.

3.1. Potential therapeutic targets in cancer- and metastasis-initiating cells and their progenies

Recent investigations have revealed that a subset of highly tumorigenic cancer stem/progenitor cells can contribute in a substantial manner to drive tumor growth and metastases at distant tissues and organs, resistance to current conventional therapies and disease recurrence. Thus, the molecular targeting of signal transduction elements that contribute to the aggressive behavior of cancer- and metastasis-initiating cells with stem cell-like properties as well as the bulk mass of cancer cells during disease progression and metastases represents new promising therapeutic strategies to improve current cancer therapies. Among

potential therapeutic targets frequently deregulated in tumorigenic cancer stem/progenitor cells and their differentiated progenies during cancer progression, there are diverse stem cell-like markers and signaling elements of the growth factor and altered metabolic pathways that play critical functions for their high self-renewal potential, survival, invasion and metastases (Fig. 2; Tables 1, 2, 3 and 4) (Dylla et al., 2008; Gilg et al., 2008; Hu et al., 2008; Loebinger et al., 2008; Mimeault et al., 2007b; Mimeault et al., 2008a; Mimeault and Batra, 2007a; Mimeault and Batra, 2007b; Mimeault and Batra, 2008b; Rappa et al., 2008; Schatton et al., 2008; van den Hoogen et al., 2011a; van der Horst et al., 2011; Yang et al., 2008). In this matter, we review here the data obtained in the last few years supporting great interest to target distinct gene products altered in cancer- and metastasis-initiating cells and their progenies as well as their local microenvironment to eradicate the total mass of cancer cells and thereby overcome treatment resistance and prevent disease relapse.

3.1.1. Targeting stem cell-like markers—Although different deregulated gene products have been identified in cancer stem/progenitor cells, only a few stem cell-like markers such as telomerase^{high}, CD133, CD44^{high}, ALDH^{high} and CD66c^{high} have been shown to contribute to their malignant behavior and/or treatment resistance, and thereby constitute potential drug targets for eradicating these immature cancer cells (Fig. 2; Table 1) (Dylla et al., 2008; Gemei et al., 2013; Gilg et al., 2008; Kim et al., 2013; Rappa et al., 2008; Schatton et al., 2008; van den Hoogen et al., 2011a; van den Hoogen et al., 2011b; Yang et al., 2008; Zhang et al., 2008b). For instance, it has been reported that the telomerase interference by co-expressing a mutated telomerase RNA construct and small interfering RNA (siRNA) directed against endogenous wild-type telomerase (MT-hTer/siRNA) induced a telomeric uncapping in integrin- $\alpha_2\beta_1$ ^{high}/CD44⁺ prostate cancer progenitor cells isolated from human tumor specimens and DU145 cells (Xu et al., 2011). The telomeric uncapping resulted in the induction of the anti-proliferative and apoptotic effects in these immature prostate cancer cells *in vitro* and prevented their tumor formation *in vivo* (Xu et al., 2011). Moreover, the down-regulation of CD133 transmembrane glycoprotein (prominin 1) by using two different short hairpin RNAs (shRNA) also reduced the number of CD133 molecules detected on the surface of human FEMX-I melanoma cells (Rappa et al., 2008). The down-regulation of CD133 surface antigen by shRNAs was also accompanied by a reduction of sphere-forming ability, growth, and mobility of human FEMX-I melanoma cells grown under stem cell-like growth conditions *in vitro* and inhibition of their metastatic spread, particularly to the spinal cord, in athymic nude mice (Rappa et al., 2008). It has also been noted that the anticarcinogenic effects induced by down-regulating CD133 in human FEMX-I melanoma cells may be mediated in part through up-regulated expression levels of genes encoding for Wingless (Wnt) signaling inhibitors (Rappa et al., 2008). Moreover, the molecular targeting of CD133 by using a monoclonal antibody (mAb) directed against epitope 1 (AC133/ W6B3C1) or epitope 2 (AC141) of this stem cell-like marker was also effective at inducing cytotoxic effects on human FEMX-I melanoma cells (Rappa et al., 2008).

Several investigations have also revealed that the CD44 transmembrane receptor is expressed at a higher level on highly tumorigenic cancer stem/progenitor cells than the bulk mass of poorly tumorigenic cancer cells (Bourguignon et al., 2008; Ghatak et al., 2002; Gilg

et al., 2008; Mimeault et al., 2010a; Mimeault et al., 2012; Misra et al., 2006; Patrawala et al., 2006; Patrawala et al., 2007; Yang et al., 2008). Of therapeutic interest, it has also been shown that the blockage of the hyaluronan (HA)-CD44 tumorigenic cascade using a specific anti-CD44 antibody or HA antagonists such as HA oligomers reduced the growth, mobility, invasion and/or metastases of cancer cells and enhanced their sensibility to the cytotoxic effects induced by chemotherapeutic drugs *in vitro* and/or *in vivo* (Bourguignon et al., 2008; Ghatak et al., 2002; Gilg et al., 2008; Misra et al., 2006; Yang et al., 2008). For instance, the down-regulation of Nanog signaling cascade by siRNA or ankyrin/cytoskeleton function using ankyrin repeat domain cDNA has been observed to inhibit HA/CD44-induced expression of multidrug resistance transporter in breast cancer cells and enhance their chemosensitivity (Bourguignon et al., 2008). In the same way, the treatment of highly tumorigenic CD90⁺/CD44⁺ cancer cell fraction from human MHCC-97L hepatocellular carcinoma cell line with an anti-CD44 antibody was also effective at inducing the apoptosis in these immature cancer cells and inhibiting their metastatic spread to the liver and lungs in immunodeficient mice (Yang et al., 2008).

In addition, it has also been observed that ALDH, which is expressed at a higher level in cancer- and metastasis-initiating cells than their differentiated progenies detected in melanoma, retinoblastoma, brain, breast, prostate, pancreatic and colorectal cancers, can be involved in the detoxification of a variety of anticancer compounds such as the bioactive metabolite of alkylating agent, cyclophosphamide (Bar et al., 2007; Boonyaratanakornkit et al., 2010; Douville et al., 2009; Dylla et al., 2008; Feldmann et al., 2007; Ishizawa et al., 2010; Seigel et al., 2005; Stuelten et al., 2010; van den Hoogen et al., 2010; van den Hoogen et al., 2011b; Wright et al., 2008). Consequently, the targeting of ALDH also may constitute a potential strategy to inhibit the tumor growth and metastases and overcome the chemoresistance. For example, it has been reported that the stimulation of TGF- β signaling cascade induced a substantial increase of ALDH7A1 activity in human PC3M-Pro4 prostate cancer cells whereas bone morphogenic protein-7 (BMP-7), which acts as an antagonist of TGF- β signaling, down-regulated the ALDH7A1 activity (van den Hoogen et al., 2011b). Moreover, the knockdown of ALDH7A1 expression by lentiviral shRNA interference was accompanied by a significant decrease in the number of integrin- α_2^{high} /integrin- α_v^{high} /CD44⁺ prostate cancer stem/progenitor cell subpopulation detected in PC3M-Pro4 cells concomitant with a reduction of expression levels of snail, twist and osteopontin (van den Hoogen et al., 2011b). It has also been noticed that the down-regulation of ALDH7A1 in PC3M-Pro4 cells inhibited their clonogenic and migratory capacities *in vitro* and intrabone growth and metastasis *in vivo* while their intraprostatic growth was not inhibited (van den Hoogen et al., 2011b).

3.1.2. Targeting growth factor signaling pathways—Among other potential molecular targets that are often altered in cancer- and metastasis-initiating cells and their progenies during cancer progression and metastases, there are diverse developmental signaling pathways initiated by different growth factors, cytokines and chemokines. These deregulated cascades include hedgehog, EGFR, Wnt/ β -catenin, Notch, TGF- β /TGFR β , interleukin (IL-4)/IL-4R receptor, IL-6/IL-6R, SDF-1/CXCR4, SCF/KIT and ECM/integrin and their downstream signaling elements such as PI3K/Akt/mTOR, MAPKs, NF- κ B and

BMI-1 (Fig. 1; Table 2) (Bourguignon et al., 2008; Chen et al., 2006; Cheng et al., 2012a; Eichhorn et al., 2012; Engl et al., 2006; Farnie et al., 2007; Feldmann et al., 2007; Ghatak et al., 2002; Hallett et al., 2012; Hart et al., 2005; Hsieh et al., 2013; Huynh et al., 2011; Jung et al., 2013; Kabashima et al., 2009; Karhadkar et al., 2004; Kucia et al., 2005; Liang et al., 2004; Mimeault and Batra, 2010a; Mimeault and Batra, 2010c; Misra et al., 2006; Neiva et al., 2005; Onoue et al., 2006; Shiozawa et al., 2011a; Sims-Mourtada et al., 2007; Singh et al., 2011; Singh et al., 2010; Singh et al., 2012b; Todaro et al., 2007; van der Horst et al., 2011; Venugopal et al., 2012; Villanueva et al., 2007; Wang et al., 2012c; Yi et al., 2012). It has been observed that the blockage of these tumorigenic pathways resulted in anti-proliferative, apoptotic, anti-invasive and anti-metastatic effects on cancer cells and/or inhibition of tumor angiogenesis *in vitro* and *in vivo* (Fig. 2; Table 2) (Bourguignon et al., 2008; Chen et al., 2006; Cheng et al., 2012a; Eichhorn et al., 2012; Engl et al., 2006; Farnie et al., 2007; Feldmann et al., 2007; Ghatak et al., 2002; Hallett et al., 2012; Huynh et al., 2011; Jung et al., 2013; Karhadkar et al., 2004; Kucia et al., 2005; Liang et al., 2004; Mimeault et al., 2007c; Mimeault et al., 2010a; Mimeault et al., 2012; Mimeault and Batra, 2010a; Mimeault and Batra, 2010c; Misra et al., 2006; Neiva et al., 2005; Onoue et al., 2006; Sims-Mourtada et al., 2007; Singh et al., 2011; Singh et al., 2010; Singh et al., 2012b; Todaro et al., 2007; Villanueva et al., 2007; Yi et al., 2012). For instance, the inhibition of EGFR tyrosine kinase activity by using gefitinib or erlotinib has been observed to induce anti-proliferative and cytotoxic effects on CD133⁺/EGFR⁺ brain tumor-initiating cells from patients with primary GBM *in vitro* (Griffero et al., 2009). The results from our recent work have also revealed that the inhibition of EGFR (gefitinib), SMO co-receptor of hedgehog (cyclopamine), NF- κ B (partenolide), pAkt (Akt inhibitor III) or macrophage inhibitory cytokine-1 (MIC-1) using anti-MIC mAb or siRNA was effective at promoting the apoptotic effect induced by docetaxel on CD133⁺ side population (SP) and CD133⁻ non-SP cells from WPE1-NB26 and PC3M-LN4 prostate cancer cells (Mimeault et al., 2010a; Mimeault et al., 2012; Mimeault et al., 2013). Moreover, the blockade of hedgehog cascade by using a specific inhibitor of SMO, such as cyclopamine, IPI-269609 or GDC-0449 or GANT-61 targeting Gli-1 and Gli-2 transcription factors also decreased the number of tumor-initiating cells and promoted the cytotoxic and anti-metastatic effects induced by current chemotherapeutic drugs on prostatic and pancreatic cancer cell lines and gliomasphere cells (Fig. 2 and Table 2) (Clement et al., 2007; Feldmann et al., 2007; Feldmann et al., 2008; Fu et al., 2013; Jimeno et al., 2009; Karhadkar et al., 2004; Singh et al., 2011). On the other hand, numerous investigations have also indicated that BMI-1 polycomb ring finger protein can play critical roles for the maintenance of self-renewal and survival of cancer stem/progenitor cells and their progenies supporting the therapeutic interest of its targeting to inhibit the cancer development and metastases and overcome treatment resistance (Fig. 2; Table 2) (Chiba et al., 2007; Chiba et al., 2008; Wang et al., 2012c). As a matter of the fact, it has been observed that the knockdown of BMI-1 protein noticeably reduced the number of self-renewing SP cells detected in both Huh7 and PLC/PRF/5 hepatocellular carcinoma cell lines and abolished the tumor-initiating ability of SP cells from these cancer cell lines implanted in non-obese diabetic/severe combined immunodeficient (NOD-SCID) mice (Chiba et al., 2008).

3.1.3. Targeting the EMT program- and drug resistance-associated molecules

—In considering the fact that cancer stem/progenitor cells and their progenies can acquire stem cell-like features and a more malignant behavior, including a migratory ability along the EMT program, the targeting EMT program-associated molecules is of great therapeutic interest to counteract cancer progression, invasion and metastatic spread (Fig. 2; Table 2) (Bao et al., 2011; Brabletz et al., 2005; Cao et al., 2012; Gulhati et al., 2011; Kabashima et al., 2009; Kuphal et al., 2005; Kurrey et al., 2009; Lamouille et al., 2012; Larue and Bellacosa, 2005; Mani et al., 2008; Mimeault et al., 2007b; Mimeault et al., 2012; Mimeault and Batra, 2007b; Mimeault and Batra, 2010b; Mimeault and Batra, 2011; Onoue et al., 2006; Rhim et al., 2012; Tso et al., 2006; Zhang et al., 2012b). The gene products that are often altered in cancer stem/progenitor cells and their progenies during the EMT process include sonic hedgehog, EGFR and TGF- β /TGF- β R and their downstream signaling elements such as PI3K/Akt/mTOR, NF- κ B, N-cadherin, CXCR4, Cripto-1, tenascin C, snail, slug and twist (Birnie et al., 2008; Dubrovskaya et al., 2009; Dubrovskaya et al., 2012a; Dubrovskaya et al., 2012b; Feldmann et al., 2007; Izumiya et al., 2012; Mimeault et al., 2007a; Mimeault et al., 2008a; Mimeault et al., 2008b; Mimeault and Batra, 2006; Mimeault and Batra, 2007b; Mimeault and Batra, 2008b; Quint et al., 2012). Some recent investigations have indicated the therapeutic benefit of targeting these signaling elements to overcome the multidrug resistance (MDR) phenotype and radioresistance of cancer cells, including cancer stem/progenitor cells (Tables 2 and 3) (Birnie et al., 2008; Dubrovskaya et al., 2009; Dubrovskaya et al., 2012a; Dubrovskaya et al., 2012b; Dylla et al., 2008; Hu et al., 2008; Mimeault et al., 2007b; Mimeault et al., 2008a; Mimeault and Batra, 2008a; Shankar et al., 2011; Zhou et al., 2007). For instance, it has been shown that a specific inhibition or inactivation of TGF- β /TGF- β R or its downstream signaling effectors such as mTORC2, TG2 and ALDH was effective at inhibiting the EMT process in different cancer epithelial cell lines and preventing their invasion and dissemination *in vitro* and *in vivo* (Cao et al., 2012; Lamouille et al., 2012; van den Hoogen et al., 2011b). In the same way, the targeting of integrin- α v by using antagonist GLPG0187 also decreased the number of ALDH^{high} PC3M-Pro4 prostate cancer stem/progenitor cells and induced a mesenchymal-epithelial transition as indicated by the increase of the E-cadherin/vimentin ratio (van der Horst et al., 2011). The data of real-time bioluminescent imaging in an orthotopically implanted PC3M-Pro4 model of prostate cancer have also indicated that the blockage of integrin- α v by GLPG0187 induced the antiresorptive and antiangiogenic effects and inhibited the *de novo* formation and progression of bone metastases (van der Horst et al., 2011). Furthermore, the blockage of the SDF-1/CXCR4 axis by using a mAb directed against SDF-1 or CXCR4 or selective CXCR4 antagonist, such as 14-mer peptide (TN14003) or AMD 3100, also inhibited the tumor growth and counteracted the migration and metastases of cancer cells to distant tissues and organs (Balkwill, 2004; Domanska et al., 2012; Dubrovskaya et al., 2012a; Dubrovskaya et al., 2012b; Engl et al., 2006; Hart et al., 2005; Kang et al., 2005; Kucia et al., 2005; Kulbe et al., 2004; Liang et al., 2004; Mimeault and Batra, 2010a; Neiva et al., 2005; Onoue et al., 2006; Rubin et al., 2003; Shiozawa et al., 2011a; Singh et al., 2010). For instance, it has been reported that a combination of AMD 3100 plus docetaxel was more effective than individual drugs at eradicating CD133⁺/CD44⁺ DU145 cell fraction and inhibiting the tumor growth of DU145 cells subcutaneously implanted in mice (Dubrovskaya et al., 2012a).

In addition, it has also been observed that the most of cancer stem/progenitor cells also express high levels of drug resistance-associated molecules, including pAkt, NF- κ B, MIC-1, Bcl-2, survivin and ABC multidrug transporters such as multidrug resistance 1 (MDR1/ABCB1) encoding P-glycoprotein (P-gp), breast cancer resistance protein-1 (BCRP-1/ABCB2) and/or multidrug resistance-associated proteins (MRPs) (An et al., 2012; Bourguignon et al., 2008; Das et al., 2008; Hamada et al., 2012; Hu et al., 2008; Loebinger et al., 2008; Ma et al., 2008b; Mimeault et al., 2007b; Mimeault et al., 2008a; Mimeault et al., 2010a; Mimeault et al., 2010b; Mimeault et al., 2012; Mimeault and Batra, 2011; Schatton et al., 2008; She et al., 2008; Shervington and Lu, 2008; Shi et al., 2008; Wang et al., 2007a). Thus, the targeting of these drug resistance-associated molecules may represent a potential therapeutic approach to inhibit drug efflux, and thereby improve the cytotoxic effects induced by diverse chemotherapeutic drugs on these immature cancer cells (Fig. 2; Table 3) (An et al., 2012; Hu et al., 2008; Loebinger et al., 2008; Ma et al., 2008b; Schatton et al., 2008). For instance, it has been observed that a treatment of CD133⁺ Huh7 liver cancer cell-induced xenografts in mice with an Akt-1 inhibitor was effective at inducing the cytotoxic effects on the total tumor mass of Huh7 cancer cells and reversing their chemoresistance (Ma et al., 2008b).

Importantly, it has also been observed that the inhibition of the NF- κ B signaling pathway using parthenolide, pyrrolidinedithiocarbamate (PDTC) and its analog diethyldithiocarbamate (DETC) preferentially inhibited the proliferation and colony formation of MCF7 mammosphere cells and verapamil-sensitive SP cell subpopulation enriched in breast cancer stem cell-like cells (Zhou et al., 2007). Furthermore, the *in vitro* treatment of CD133⁺ primary prostatic cancer cells and their progenies with parthenolide significantly reduced their viability while normal primary prostatic epithelial cells from a patient with benign prostatic hyperplasia (BPH) were insensitive to this treatment type (Birnie et al., 2008). In the same way, the dietary agent resveratrol has also been reported to inhibit the expression levels of pluripotency-associated transcription factors (Myc, Oct-3/4, Nanog and Sox-2) and EMT process- and drug resistance-associated molecules (zeb-1, slug, snail and ABCG2 multidrug transporter) in human pancreatic cancer stem/progenitor cells isolated from primary pancreatic tumors of patients and K-Ras^{G12D} transgenic mouse model of pancreatic cancer (Shankar et al., 2011). Resveratrol was also effective at inhibiting the self-renewal, migratory and invasive capacities and inducing apoptosis through the activation of capases-3/7 and down-regulation of the expression of Bcl-2 and X-linked inhibitor of apoptosis protein (XIAP) in CD133⁺/CD24⁺/CD44⁺/CD24⁺/ALDH⁺/epithelial specific antigen (ESA⁺) pancreatic cancer stem/progenitor cells (Shankar et al., 2011). Moreover, resveratrol prevented the development of pancreatic intraepithelial neoplastic (PanIN) lesions in K-Ras^{G12D} mice (Shankar et al., 2011). In addition, a treatment of the SP cell fraction from human MHCC-97L hepatocellular carcinoma cell line with an ABCG2 transporter inhibitor, fumitremorgin C has also been observed to sensitize these SP cells to the cytotoxic effects induced by chemotherapeutic drug, doxorubicin by inhibiting ABCG2-mediated drug efflux (Hu et al., 2008). The targeting of ABCB5 multidrug transporter with a mAb directed against this stem cell-like surface marker also reversed the resistance of human G3361 melanoma cells to doxorubicin and inhibited the tumor growth derived from melanoma-initiating cells xenografted in nude mice (Schatton et al., 2008).

3.1.4. Targeting altered metabolic pathway-associated molecules

3.1.4.1. Targeting HIFs and glycolytic pathway: In view of the fact that the up-regulation of HIF-1 α and HIF-2 α may contribute of a major fashion to the high self-renewal ability, enhanced glycolysis and survival of cancer stem/progenitor cells and their progenies under stressful conditions, including hypoxia and nutrient deprivation, some new therapeutic strategies have been developed to inhibit HIF activities and the expression of their target gene products (Mimeault and Batra, 2013). It has been observed that the down-regulation of the expression and/or activities of HIF-1 α and/or HIF-2 α proteins by using RNA interference, guanine-rich oligodeoxynucleotides (G-rich ODNs), chemical compounds and chemotherapeutic drugs inhibited the expression of their target genes and glycolysis and induced the apoptotic death of cancer stem/progenitor cells *in vitro* (Table 4) (Bao et al., 2012a; Dunn et al., 2009; Giannoni et al., 2011; Guan et al., 2010; Heddleston et al., 2012; Kawakami et al., 2012; Lee et al., 2009; Li et al., 2009; Mimeault and Batra, 2013; Reddy et al., 2011; Schwartz et al., 2010; Soeda et al., 2009; Spinella et al., 2010; Xu et al., 2005). Moreover, the targeting of HIF-1 α and/or HIF-2 α proteins was also effective at inhibiting tumor growth and angiogenesis and/or preventing metastatic spread of cancer cells at distant sites *in vivo* (Table 4) (Bao et al., 2012a; Dunn et al., 2009; Giannoni et al., 2011; Guan et al., 2010; Heddleston et al., 2012; Kawakami et al., 2012; Lee et al., 2009; Li et al., 2009; Mimeault and Batra, 2013; Reddy et al., 2011; Schwartz et al., 2010; Soeda et al., 2009; Spinella et al., 2010; Xu et al., 2005). For instance, it has been reported that a treatment of AsPC1 and MiaPaCa-2 pancreatic cancer cells with a curcumin-derived fluorinated synthetic analog designated as CDF down-regulated the expression of Nanog, Oct-4, EZH2 mRNAs, miR-21 and miR-210 and the production of VEGF and IL-6 as well as their clonogenic growth, pancreatosphere-forming ability, migration, invasion and survival under hypoxia (Bao et al., 2012a). CDF was also effective at reducing expression levels of stem cell-like gene signature, miR-21, miR-210, IL-6, HIF-1 α and VEGF in pancreatic tumors induced by an orthotopic implantation of human pancreatic cancer cells (Bao et al., 2012a). On the other hand, the pharmacological blockade of PPAR- γ by using PPAR- γ antagonist GW9662 also decreases the viability of pzp53med medulloblastoma cells and promoted the survival of medulloblastoma-bearing *NeuroD2-SmoA* mice *in vivo* at least in part through the inhibition of glycolysis in tumors (Bhatia et al., 2012).

3.1.4.2. Targeting enzymes involved in the cholesterol synthesis and lipogenesis: Several natural compounds such as resveratrol or pharmacological agents targeting key enzymes that regulate the cholesterol synthesis and lipogenesis, including inhibitors of HMG-CoA reductase (atorvastatin), ACC (TOFA) and FASN (cerulenin and its synthetic analogue, C75, C93 and FAD-approved antiobesity drug, orlistat) have been shown to inhibit these metabolic pathways in cancer cells (Fig. 2; Table 4) (Bhatia et al., 2011; Guo et al., 2009a; Kridel et al., 2004; Menendez et al., 2005; Orita et al., 2008; Pandey et al., 2012; Sankaranarayanapillai et al., 2013; Scott et al., 2012; Seguin et al., 2012). Moreover, these anticancer agents also induced chemopreventive and/or anti-proliferative, anti-metastatic, anti-angiogenic and apoptotic effects on diverse cancer cell lines, including cutaneous melanoma cells and NSCLC, pancreatic, breast and prostate cancer cells *in vitro* and *in vivo* (Table 4) (Bhatia et al., 2011; Guo et al., 2009a; Kridel et al., 2004; Menendez et al., 2005; Orita et al., 2008; Pandey et al., 2012; Scott et al., 2012; Seguin et al., 2012). For instance, it

has been reported that the natural compound resveratrol inhibited the lipogenic gene expression in BCSCs and significantly suppressed their ability to generate DCIS in animals supporting the therapeutic interest to use resveratrol as chemopreventive agent to counteract the development of DCIS (Pandey et al., 2012). Moreover, the treatment of Pzp53med cells derived from a *Ptc^{+/-}/p53^{-/-}* transgenic mouse model of medulloblastoma with the cyclin-dependent kinase inhibitor roscovitine, which reduced levels of E2F1 and FASN, or a pharmacological inhibitor of FASN activity, C75 also inhibited the lipogenesis and proliferation, and induced a caspase-dependent apoptotic death in the Pzp53med cells (Bhatia et al., 2011). A treatment of *NeuroD2-SmoA1*-transgenic mice bearing medulloblastomas with C75 was also effective at increasing the survival of these animals as compared to vehicle-treated mice (Bhatia et al., 2011). The pharmacological inhibition of ACC by TOFA also reduced the ability of Snb19 glioblastoma cells, MDA-MB-231 breast cancer cells, and PC3 prostate cancer cells to invade through matrigel suggesting a lipid-dependent regulation of tumor cell invasion (Scott et al., 2012). Moreover, a treatment with a combination of inhibitors of HMG-CoA reductase and ACC, atorvastatin and TOFA, which are involved in the synthesis of HMG-CoA and malonyl-CoA respectively, significantly suppressed the proliferation of EGFR-activated glioblastoma cells while only minimal anti-proliferative effects were induced on non-EGFR activated cell lines (Guo et al., 2009a). Importantly, the activation of AMP-activated protein kinase (AMPK), which regulate the cellular metabolism modulated by different growth factors, in part by negatively regulating mTOR, ACC and HMG-CoA reductase that are involved in the regulation of fatty acid and cholesterol synthesis, also may constitute an alternative approaches to kill cancer cells. As a matter of the fact, it has been observed that a treatment of U87 glioma cells expressing the constitutively activated EGFR mutant, EGFRvIII with an activated AMPK adenovirus or AMPK-agonist AICAR inhibited the lipogenesis and glycolysis and the tumor growth derived from these cancer cells *in vitro* and *in vivo* (Guo et al., 2009a). In the same way, the treatment of GBM cell lines with a specific inhibitor of EGFR (erlotinib), PI3K (LY294002), Akt (Akti-1/2) or SREBP processing (hydroxycholesterol, 25-HC) also blocked EGF-induced SREBP-1 cleavage into its mature form while the inhibition of mTORC1 by rapamycin had no effect (Guo et al., 2009b). The treatment of U87 and U87/EGFRvIII GBM cell lines with SREBP-1 shRNA, C75 or atorvastatin was also effective at inducing their apoptotic death *in vitro* and *in vivo* (Guo et al., 2009b). These data suggest that the wild-type EGFR and EGFRvIII mutant-induced PI3K/Akt/SREBP signaling elements can play critical roles in the metabolic reprogramming of glioma cells through the coordinate modulation of aerobic glycolysis and its interconnections with the lipogenic pathways.

In addition, it has been observed that the knock-down of FASN was also effective at reversing the resistance of pancreatic cancer cell lines to both gemcitabine and radiation treatments (Yang et al., 2011a). In counterbalance, it has however been noted that the targeting of FASN by using specific inhibitors such as orlistat was associated with adaptive changes in the metabolic pathways of cancer cells in response to drug treatment (Lemmon et al., 2011; Sankaranarayananpillai et al., 2013). The protective responses that may restrain the cytotoxic effects of drugs targeting FASN include the accumulation of intermediary metabolites of fatty acid synthetic pathway and induction of alternative metabolic pathways,

such as ketone metabolism and glutaminolysis as well as the activation of anti-apoptotic factor including NF- κ B (Lemmon et al., 2011; Sankaranarayanapillai et al., 2013).

3.1.4.3. Targeting autophagy: Numerous investigations have indicated that the expression levels and/or activities of key regulators of autophagy are often altered in cancer cells during cancer progression (Galavotti et al., 2012; Mimeault and Batra, 2013; Sotelo et al., 2006). More particularly, some chemotherapeutic drugs and radiation therapy may up-regulate autophagy in cancer stem/progenitor cells and their progenies through the induction of cellular stress and/or inhibition of Akt/mTOR which in turn may promote their survival and therapy resistance (Galavotti et al., 2012; Lee et al., 2011; Mimeault and Batra, 2013; Sotelo et al., 2006). Importantly, it has been shown that the repression of the late stage of autophagy by using a specific inhibitor such as bafilomycin A1, chloroquine or hydrochloroquine sensitized cancer stem/progenitor and their progenies to cytotoxic effects induced by different drugs such as an inhibitor of aurora kinase A (VX-680), orally bioavailable AMPK activator designated as OSU-53, chemotherapy and radiation (Lee et al., 2011; Mimeault and Batra, 2013; Singh et al., 2012a; Sotelo et al., 2006; Yang et al., 2011b; Zou et al., 2012). Moreover, the suppression of autophagy by using 3-methyladenine or monensin, also decreased the expression of stem cell-like markers, clonogenicity, spheroid formation, migratory activity and tumorigenicity of MiaPaCa-2 pancreatic cancer cells in mice and sensitized these cancer cells to apoptosis (Rausch et al., 2012). The protein kinase C- δ inhibitor, rottlerin has also been observed to induce the formation of cytoplasmic vacuolations in human pancreatic cancer stem/progenitor cells expressing stem cell markers such as CD133, CD44, CD24, ALDH and ESA derived from patients with primary pancreatic tumors which was followed by the apoptotic cell death after a long exposure to drug (Singh et al., 2012a). The late apoptosis induced by rottlerin was mediated, in part, *via* the inhibition of PI3K/Akt/mTOR pathway, down-regulation of anti-apoptotic factors (XIAP, cellular inhibitor of apoptosis proteins “cIAP-1”, Bcl-2 and Bcl-X_L), induction of Bax, activation of caspases-3 and -9 concomitant with a degradation of poly(ADP-ribose) polymerase (PARP) in human pancreatic cancer stem/progenitor cells (Singh et al., 2012a). Interestingly, it has also been noted that the silencing of autophagy-associated proteins, Atg7 and beclin-1, or a co-treatment of pancreatic stem/progenitor cells with autophagosome inhibitor, 3-methyladenine, inhibited rottlerin-induced autophagy and accelerated rottlerin-induced apoptosis in these immature cancer cells (Singh et al., 2012a). In the same way, the silencing of beclin-1 by shRNA in MCF-7 adherent or mammospheric MCF-7 cells was also effective at inhibiting the tumor growth derived from these immature cancer cells (Gong et al., 2013). Importantly, it has also been observed that the MCF-7 breast cancer cell subpopulation expressing stem cell-like markers such as ALDH1 and CD44 and autophagy markers, including Atg5, Atg12, and LC3-B undertaken a reversible state of dormancy following a treatment with farnesyl transferase inhibitors (FTIs) *via* an activation of RhoC GTPase and c-jun NH2 terminal kinase (JNK/SAPK) (Chatterjee and van Golen, 2011). A treatment of FTI-treated cells with an autophagy inhibitor, 3-methyladenine was however effective at reversing the dormancy phenotype of MCF-7 breast cancer cells while it did not lead to apoptosis of FTI-treated cells (Chatterjee and van Golen, 2011). These observations suggest that a combination of FTIs, autophagy inhibitor with other cytotoxic drugs could be required to induce the apoptotic death of dormant MCF-7 cancer cells.

Of particular interest, some recent studies have also indicated that the first-line drug for type I diabetes, metformin (*N,N*'-dimethylbiguanide) inhibited the self-renewal ability and induced the cytotoxic effects on cancer stem/progenitor cells, inhibited tumor growth and improved the anticarcinogenic effects of different chemotherapeutic drugs or radiation on melanoma, brain, thyroid, lung, hepatocellular, pancreatic, breast, ovarian and prostate cancer cells (Tables 2 and 3) (Bao et al., 2012b; Ben Sahra et al., 2010; Ben Sahra et al., 2011; Chen et al., 2012; Colquhoun et al., 2012; Cufi et al., 2012; Gou et al., 2013; Hirsch et al., 2009; Hirsch et al., 2013; Iliopoulos et al., 2011; Jung et al., 2011; Liu et al., 2012; Menendez et al., 2012; Petrushev et al., 2012; Sato et al., 2012; Shank et al., 2012; Sinnett-Smith et al., 2013; Song et al., 2012; Vazquez-Martin et al., 2010; Vazquez-Martin et al., 2011; Wurth et al., 2012; Zakikhani et al., 2008). The anticarcinogenic effects of metformin may be mediated through different mechanisms, including the inhibition of Akt/mTOR, NF- κ B and/or STAT3, prevention of EMT process, reduction of ATP synthesis *via* the inhibition of complex I of the mitochondrial respiratory chain, activation of AMPK, generation of ROS and modulation of autophagy in a dose- and cancer cell type-dependent manner (Tables 2 and 3) (Bao et al., 2012b; Ben Sahra et al., 2010; Ben Sahra et al., 2011; Chen et al., 2012; Colquhoun et al., 2012; Cufi et al., 2012; Gou et al., 2013; Hirsch et al., 2009; Hirsch et al., 2013; Iliopoulos et al., 2011; Jung et al., 2011; Liu et al., 2012; Menendez et al., 2012; Petrushev et al., 2012; Sato et al., 2012; Shank et al., 2012; Sinnett-Smith et al., 2013; Song et al., 2012; Vazquez-Martin et al., 2010; Vazquez-Martin et al., 2011; Wurth et al., 2012; Zakikhani et al., 2008). More specifically, it has been observed that metformin preferentially eradicated the CD44⁺/CD24^{+/-} breast cancer stem cell subpopulation isolated from metastatic JIMT-1 breast cancer cell line established from a patient showing resistance to trastuzumab treatment and reversed resistance of JIMT-1 xenografts expressing HER2 (erbB2) oncogene to trastuzumab *in vivo* (Cufi et al., 2012). Metformin was also more effective at inducing the cytotoxic effects on CD44⁺/CD24^{+/-} MCF-7 breast cancer stem cell subpopulation than the bulk mass of non-cancer stem cells and increased the efficacy of radiation to suppress the FSaII tumor growth *in vivo* through the AMPK activation and mTOR inactivation (Song et al., 2012). Moreover, it has also been reported that metformin significantly inhibited the formation of tumor spheres derived from ovarian cancer cell lines and patient ovarian tumor cells and reduced ALDH⁺ ovarian cancer stem cell subpopulation *in vitro* (Shank et al., 2012). Metformin was also effective at inhibiting the growth and promoting the anti-tumoral effect induced by cisplatin on tumor xenografts derived from SKOV3 tumor cell line and ALDH⁺ ovarian cancer stem cells *in vivo* (Shank et al., 2012). Importantly, it has also been noted that the *in vitro* treatment of PANC-1 and MiaPaCa-2 pancreatic cancer cells with low doses of metformin under physiological low-glucose conditions repressed mTORC1 activation and DNA synthesis through an AMPK-dependent mechanism and inhibited their proliferation as compared to these pancreatic cancer cells cultured in a medium containing high-glucose concentrations (Sinnett-Smith et al., 2013). Furthermore, the treatment of MCF-7 breast cancer cells engineered to overexpress HER2 with low doses of metformin under glucose starvation conditions also caused a substantial rate of apoptotic cell death as compared to these cancer cells cultured under supraphysiological concentrations of glucose (Menendez et al., 2012). These data suggest that the anticarcinogenic effects of metformin on the bulk mass of cancer cells may be promoted in presence of low glucose concentrations which are typically

prevalent in the areas of solid tumors *in vivo* that are poorly perfused. Hence, these pre-clinical results support great interest to use metformin, alone or in combination with current chemotherapeutic drugs, to eradicate cancer stem/progenitor cells and thereby prevent tumor development and disease relapse of diverse aggressive cancers.

Collectively, these data have underlined the critical importance to combine different anticancer drugs targeting distinct master regulators of glycolytic and lypogenic pathways and autophagy to prevent the development of treatment resistance that may result from the activation of alternative metabolic pathways that provide the energy and nutrients necessary to the survival of cancer cells.

3.2. Potential therapeutic targets in the local microenvironment of cancer- and metastasis-initiating cells

In view of the fact that the local microenvironment of cancer stem/progenitor cells and their differentiated progenies plays an active role in their malignant transformation at the primary and secondary neoplasms and angiogenic process, many efforts are being made to counteract cancer progression by targeting the host stromal cells. In particular, the targeting of myofibroblasts and immune cells that support the malignant transformation of cancer stem/progenitor cells as well as the use of anti-angiogenic agents targeting endothelial cells involved in tumor neo-angiogenic process may constitute the adjuvant treatments. These therapeutic strategies may be effective to improve current cancer treatments and counteract cancer progression to locally invasive and metastatic disease states (Calabrese et al., 2007; Folkins et al., 2007; Ganss, 2006; Keung et al., 2013; Langley and Fidler, 2007; Mimeault et al., 2007a; Mimeault et al., 2007b; Mimeault et al., 2008a; Mimeault and Batra, 2006; Mimeault and Batra, 2007a; Mimeault and Batra, 2007b; Mimeault and Batra, 2008b; Mitchem et al., 2013; Moreira et al., 2007; Rafii et al., 2002; Rosa et al., 2008; van den Hoogen et al., 2011b). For instance, it has also been shown that the targeting of tumor-infiltrating macrophages and inflammatory monocytes by inhibiting either the myeloid cell receptors, colony stimulating factor-1 receptor (CSF1R) or chemokine (C-C motif) receptor 2 (CCR2) decreased the number of tumor-initiating cells in pancreatic tumors (Mitchem et al., 2013). This treatment type was also effective at improving the efficacy of current chemotherapeutic drug, gemcitabine, inhibiting metastasis and increasing antitumoral T-cell responses (Mitchem et al., 2013). Moreover, the combinations of cytotoxic agents targeting cancer stem/progenitor cells and their progenies plus inhibitors of HIF-1 α and HIF-2 α , COX-1 or -2, NF- κ B and/or VEGF-VEGFRs that inhibit tumor angiogenesis have also been shown to be effective strategies to prevent primary and secondary tumor growth and disease recurrence (Calabrese et al., 2007; Mimeault and Batra, 2013; Vosseler et al., 2005). It has been observed that the treatment of U87 glioma cell xenografts orthotopically implanted in mice with an anti-VEGF mAb, bevacizumab reduced the microvasculature density and number of vessel-associated CD133⁺/nestin⁺ brain cancer-initiating cells and tumor growth (Calabrese et al., 2007).

In addition, the use of pharmacological agents that are able to interfere with the formation of pre-metastatic niches, recruitment, homing and/or adhesion of tumorigenic and migrating cancer stem/progenitor cells on the endothelial surface and stromal components at distant

metastatic sites also constitute potential cancer therapies (Peinado et al., 2012). These therapeutic strategies could eradicate circulating cancer cells and metastasis-initiating cells with stem cell-like properties and prevent secondary tumor initiation and disease relapse. In this matter, it has been reported that the interference with the regulators of membrane trafficking and exosome formation may prevent the formation of pre-metastatic niches and metastases. For example, it has been observed that MET was highly expressed on circulating CD45⁻/KIT^{low/+}/tyrosine kinase with immunoglobulin-like and EGF-like domains 1 (TIE2⁺) bone marrow-derived progenitors from patients with metastatic melanoma while the down-regulation of MET expression in exosomes diminished the pro-metastatic behavior of these circulating cells (Peinado et al., 2012). Similarly, the down-regulation of Rab27A, which is highly expressed in melanoma cells, by siRNA also decreased the exosome production by melanoma cells, and thereby prevented the education of bone marrow cells by exosomes and reduced the tumor growth and metastases of melanoma cells (Peinado et al., 2012).

On the other hand, the blockade of chemoattractant gradient systems involved in the preferential migration of cancer cells at distant metastatic sites also may constitute potential therapeutic strategies to counteract metastases. These chemoattractant systems includes SDF-1/CXCR4, monocyte chemoattractant protein-1 (MCP-1/CCL2) and its high affinity receptor CCR2, and endothelial E-selectine and its P-selectine glycoprotein ligand-1 expressed on circulating cancer cells (Hart et al., 2005; Hartmann et al., 2005; Kang et al., 2005; Kucia et al., 2005; Kulbe et al., 2004; Liang et al., 2004; Loberg et al., 2006; Mimeault et al., 2007a; Mimeault et al., 2007b; Mimeault et al., 2008b; Mimeault and Batra, 2006; Mimeault and Batra, 2007a; Mimeault and Batra, 2007b; Mimeault and Batra, 2008a; Neiva et al., 2005; Onoue et al., 2006; Witz, 2006). More specifically, the use of a mAb directed against chemokine SDF-1 or its cognate receptor CXCR4 expressed on migrating cancer cells or a selective CXCR4 antagonist, such as 14-mer peptide (TN14003) or AMD 3100, has been shown to inhibit the migration of cancer cells at distant sites and metastasis formation (Table 2; Fig. 1) (Hart et al., 2005; Hartmann et al., 2005; Kang et al., 2005; Kucia et al., 2005; Kulbe et al., 2004; Liang et al., 2004; Mimeault et al., 2007a; Mimeault et al., 2007b; Mimeault et al., 2008b; Mimeault and Batra, 2006; Mimeault and Batra, 2007a; Mimeault and Batra, 2007b; Mimeault and Batra, 2008a; Neiva et al., 2005; Onoue et al., 2006). For instance, it has been shown that disseminated PC3 cells homing at the hypoxic endosteal niche of HSCs in the bone marrow in a mouse model of metastasis may be mobilized out of the niche into the peripheral circulation by a treatment of mice with the mobilizing agent AMD 3100 targeting CXCR4 expressed by PC3 cells (Shiozawa et al., 2011a). In this matter, in view of the fact that HIF-1 α and NF- κ B may cooperate to induce an up-regulation of CXCR4 transcriptional expression and migration of cancer cells, the co-targeting of these signaling elements also may represent potential anti-metastatic treatments (Helbig et al., 2003; Schioppa et al., 2003). Moreover, in considering the fact that several epithelial cancers such as lung, breast, prostate, pancreas and kidney cancers typically display a pre-disposition to metastasize at bones and induce the formation of osteoblastic and/or osteolytic lesions, the use of inhibitors that are able to interfere with the development of these types of bone damages may constitute an adjuvant therapy. Among these agent types, there are bisphosphates such as zoledronate that can inhibit osteoclast activity, and

thereby reduce the osteolytic bone resorption (Winter et al., 2008). These adjuvant treatments could prevent severe bone complications including intense pain resulting from osteoblastic lesions that are associated with bone remodeling mediated by osteoblast-driven new bone overgrowth as well as osteolytic lesions implicating bone lysing.

4. Conclusions and perspectives

Taken together, these recent studies have revealed that the most cancers may derive from the accumulation of genetic and/or epigenetic alterations in adult stem/progenitor cells endowed with a high self-renewal capacity or their early progenies that acquire stem cell-like properties and which lead to their malignant transformation into cancer stem/progenitor cells. Importantly, it has also been observed that the alterations in distinct oncogenic products occurring in cancer stem/progenitor cells with aberrant differentiation potential during cancer initiation and progression may result in the development of different cancer subtypes that exhibit different responses to cancer therapies. Consequently, the combined use of different gene products altered in cancer stem/progenitor cells and their differentiated progenies as molecular biomarkers and therapeutic targets may represent potential strategies for improving the accuracy of current diagnostic and prognostic methods and efficacy of current treatments of cancer patients in the clinics.

Additional investigations are however required to further establish stem cell-like markers and signal transduction elements deregulated in cancer- and metastasis-initiating cells and their local microenvironment that are involved in the regulation of their high self-renewal ability, survival and/or treatment resistance in considering the cancer heterogeneity. More particularly, it will be important to delineate of gene products that may cooperate to the malignant reprogramming and acquisition of a more aggressive behavior and survival advantages by cancer stem/progenitor cells during the EMT process under normoxic and hypoxic conditions. Moreover, the determination of specific biomarkers expressed by exosomes produced by cancer cells and circulating tumor cells also should help to design novel cancer therapies to prevent the metastases at distant sites. Hence, these future investigations should result in the validation of distinct therapeutic targets in cancer stem/progenitor cells and their differentiated progenies that could be exploited to develop novel multitargeted therapies for reversing their multidrug resistance phenotypes and treating and even curing the patients diagnosed with locally advanced, metastatic, recurrent and lethal cancers.

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Abbreviations

ABC	ATP-binding cassette
ABCG2/BCRP	breast cancer related protein
ACC	acetyl-CoA carboxylase
ALDH	aldehyde dehydrogenase
AMPK	AMP-activated protein kinase
BMI-1	B lymphoma Mo-MLV insertion region 1 homolog
CAIX	carbonic anhydrase IX
COX-2	cyclooxygenase-2
CPA	cyclophosphamide
CXCR4	CXC chemokine receptor 4
DEAB	diethylaminobenzaldehyde
DETC	diethyldithiocarbamate
DRAM1	DNA-damage regulated autophagy modulator protein 1
ECM	extracellular matrix
EGFR	epidermal growth factor receptor
EMT	epithelial-mesenchymal transition
ESA	epithelial-specific antigen
FASN	fatty acid synthase
Fzd	Frizzled receptor
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GBM	glioblastoma multiforme
GLUTs	glucose transporters
GPI	glucose-6-phosphate isomerase
HA	hyaluronan
HGF	hepatocyte growth factor
HIFs	hypoxia-inducible factors

HK	hexokinase
HMG-CoA reductase	3-hydroxy-3-methyl-glutaryl-CoA reductase
Hsp	heat shock protein
IGF-1R	insulin-like growth factor 1-receptor
IL	interleukin
KIT	stem cell factor receptor
LDH	lactate dehydrogenase
MAPKs	mitogen-activated protein kinases
MET	hepatocyte growth factor/scatter factor receptor
MIC-1	macrophage inhibitory cytokine-1
MMPs	matrix metalloproteases
Myc	V-myc myelocytomatosis viral oncogene homolog (avian)
mTOR	molecular target of rapamycin
NSCLC	non-small cell lung cancer
NF-κB	nuclear factor-kappaB
NOD-SCID	non-obese diabetic-severe combined immunodeficient
Oct-3/4	octamer-binding transcription factor-3/4
PDGF	platelet-derived growth factor
PDGFRs	platelet-derived growth factor receptors
PDTC	pyrrolidinethiocarbamate
PEP	phosphoenolpyruvic acid
PFK	phosphofructokinase
2-PG	glycerate 2-phosphate
3-PG	glycerate 3-phosphate
P-gp	P-glycoprotein
PGM	phosphoglyceromutase
PI3K	phosphatidylinositol 3'-kinase
PK	pyruvate kinase
PTCH	pathched receptor
PTEN	phosphatase and tensin homolog deleted on chromosome 10
ROS	reactive oxygen species
SCC	squamous cell carcinoma

SCF	stem cell factor
SDF-1	stromal cell-derived factor-1
SHH	sonic hedgehog ligand
shRNA	small hairpin RNA
siRNA	small interfering RNA
SMO	smoothened
SP	side population
SREBPs	transcription factor sterol regulatory element-binding proteins
SRY or SOX-2	sex determining region Y-box 2
TG2	tissue transglutaminase
TGF-β	transforming growth factor- β
TGF-βR	transforming growth factor-beta receptor
TRAIL	apoptotic tumor necrosis factor-related apoptosis-inducing ligand
uPA	urokinase plasminogen activator
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor and Wnt, Wiggless ligand

Fig 1. Altered growth factor and metabolic signaling pathways involved in aggressive behavior and multidrug resistance phenotypes of cancer stem/progenitor cells and their progenies

The scheme shows the molecular events associated with the malignant transformation of cancer cells that may be induced through the genetic and/or epigenetic alterations and the changes in their local microenvironments during cancer progression. More specifically, the sustained activation of diverse growth factors in cancer cells which may lead to an enhanced expression of pluripotency-associated transcription factors (Myc, Oct-3/4, Sox-2, Nanog), epithelial-mesenchymal transition (EMT) associated molecules (N-cadherin, vimentin, twist, snail and/or slug) and drug resistance-related proteins (aldehyde dehydrogenase “ALDH”, Bcl-2, survivin and macrophage inhibitory cytokine “MIC-1”) that are involved in their high selfrenewal ability, acquisition of migratory phenotype and invasive capacity, metastases and treatment resistance, are indicated. The potential metabolic pathways including glycolysis, lipogenesis and autophagy that may be altered in cancer cells under oxygen and nutrient deprivation are also illustrated.

Fig 2. Novel cancer therapies by targeting distinct growth factor and metabolic signaling pathways and multidrug resistance transporters in cancer stem/progenitor cells and their progenies

The scheme shows the potential cytotoxic effects induced by the inhibitors of tyrosine kinase activity such as EGFR (gefitinib or erlotinib), SMO hedgehog signaling element (cyclopamine, PI-269609 or GDC-0449) and Gli-1 and Gli-2 (GANT-61) as well as monoclonal antibody (mAb) directed against CD133 and CD44 stem cell-like markers and SDF-1, Wnt and SHH ligands. The therapeutic interest to combine a specific inhibitor of PI3K/Akt/mTOR cascade such as LY294002, Akt inhibitor or NVP-BEZ235, which may induce the autophagy, with a pharmacological agent (3-methyladenine, monensin, bafilomycin A1, chloroquine or hydrochloroquine) blocking autophagy is also indicated. Moreover, the inhibition of cholesterol and fatty acid synthesis by using a specific inhibitor of HMG-CoA reductase (arvastatin), acetyl-CoA carboxylase "ACC" (TOFA) and fatty acid synthase "FASN" (cerulenin, C75, C93 or orlistat), alone or in combination, is also indicated. In addition, the inhibition of different ABC multidrug transporters by using specific transporter inhibitors or antibodies and which result in the intracellular accumulation of cytotoxic drugs is also illustrated.

Table 1

Targeted strategies directed against stem cell-like markers of cancer stem/progenitor cells and their differentiated progenies in diverse cancer types

Therapeutic target	Inhibitory agent	Cancer type	Therapeutic effects	Ref.
Telomerase	MT-hTer/siRNA	Prostate cancer	The telomerase interference using MT-hTer/siRNA strategy induced anti-proliferative and apoptotic effects on the integrin- $\alpha_3\beta_1^{\text{high}}$ /CD44 ⁺ prostate cancer progenitor cell subpopulation from freshly-resected human PC specimens and DU145 cells. The MThTer/siRNA treatment was also effective at counteracting the tumor formation by $\alpha_3\beta_1^{\text{high}}$ /CD44 ⁺ DU145 cell fraction <i>in vivo</i> .	(Xu et al., 2011)
Telomerase	Telomerase-specific oncolytic adenoviral vector carrying TRAIL ligand and E1A gene (Ad/TRAIL-E1)	Esophageal carcinoma cells	Ad/TRAIL-E1 preferentially targeted radioresistant esophageal carcinoma cells with stem cell-like properties and induced significant tumor growth suppression and longer survival in radioresistant cancer cells-bearing mice.	(Zhang et al., 2008b)
CD133	Anti-CD133 mAbs	Melanoma	Cytotoxic effects in FEXM-1 human melanoma cells <i>in vitro</i> .	(Rappa et al., 2008)
CD133	shRNA	Melanoma	Reduction of the growth, sphere-forming capacity and mobility of FEXM-1 human melanoma cells <i>in vitro</i> and inhibition of their metastatic capacity <i>in vivo</i> .	(Rappa et al., 2008)
CD44	Anti-CD44 mAb	Liver cancer	Induction of the apoptotic effect on the total cancer cell mass, including CD90 ⁺ and CD90 ⁻ cell fractions from MHCC-97L liver cancer cell line <i>in vitro</i> . Growth inhibitory effect on tumors derived from CD90 ⁺ MHCC-97L cell subpopulation subcutaneously or orthotopically implanted in nude mice.	(Yang et al., 2008)
CD44	Small oligosaccharides directed against CD44 ligand, HA	Brain cancer	Growth inhibitory effect on spinal cord gliomas formed after engraftment of the SP cell fraction from C6 glioma cell line in rats.	(Gilig et al., 2008)
CD44	Anti-CD44 mAb	Breast cancer	The CD44 down-regulation in breast cancer stem cells by RNA interference induced their differentiation and inhibited the expression levels of different stem cell-like markers, oncogenes and anti-apoptotic factors such as lymphoid enhancer-binding factor-1 (LEF-1), Myc, EGFR, mucin-1, FASN and Bel-2 and thereby sensitized these immature cancer cells to the anti-tumoral effect induced by doxorubicin.	(Pham et al., 2011; Van Phuc et al., 2011)
CD66c	shRNA	Colorectal cancer	The silencing of carcinoembryonic antigen-related cell adhesion molecule 66c, CD66c, which is expressed at a high level on CD133 ⁺ colon cancer cells, by shRNA inhibited the proliferation and clonogenic potential of Caco2 colon cancer cells <i>in vitro</i> and the tumor growth of Caco2 cell-derived xenografts <i>in vivo</i> .	(Gemei et al., 2013)
ALDH7A	shRNA	Prostate cancer	The ALDH7A down-regulation by shRNA reduced the number of integrin- $\alpha_2\beta_1^{\text{high}}$ /integrin- $\alpha_3\beta_1^{\text{high}}$ /CD44 ⁺ prostate cancer stem/progenitor cells detected in the PC3M-Pro4 cell line and inhibited their migratory ability <i>in vitro</i> and intra-bone growth and metastasis formation <i>in vivo</i> .	(van den Hoogen et al., 2011b)
ALDH1	Diethylaminobenzaldehyde (DEAB)	Colorectal cancer	The inhibition of ALDH1 activity with DEAB sensitized human colorectal tumor lines to cytotoxic effects induced by bioactive metabolite byproduct, 4-hydroxycyclophosphamide (CPA) of chemotherapeutic drug, CPA.	(Dylla et al., 2008)
ALDH1	shRNA	Colorectal cancer	The ALDH1 down-regulation by shRNA reduced the number of ESA ⁺ /CD44 ⁺ /CD166 ⁺ colon cancer stem cells detected in human colorectal tumor line-derived xenografts subcutaneously established in nude mice after CPA treatment.	(Dylla et al., 2008)
ALDH1	DEAB	Breast cancer	The inhibition of ALDH activity with DEAB reduced the tumorsphere-forming ability of diverse human and mouse breast cancer cell lines including 4T1 mouse	(Kim et al., 2012)

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and suppressed their *in vitro* self-renewal capacity under hypoxic conditions and tumor-initiating ability *in vivo*.

Table 2

Targeted strategies directed against growth factor signaling elements and EMT program-associated molecules in cancer stem/progenitor cells and their differentiated progenies in diverse cancer types

Therapeutic target	Inhibitory agent	Cancer type	Therapeutic effects	Ref.
EGFR	Gefitinib or erlotinib	Brain cancer	Gefitinib or erlotinib induced antiproliferative effects on EGFR ⁺ /CD133 ⁺ tumor-initiating cells (TICs) from 5 patients with glioblastomas. It has however been noted that two GBM TICs among seven glioma specimens analyzed, which displayed a high level of Akt activation, were insensitive to this treatment type.	(Griffith et al., 2009)
EGFR	Gefitinib	Prostate cancer	A treatment with gefitinib inhibited the prostasphere-forming ability of SP and non-SP cell fractions from invasive and tumorigenic WPE1-NB26 prostate cancer cell line. Gefitinib also induced the prostasphere disintegration and apoptosis <i>via</i> the caspase pathway on SP and non-SP WPE1-NB26 cells.	(Mimeault et al., 2012)
EGFR(HER-1)/erbB2 (HER-2)	Oral dual tyrosine kinase inhibitor of EGFR and erbB2 (lapatinib) plus anti-erbB2 mAb (trastuzumab)	Breast cancer	A treatment of 40 women with erbB2-positive breast cancer with lapatinib alone for 6 weeks followed by 6 weeks with standard chemotherapy and anti-erbB2 mAb, trastuzumab resulted in a complete tumor regression in 63% patients. These data indicate that this combination therapy may eradicate CD44 ⁺ /CD24 ^{-low} breast cancer-initiating cells.	(Schmidt, 2008)
erbB2 (HER-2)	Trastuzumab plus metformin	Breast cancer	Metformin preferentially killed CD44 ⁺ /CD24 ^{-low} breast cancer-initiating cells and sensitized JIMT-1 xenograft mouse models to the growth inhibitory effect of trastuzumab.	(Cufi et al., 2012)
Hedgehog	Cyclopamine	Brain cancer	The blockade of hedgehog pathway by using cyclopamine prevented GBM-derived neurospheres and reduced or eliminated the glioma stem cell-like fraction in gliomas characterized by both aldehyde dehydrogenase activity and SP cell fraction. Moreover, the hedgehog blockade in viable GBM cells also prevented the tumor formation in athymic mice, indicating that the glioma stem cell population had been eradicated.	(Bar et al., 2007)
Hedgehog	Cyclopamine	Brain cancer	A long-term treatment with cyclopamine eradicated CD133 ⁺ gliomasphere cells in GBM tumor samples from patients in culture <i>in vitro</i> and inhibited the formation of gliomasphere cell-derived tumors <i>in vivo</i> . Additive or synergistic antiproliferative and apoptotic effects were also induced by low doses of cyclopamine plus temozolomide on human gliomasphere cells <i>in vitro</i> .	(Clement et al., 2007)
Hedgehog	Cyclopamine	Prostate cancer	Cyclopamine induced a complete regression of the tumors derived from human CWR22RV1 and PC3 cells xenografted in athymic nude mice without apparent tumor re-growth after 58 and 148 days of treatment cessation, respectively. These results suggest that the total tumor cell mass including prostate cancer-initiating cells may be eliminated by a cyclopamine treatment.	(Karhadkar et al., 2004)
Hedgehog	Cyclopamine	Pancreatic cancer	Cyclopamine decreased the number of E3LZ10.7 pancreatic cancer cells expressing cancer stem cell-like marker ALDH <i>in vitro</i> and inhibited the metastatic spread of E3LZ10.7 cells orthotopically implanted in nude mice.	(Feldmann et al., 2007)
Hedgehog	Cyclopamine	Pancreatic cancer	Cyclopamine induced a decreased expression of BMI-1 and ABCG2 in PANC-1 tumorspheres which was accompanied by an inhibition of the proliferation of PANC-1 tumorspheres <i>via</i> a blockade in G1 phase and their enhanced sensitivity to gemcitabine.	(Huang et al., 2012a)

Therapeutic target	Inhibitory agent	Cancer type	Therapeutic effects	Ref.
Hedgehog	Cyclopamine	Pancreatic cancer	A treatment with cyclopamine and gemcitabine of Panc185 pancreatic cancer cells xenografted in athymic nude mice was more effective as compared to single drugs at inducing the tumor regression and decreasing the number of Panc185 cells expressing cancer stem cell-like markers CD24 and ALDH.	(Jimeno et al., 2009)
Hedgehog	Oral active SMO inhibitor, IPI-269609	Pancreatic cancer	A treatment with IPI-269609 decreased the number of E3LZ10.7 pancreatic cancer cells expressing a high ALDH activity detected in primary tumors derived from orthotopic implantation of these cancer cells <i>in vivo</i> and inhibited their metastatic spread.	(Feldmann et al., 2008)
Hedgehog	Oral active SMO inhibitor, GDC-0049 or suppression of both Gli-1 plus Gli-2 by shRNA	Pancreatic cancer	GDC-0499 (Vismodegib) inhibited the sphere forming-ability and cell viability and induced apoptosis in three pancreatic cancer cell lines and pancreatic stem/progenitor cells through the caspase-3 activation and poly (ADP-ribose) polymerase (PARP) cleavage. GDC-0449-induced apoptosis was also accompanied by an increase of Fas and tumor necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAIL-R1)/DR4 and TRAIL-R2/DR5 expression while PDGFR α and Bcl-2 was down-regulated.	(Singh et al., 2011)
Hedgehog	(N-(2-hydroxypropyl)methyl methacrylamide) copolymer-based cyclopamine delivery system	Prostate cancer	The treatment of RC-929/human telomerase reverse transcriptase (TERT) prostate cancer epithelial cells with a drug delivery system consisting of (N-(2-hydroxypropyl)methyl methacrylamide) copolymer-conjugated cyclopamine reduced the expression level of CD133 stem cell marker and viability of prostate cancer stem cells.	(Zhou et al., 2012)
Hedgehog	Cyclopamine	Prostate cancer	A treatment with cyclopamine plus paclitaxel was more effective as single drugs at inducing the apoptotic effect on DU145-TXR and PC3-TXR cells made resistance to paclitaxel and reducing the viability of SP cell fraction detected by Hoechst dye efflux assay.	(Singh et al., 2012b)
Hedgehog	Gli transcription factor inhibitor, GANT-61 or shRNA directed against Gli-1 and Gli-2	Pancreatic cancer	The inhibition of Gli-1 and Gli-2 by using GANT-61 or shRNA suppressed the expression of pluripotency-associated transcription factors (Nanog, Oct-4, Sox-2 and c-Myc) and EMT program-associated molecules (N-cadherin and transcription factors Snail, Slug and Zeb1) in CD133 ⁺ /CD44 ⁺ /CD24 ⁺ /ESA ⁺ human pancreatic cancer stem cells from primary tumors. Moreover, GANT-61 inhibited the viability and sphere-forming ability and induced the apoptosis by activation of caspase-3 and cleavage of PARP in these immature cancer cells <i>in vitro</i> and inhibited their tumor growth in NOD/SCID IL2R γ null mice.	(Fu et al., 2013)
EGFR and hedgehog	Gefitinib plus cyclopamine	Prostate cancer	A combination of low doses of gefitinib and cyclopamine plus mitoxantrone induced supra-additive cytotoxic effects on CD44 ⁺ -high and CD44 ⁺ -low cell fractions from DU145 and PC3 prostate cancer cells as compared to drugs alone or double combination therapy.	(Mimeault et al., 2007c)
EGFR and hedgehog	Gefitinib plus cyclopamine	Prostate cancer	A combination of low doses of gefitinib and cyclopamine plus docetaxel induced supra-additive or additive antiproliferative and apoptotic effects on SP and non-SP cell fractions from WPE1-NB26 prostate cancer cell line <i>via</i> the activation of caspase pathway as compared to drugs alone or combinations of two drugs.	(Mimeault et al., 2010a)
Wnt/ β -catenin	Small molecule antagonist of Wnt/ β -catenin, PKH 118-310	Breast cancer	PKH 118-310 inhibited the sphere-forming capacity of primary breast tumor cells and primary mammary epithelial cells and self-renewal ability of mammary epithelial stem/progenitor cells <i>in vitro</i> and tumor growth of primary tumor cells <i>in vivo</i> .	(Hallett et al., 2012)

Therapeutic target	Inhibitory agent	Cancer type	Therapeutic effects	Ref.
Wnt/ β -catenin	miR-320 overexpression	Prostate cancer	miR-320 overexpression in prostate cancer cells significantly decreased their expression level of β -catenin and prostate cancer stem cell-like markers (CD44, Nanog and nestin), inhibited their prosphere-forming ability and tumorigenic capacity and enhanced their sensitivity to cytotoxic effects induced by cisplatin, carboplatin or paclitaxel.	(Hsieh et al., 2012)
Notch	γ -secretase inhibitor, RO4929097	Melanoma	RO4929097 decreased the expression level of Notch transcriptional target HES-1 in human primary melanoma cells, reduced their proliferation and impaired their ability to form colonies in soft agar and to organize in tridimensional spheres. RO4929097 was also effective at inhibiting the growth of human primary melanoma xenografted in NOD/SCID/IL-2 γ R $^{-/-}$ mice as well as their subsequent tumor formation in a serial xenotransplantation model. These data suggest that the blockade of Notch cascade may suppress the tumor-initiating potential of melanoma cells.	(Huynh et al., 2011)
Notch	γ -secretase inhibitor, N-[N-(3,5-difluorophenyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT) or a Notch 4-neutralizing antibody	Breast cancer	Inhibition of mammosphere-forming ability of primary breast cancer cells from ductal carcinoma <i>in situ</i> .	(Farnie et al., 2007)
Notch	γ -secretase inhibitor IX (GSI IX)	Pancreatic cancer	GSI IX inhibited the growth and induced apoptosis in CD44 $^{+}$ /ESA $^{+}$ pancreatic cancer-initiating cells from human KP3 pancreatic cell line <i>in vitro</i> and xenograft mouse model <i>in vivo</i> .	(Palagani et al., 2012)
Notch	DAPT	Pancreatic cancer	The blockade of delta-like ligand (DLL4)-Notch pathway by DAPT decreased the viability and number of CD133 $^{+}$ cells in BxPC-3 pancreatic cancer cells engineered for overexpressing DLL4 ligand and improved their sensitivity to gemcitabine treatment.	(Kang M and Wu Y, 2012)
Notch1	shRNAs or DAPT	Lung cancer	The treatment of H460 and H661 lung cancer cell lines with Notch1 shRNA or DAPT substantially reduced the cisplatin-induced enrichment of CD133 $^{+}$ lung cancer cells and ABCG2 and ABCB1 expression and increased their sensitivity to doxorubicin and paclitaxel. Furthermore, the intratumoral injection of DAPT combined with a cisplatin treatment significantly reduced the number of CD133 $^{+}$ lung cancer cells <i>in vivo</i> .	(Liu et al., 2013)
Hedgehog and Notch	Cyclopamine or GDC0449 plus γ -secretase inhibitor, dibenzazepine (DBZ) and compound E	Prostate cancer	The treatment with the chemical inhibitors, including cyclopamine or GDC0449 plus γ -secretase inhibitor, DBZ and compound E of DU145 and CW22Rv1 prostate cancer cell lines made resistant to docetaxel, which lacked differentiation markers and exhibited potent tumor-initiating capacity, depleted these cancer cells through the inhibition of the survival molecules Akt and Bcl-2 and improved the cytotoxic effects of docetaxel on <i>in vitro</i> and <i>in vivo</i> .	{Domingo-Domenech, 2012 11047 /id}
CXCR4	Anti-CXCR4 antibody or CXCR4 antagonist, AMD3100	Prostate cancer	Anti-CXCR4 antibody inhibited the prosphere-forming ability of DU145 cells and decreased the number of CD133 $^{+}$ /CD44 $^{+}$ DU145 progenitor cell fraction. CXCR4 antagonist, AMD3100, also decreased the number of CD133 $^{+}$ /CD44 $^{+}$ DU145 progenitor cell fraction and inhibited the tumor growth of DU145 xenograft tumors at least in part through the inhibition of PI3K/Akt pathway. Moreover, a combination of AMD3100 with docetaxel targeting the total mass of DU145 cells was also more effective than single drugs at inhibiting tumor growth and inducing tumor regression.	(Domanska et al., 2012)
CXCR4	AMD 3100	Prostate cancer	A combination of AMD3100 targeting CXCR4 expressed by CD133 $^{+}$ /CD44 $^{+}$ DU145 cancer stem/progenitor cells plus docetaxel was more effective than single drugs at inducing the tumor growth inhibitory effect on DU145 cell	(Dubrovskaya et al., 2012a)

Therapeutic target	Inhibitory agent	Cancer type	Therapeutic effects	Ref.
CXCR4	AMD 3100	Breast cancer	xenografts in NOD/SCID mice and preventing the tumor re-growth after treatment cessation. xenografts in NOD/SCID mice and preventing the tumor re-growth after treatment cessation.	(Dubrovská et al., 2012b)
CXCR4	siRNA or AMD3100	Lung cancer	The down-regulation of CXCR4 signaling pathway by using AMD3100 antagonist specifically inhibited the growth of progenitor cell population in tamoxifen-resistant MCF-7 breast cancer cells both <i>in vitro</i> and <i>in vivo</i> .	(Jung et al., 2013)
Integrin- α v	shRNA against integrin- α v	Prostate cancer	The inhibition of CXCR4 by CXCR4-siRNA or using AMD3100 antagonist significantly suppressed the sphere-forming ability and oestrogenicity of parental A549 and gefitinib-resistant A549/GR lung cancer cells and various non-small cell lung cancer (NSCLC) cell lines.	(van den Hoogen et al., 2011a)
Integrin- α v	shRNA against integrin- α v	Prostate cancer	The integrin- α v down-regulation by shRNA reduced the expression of the EMT program-associated molecules (snail, snail2 and twist) and number of the ALDH ^{high} /CD44 ⁺ /integrin- α v ^{high} /integrin- α 2 ^{high} prostate cancer stem/progenitor cell fraction detected in PC3M-Pro4 human prostate cancer cell line. The integrin- α v down-regulation also inhibited the clonogenic and migratory potentials of PC3M-Pro4 cells <i>in vitro</i> and decreased their tumorigenicity and formation of bone metastases <i>in vivo</i> .	(van der Horst et al., 2011)
Integrin- α v	Integrin- α v antagonist, GLPG0187	Prostate cancer	The inhibition of integrin- α v by GLPG0187 reduced the expression of the EMT program-associated molecules and number of the ALDH ^{high} prostate cancer stem/progenitor cell fraction detected in PC3M-LN4 human prostate cancer cell line. The inhibition of integrin- α v also reduced the <i>de novo</i> formation and progression of bone metastases by PC3M-LN4 prostate cancer cells <i>via</i> the induction of antitumoral, antiresorptive, and anti-angiogenic effects.	(van der Horst et al., 2011)
IL-4R	Anti-IL-4R mAb or IL-4R α antagonist	Colon cancer	A treatment with an anti-IL-4R mAb or IL-4R α antagonist enhanced the sensitivity of tumorigenic CD133 ⁺ cancer stem/progenitor cells from human colon carcinoma to the antitumoral effects induced by standard chemotherapeutic drugs <i>in vitro</i> and <i>in vivo</i> .	(Todoraro et al., 2007)
IL-6/IL-6R	siRNAs or anti-IL-6 and IL-6R mAbs	Lung cancer	The inhibition of IL-6/IL-6R signaling pathways by siRNA or mAbs significantly inhibited the growth of sphere-forming cells from the H460 non-small cell lung cancer cell line.	(Yi et al., 2012)
VEGF	Anti-VEGF mAb (bevacizumab)	Brain cancer	Bevacizumab induced anti-angiogenic effect and tumor growth inhibition of vessel-associated CD133 ⁺ /nestin ⁺ brain cancer stem cells from U87 glioma cells orthotopically implanted in nude mice.	(Calabrese et al., 2007)
VEGFR-2	DC101, a rat anti-mouse flk-1 mAb	Brain cancer	The treatment with an antiangiogenic agent, DC101 plus cyclophosphamide reduced the fraction of tumor sphere-forming units (SFU) in tumor xenografts of the C6 rat glioma cell line subcutaneously implanted in mice while single drugs were not effective.	(Folkens et al., 2007)
VEGFR-1	shRNA	Melanoma	Melanoma-specific shRNA-mediated knockdown of VEGFR-1 blocked the development of vasculogenic mimicry by ABCB5 ⁺ /VEGFR-1 ⁺ melanoma-initiating cell subpopulations and markedly suppressed the tumor growth <i>in vivo</i> .	(Frank et al., 2011)

Table 3

Targeted strategies directed against drug resistance-associated molecules in cancer stem/progenitor cells and their differentiated progenies in diverse cancer types

Therapeutic target	Inhibitory agent	Cancer type	Therapeutic effects	Ref.
Akt	Akt inhibitor VIII	Prostate cancer	Akt inhibitor VIII inhibited the prosphere-forming ability of SP and non-SP cell WPE1-NB26 fractions. Akt inhibitor VIII was also effective at inducing the prosphere disintegration and apoptosis <i>via</i> the caspase pathway on SP and non-SP WPE1-NB26 cells	(Mimeault et al., 2012)
PI3K/Akt/mTOR	PI3K (LY294002), mTOR (rapamycin) or siRNA-Akt-1	Liver cancer	The inhibition of the PI3K/Akt/mTOR signaling elements by LY294002 or siRNA-Akt-1 in the SP cell fraction sorted from MHCC-97L hepatocellular carcinoma cell lines induced an intracellular translocation of the ABCG2 transporter, attenuated the cellular efflux of doxorubicin and increased doxorubicin-induced cytotoxicity. siRNA-Akt-1 was also effective at reducing the number of SP cells detected by Hoechst 33342 efflux technique.	(Hu et al., 2008)
Akt	Akt-1 inhibitor	Liver cancer	The treatment of HCC cells from CD133 ⁺ Huh7 liver cancer cell-induced xenograft tumor with an Akt-1 inhibitor induced the cytotoxic effects on total tumor cell mass and reversed the chemoresistance of CD133 ⁺ HCC cells.	(Ma et al., 2008b).
PI3K/Akt/mTOR	PI3K (LY294002), mTOR (rapamycin) or siRNA-Akt-1	Liver cancer	The inhibition of the PI3K/Akt/mTOR signaling elements by LY294002 or siRNA-Akt-1 in the SP cell fraction sorted from MHCC-97L hepatocellular carcinoma cell lines induced an intracellular translocation of the ABCG2 transporter, attenuated the cellular efflux of doxorubicin and increased doxorubicin-induced cytotoxicity. siRNA directed against Akt-1 was also effective at reducing the number of SP MHCC-97L cells detected by Hoechst 33342 efflux technique.	(Hu et al., 2008)
PI3K/mTOR	PI3K inhibitor, LY294002 or dual PI3K/mTOR inhibitor, NVP-BEZ235	Prostate cancer	The treatment of DU145 or PC3 cells with LY294002 or NVP-BEZ235 inhibited their prosphere-forming ability and decreased the number of CD133 ⁺ /CD44 ⁺ PC progenitor cells in a dose-dependent manner <i>in vitro</i> .	(Dubrovskaya et al., 2009)
Akt	Metformin	Brain cancer	A treatment with metformin reduced the spherogenesis and proliferation rate of tumor-initiating cells isolated from four human glioblastomas <i>via</i> a marked inhibition of Akt-dependent cell survival pathway.	(Wurth et al., 2012)
AMPK and mTOR	Metformin	Breast cancer	Metformin preferentially induced the cytotoxic effects on CD44 ^{high} /CD24 ^{low} MCF-7 breast cancer stem cells relative to non-cancer stem cells and increased their radiosensitivity <i>in vitro</i> through the activation of AMPK, which induced the inactivation of mTOR and suppression of its downstream effectors such as ribosomal S6 protein kinase 1 (S6K1) and eIF-4E binding protein (4EBP1).	(Song et al., 2012)
mTOR	Metformin	Pancreatic cancer	Low concentrations of metformin selectively inhibited the proliferation of CD133 ⁺ pancreatic cancer cells <i>via</i> a reduction of phosphorylation of Erk and mTOR but independently of Akt and AMPK phosphorylation. Low concentrations of metformin also reduced the <i>in vitro</i> invasion and <i>in vivo</i> tumor formation by pancreatic cancer cells.	(Gou et al., 2013)
NF-κB	Partenolide	Prostate cancer	Partenolide inhibited the prosphere-forming ability of SP and non-SP cell WPE1-NB26 fractions. Partenolide was also effective at inducing the prosphere disintegration and apoptosis <i>via</i> the caspase pathway on SP and non-SP WPE1-NB26 cells.	(Mimeault et al., 2012)

Therapeutic target	Inhibitory agent	Cancer type	Therapeutic effects	Ref.
NF- κ B	Partenolide	Prostate cancer	Partenolide decreased the number of CD133 ⁺ primary prostate tumor cells while no cytotoxic effect was observed on CD133 ⁺ normal prostate cells from benign hyperplasia.	(Birnie et al., 2008)
NF- κ B	Partenolide, pyrrolidinedithiocarbamate (PDTIC) and its analog diethylthiocarbamate (DETC)	Breast cancer	The inhibition of NF- κ B by using partenolide, PDTIC or DETC preferential inhibited the proliferation and colony formation by MCF-7 sphere cells or SP cells relative to the parental MCF-7 cells and non-SP MCF-7 cell fraction.	(Zhou et al., 2007)
BMI-1	siRNA	Liver cancer	The BMI-1 down-regulation reduced the number of self-renewing SP cells detected in both Huh7 and PLC/PRE/5 HCC cells and tumor-initiating ability of SP cells implanted in NOD-SCID mice.	(Chiba et al., 2008)
BMI-1 or sonic hedgehog	BMI-1-siRNA or KAAD-cyclopamine	Brain cancer	The knockdown experiments of BMI-1 <i>in vitro</i> and <i>in vivo</i> demonstrate that hedgehog and BMI-1 are mutually indispensable pathways in the maintenance of childhood medulloblastoma-initiating cells.	(Wang et al., 2012d)
MIC-1	siRNA	Prostate cancer	The MIC-1 down-regulation by siRNA plus docetaxel reduced the number of viable SP and non-SP WPE1-NB26 cells detected by Hoechst 33342 fluorescent dye efflux technique.	(Mimeault et al., 2013)
MUC-4	shRNA	Pancreatic cancer	The MUC-4 down-regulation by shRNA plus gemcitabine reduced the number of viable SP and non-SP WPE1-NB26 cells detected by Hoechst 33342 fluorescent dye efflux technique.	(Mimeault et al., 2010b)
ABCB2	Fumitremorgin C	Liver cancer	Fumitremorgin C sensitized the SP cell fraction from MHCC-97L hepatocellular carcinoma cells to the cytotoxic effects induced by chemotherapeutic drug, doxorubicin by inhibiting ABCG2-mediated drug efflux.	(Hu et al., 2008)
ABCB2	Gefitinib	Head and neck SCC	The treatment of aerodigestive squamous cell carcinoma (SCC) cell lines with EGF increased the number of SCC-SP cells while gefitinib decreased the SCC-SP cell fraction at least in part by inhibiting ABCG2 expression at the cancer cell surface.	(Loebinger et al., 2008)
ABCB5	Anti-ABCB5 mAb	Melanoma	Anti-ABCB5 mAb reduced the number of ABCB5 ⁺ melanoma-initiating cells detected in primary melanoma cell-derived xenografts established in nude mice and inhibited the tumor growth.	(Schatton et al., 2008)

Table 4

Targeted strategies directed against hypoxia- and altered metabolism-associated molecules in cancer stem/progenitor cells and their differentiated progenies in diverse cancer types

Therapeutic target	Inhibitory agent	Cancer type	Therapeutic effects	Ref.
HIF-1 α or HIF-2 α	shRNA	Brain cancer	The down-regulation of HIF-1 α or HIF-2 α by shRNA in CD133 ⁺ GSCs from a patient's glioblastoma specimen inhibited their neurosphere-forming capacity and proliferation and induced the caspase-dependent apoptotic effect on immature cancer cells <i>in vitro</i> and attenuated their tumorigenic capacity <i>in vivo</i> .	(Li et al., 2009; Soeda et al., 2009)
Hsp27	shRNAs or Hsp27 inhibitor, quercetin or KRIBB3	Colorectal cancer	The inhibition of Hsp27 by shRNA, quercetin or KRIBB3 sensitized CD133 ⁺ colorectal cancer-initiating cells from HT-29 cells to hypoxia and serum depletion-induced apoptosis.	(Lin et al., 2012)
CAIX	Novel specific small-molecule inhibitors of CAIX, U-104 and GC-205	Breast cancer	The inhibition of CAIX expression or activity with U-104 and GC-205 inhibited the expansion of breast cancer stem cell (BCSCs) in breast cancer cell lines and primary metastatic breast cancer cells <i>in vitro</i> and significantly reduced the number of BCSCs in mice bearing orthotopic breast tumors. Furthermore, a combination of U-104 with paclitaxel was also more effective than single drugs at inhibiting the tumor growth and lung metastases.	(Lock et al., 2012)
SREBPs or HMG-CoA reductase	25-hydroxycholesterol (25-HC) or compactin	Melanoma	The inhibition of SREBP processing by 25-HC which maintains the SREBP/SREBP cleavage-activating protein (SCAP) complex in the endoplasmic reticulum or inhibition of HMG-CoA reductase with compactin inhibited the proliferation and tumorigenicity of SK-MEL-28 melanoma cells <i>in vitro</i> and <i>in vivo</i> .	(Yamauchi et al., 2011)
SREBP-1, FASN and HMG-CoA reductase	SREBP-1 shRNA or C75 plus atorvastatin	Brain cancer	The treatment of U87 and U87/EGFRvIII GBM cell lines with SREBP-1 shRNA induced their apoptotic death <i>in vitro</i> . Moreover, the co-treatment of U87 and U87/EGFRvIII GBM cell lines with C75 and atorvastatin was more effective as single drugs at inducing the apoptotic cell death <i>in vitro</i> and <i>in vivo</i> .	(Guo et al., 2009b)
Glycine decarboxylase/glycine-serine metabolism	siRNA	Non-small cell lung cancer	The knock-down of glycine decarboxylase by siRNA in tumor sphere cells established from primary lung adenocarcinoma specimens of patients inhibited their growth and tumorigenicity <i>in vivo</i> .	(Zhang et al., 2012a)
DNA-damage regulated autophagy modulator protein 1 (DRAM1) or p62 encoded by sequestosome 1 (<i>SQSTM1</i>)	shRNA	Brain cancer	The down-regulation of DRAM1 or p62 by shRNA in GSCs attenuated autophagy and metabolic energy, including ATP and lactate levels, and reduced their migration and invasion.	(Galavotti et al., 2012)
Autophagy	3-methyladenine or monensin	Pancreatic cancer	Inhibition of the autophagy by using 3-methyladenine or monensin sensitized Mia-PaCa2 pancreatic cancer cells with stem cell-like properties (CSC ^{high}) to apoptosis and diminished their clonogenicity, spheroid-forming ability, migratory activity and tumorigenicity in mice under hypoxia and nutrient deprivation.	(Rausch et al., 2012)

Therapeutic target	Inhibitory agent	Cancer type	Therapeutic effects	Ref.
PI3K/Akt/mTOR and autophagy	PKC- δ inhibitor, rottlerin plus autophagy inhibitor 3-methyladenine	Pancreatic cancer	The combination of rottlerin plus autophagosome inhibitor, 3-methyladenine, which represses rottlerin-induced autophagy has been shown to accelerate the apoptosis induced by rottlerin through the mitochondria-dependent caspase activation in human pancreatic human CD44 ⁺ /CD24 ⁺ /ESA ⁺ pancreatic cancer stem/progenitor cells <i>in vitro</i> .	(Singh et al., 2012a)