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Novel Therapies Against Aggressive and Recurrent Epithelial Cancers by Molecular Targeting Tumor- and Metastasis-Initiating Cells and Their Progenies

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Abstract

A growing body of experimental evidence has revealed that the highly tumorigenic cancer stem/progenitor cells endowed with stem cell-like properties might be responsible for initiation and progression of numerous aggressive epithelial cancers into locally invasive, metastatic and incurable disease states. The malignant transformation of tissue-resident adult stem/progenitor cells or their progenies into tumorigenic and migrating cancer stem/progenitor cells and their resistance to current cancer therapies have been associated with their high expression levels of specific oncogenic products and drug resistance-associated molecules. In this regard, we describe the tumorigenic cascades that are frequently activated in cancer stem/progenitor cells *versus* their differentiated progenies during the early and late stages of the epithelial cancer progression. The emphasis is on the growth factor signaling pathways involved in the malignant behavior of prostate and pancreatic cancer stem/progenitor cells and their progenies. Of clinical interest, the potential molecular therapeutic targets to eradicate the tumor- and metastasis-initiating cells and their progenies and develop new effective combination therapies against locally advanced and metastatic epithelial cancers are also described.

Keywords

Epithelial cancers; Prostate cancer stem/progenitor cells; Pancreatic cancer stem/progenitor cells; Treatment resistance; Targeted therapy; Combination cancer therapy

INTRODUCTION

Recent progresses in cancer research have indicated that the most human tumors including the epithelial cancers, might arise from the malignant transformation of tissue-resident adult stem cells and/or their progenitors into tumorigenic cancer stem/progenitor cells, also designated as cancer- and tumor-initiating cells (Fig. 1) [1–27]. The epithelial cancers harboring a small subpopulation of cancer stem/progenitor cells include lung, skin, head and neck, thyroid, cervical, renal, hepatic, esophageal, gastrointestinal, colorectal, bladder, pancreatic, prostate, mammary and ovarian cancers [1–27]. More specifically, it has been shown that the immature cancer stem/progenitor cells isolated from primary patient's tumors or established cancer cell lines, which possess the capacity to self-renew and aberrant differentiation ability were able to give rise to the bulk mass of more differentiated cancer

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cells *in vitro*, and to reconstitute the patient original cancer-like tumors in mice *in vivo* [1–27].

In addition, the accumulation of genetic and/or epigenetic alterations resulting in the sustained activation of diverse developmental cascades in cancer stem/progenitors may also lead to their acquisition of a more malignant behavior along cancer progression (Fig. 1) [11,12,14,25,28–42]. The tumorigenic cascades include hedgehog, epidermal growth factor (EGFR), Wnt/ β -catenin, Notch, stem cell factor (SCF)/KIT, bone morphogenic proteins (BMPs) and/or stromal cell-derived factor-1 (SDF-1)/CXC chemokine receptor 4 (CXCR4) (Fig. 2) [14,17,21,25,^{30,31},36,37,41–51]. Especially, the acquisition of a migratory phenotype by tumorigenic cancer stem/progenitor cells during epithelial-mesenchymal transition (EMT) may promote the development of more invasive and metastatic epithelial cancers [12,25,28,35,36,45,52–62]. In fact, the tumorigenic and migrating cancer stem/progenitor cells, also termed as metastasis-initiating cells, can evade the primary malignant neoplasm, migrate *via* the peripheral circulation at distant tissues and organs, and thereby contribute to metastasis formation by giving rise to a heterogeneous cancer cell population at secondary sites (Fig. 1). In support with this concept of carcinogenesis, the immature tumorigenic cancer cells with stem cell-like properties have been identified in peripheral blood and metastatic tissues of cancer patients and cell lines [3,4,11,12,^{15,18,35},50,52,63–72].

Importantly, it has also been shown that the cancer stem/progenitor cells may be more resistant than their differentiated progenies to current anti-cancer therapies, and thereby contribute to the tumor re-growth and disease relapse [2,12,14,15,^{17,24,27,29,33,37},60,68,73–84]. Thus, on the basis of these observations, it appears that the elimination of this minority of tumor- and metastasis-initiating cells, which are responsible of the primary tumor formation and metastases at distant tissues, is essential for the development of more effective curative treatments against aggressive and recurrent epithelial cancers. Therefore, the use of the combinations of drugs that are able to induce the cytotoxic effects on cancer stem/progenitor cells and their more differentiated progenies should eliminate the total cancer cell mass, and thereby lead to a more complete remission without chance of relapse.

In this context, we describe the recent advancements on the establishment of deregulated signaling cascades, which are frequently associated with the malignant transformation of tissue-resident adult stem/progenitor cells into tumorigenic, migrating and metastatic cancer progenitor cell subpopulations during epithelial cancer progression. Of clinical interest, we also describe new therapeutic strategies for the development of more effective treatments against aggressive epithelial cancers by molecular targeting cancer stem/progenitor cells and their differentiated progenies as well as their local microenvironment.

CRITICAL FUNCTIONS OF CANCER STEM/PROGENITOR CELLS IN EPITHELIAL CANCER INITIATION AND PROGRESSION

The cancer initiation is generally associated with the inactivation of distinct tumor suppressor gene products such p53, p16^{INK4A} and retinoblastoma (Rb) and phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and occurrence of oncogenic events in tissue-resident adult stem/progenitor cells and/or their more committed progenies (Fig. 1) [7,17,31,48,49,85–91]. These genomic alterations include the mutations, deletions, amplifications, chromosomal rearrangements and changes in DNA methylation [7,17,31,48,49,85–91]. Particularly, cumulative telomere shortening, sustained oxidative stress, chronic inflammatory and/or fibrosis, occurring during chronological aging may culminate to a genomic instability and malignant transformation of these immature cells [7,17,31,42,48,49,85–94]. Moreover, the persistent activation of diverse developmental

cascades frequently occurs in the cancer stem/progenitor cells and their progenies during the etiopathogenesis and progression of localized cancers to aggressive and metastatic disease states [14,17,21,25,^{30,31,36,37,41–48,50}]. Therefore, the molecular targeting of deregulated signaling elements in tumor- and metastasis-initiating cells is of major interest to counteract the epithelial cancer development and improve the current cancer therapies.

NOVEL CANCER THERAPIES BY TARGETING OF CANCER STEM/PROGENITOR CELLS AND THEIR PROGENIES

Several current therapies used in the clinics for the treatment of aggressive, localized advanced and metastatic epithelial cancers are based on the use of drugs showing antitumorigenic properties and which are able to eliminate cancer cells. However, these treatments generally target the global tumor cell population and no distinction is usually made between highly tumorigenic cancer stem/progenitor cells and their more differentiated progenies (Fig. 1). Therefore, despite the combinations of diverse strategies by using surgery, radiotherapy, anti-hormonal therapy and/or chemotherapy might be effective for eliminating the bulk mass of differentiated cancer cells, the possibility that the cancer stem/progenitor cells with stem cell-like properties can resist to these treatment types must also be considered.

Indeed, numerous accumulating lines of evidence have revealed that the unique intrinsic and extrinsic properties of the cancer stem/progenitor cells *versus* their more committed and differentiated progenies may contribute to treatment resistance and disease relapse [2,12,14,15,^{17,24,27,29,33,37,60,68,73–84}]. Similar to normal tissue-resident adult stem/progenitor cells, cancer stem/progenitor cells principally exist under a quiescent or slow-cycling and less metabolically active state [17,46,78,95–97]. Thereby, these immature cancer cells may be more resistant than their differentiated progenies to chemo- and/or radiotherapies targeting proliferative cancer cells. Moreover, the cancer stem/progenitor cells typically express high levels of diverse anti-apoptotic factors such as Bcl-2, ATP-binding cassette (ABC) multidrug transporters, including ABCG2 and multidrug resistance-1 (MDR-1) encoding p-glycoprotein (P-gp) and DNA repair and detoxifying enzymes such as aldehyde dehydrogenase (ALDH) [2,14,17,26,^{37,47,78,81–84,89,97–107}]. Thus, in considering the fact that the resistance of locally invasive and metastatic cancers to current therapies represents one of the major causes of cancer related deaths, the molecular targeting of tumor- and metastasis-initiating cells is of great clinical interest [17,45–49,78,97,103,108]. In this regard, we reviewed novel targeting strategies designed to eradicate the total tumor cell mass consisting of tumorigenic and migrating cancer stem/progenitor cells and their progenies, and which could be used for improving the current treatments against aggressive and recurrent epithelial cancers.

NOVEL THERAPEUTIC MOLECULAR TARGETS IN TUMOR- AND METASTASIS-INITIATING CELLS AND THEIR PROGENIES

The molecular targeting of stem cell-like markers and deregulated signaling elements that mediate the transforming events occurring in tumorigenic and migrating cancer stem/progenitor cells and their differentiated progenies during cancer progression represents new promising therapeutic strategies to improve the current cancer therapies [17,45–49,78,103,109,110]. Among the potential molecular targets often altered in tumor- and metastasis-initiating cells and their differentiated progenies during cancer progression, there are diverse developmental signaling pathways. These deregulated pathways include hedgehog, EGFR, ErbB2 “HER2”, Wnt/ β -catenin, Notch, hyaluronan (HA)/CD44, interleukin-6 (IL-6)/IL-6R, BMI-1, stem cell factor (SCF)/KIT, extracellular matrix (ECM)

component/integrin and/or SDF-1/CXCR4 signaling elements (Fig. 2 and Table 1) [11,17,45–49,^{51,97,103,106,111–126}]. It has been reported that the blockade of these tumorigenic pathways by using a monoclonal antibody (mAb), antisense oligonucleotides (As) or a specific inhibitor or antagonist, led to a growth inhibition, a reduction of invasiveness or metastatic spread and/or apoptotic death of cancer cells with stem cell-like properties *in vitro* or in animal models *in vivo* (Fig. 2 and Table 1) [11,17,45–49,^{97,103,111–121,127–129}].

Other potential molecular therapeutic targets also comprise the gene products that are frequently involved in sustained growth, enhanced survival and invasion during the EMT process and/or drug resistance of cancer stem/progenitor cells and their differentiated progenies [24,45,48,49,^{56,78,98,106,116,130–134}]. These cellular signaling effectors include telomerase reverse transcriptase (TERT), Cripto-1, tenascin C, nuclear factor-kappaB (NF- κ B), phosphatidylinositol 3-kinase (PI₃K)/Akt/mTOR, interleukin-4 (IL-4)/IL-4R α , Bcl-2, survivin, snail, slug, twist, ABC multidrug efflux pumps and/or ALDH [24,45,48,49,^{56,78,97,98,106,116,130–136}]. Importantly, some recent investigations have also indicated the potential benefit to target these signaling elements to prevent tumor growth and/or overcoming MDR phenotype and radioresistance of cancer stem/progenitor cells and their progenies (Fig. 2 and Table 2) [15,17,24,62,^{77,78,83,103,106,137–140}].

More particularly, it has been shown that the inhibition of ALDH activity by using ALDH1-specific inhibitor, diethylaminobenzaldehyde (DEAB) or ALDH1A-targeted small hairpin RNA (shRNA) enhanced the sensibility of ESA⁺/CD44⁺/CD166⁺ colorectal cancer stem cells and their progenies to the active metabolite (4-HC) of cyclophosphamide (CPA) *in vitro* and *in vivo* [83]. Moreover, in view of fact that the enzymatic catalytic subunit, TERT, which is involved in telomere maintenance, is re-expressed in the majority of human tumors including in tumor-initiating cells, numerous strategies have been developed based on its molecular targeting. Among them, there are the use of TERT-specific oncolytic adenoviruses, gene therapy and cancer immunotherapy (Figs. 1 and 2) [97,135,141]. For instance, it has been observed that the adoptive cell therapy (ACT) with human TERT-specific T lymphocytes inhibited tumor growth and/or metastatic spread of a variety of human cancer cell models *in vivo* and resulted in a decreased survival and complete cure in some mice [135]. Interestingly, it has also been observed that the colon cancer stem cells from patient's colon adenocarcinoma specimens did not grow in mice treated with human TERT_{865–873}-specific T cells (Table 1) [135]. In this regard, other attractive adjuvant therapies against aggressive and relapsed epithelial cancers also include cancer vaccines and transplantation of hematopoietic stem cells (HSCs) or activated B and T cells (Figs. 1 and 2) [17,47,97,142–144].

Of therapeutic interest, the induction of the differentiation of cancer stem/progenitor cells by using agents such as retinoic acid and its synthetic analogues, interferons (IFNs) or histone deacetylase inhibitor, also may represent a promising adjuvant cancer treatment [145–148]. For instance, it has been reported that the IFN- α treatment caused a dramatic reduction in the verapamil-sensitive side population (SP) cell fraction from diverse ovarian cancer cell lines [148]. Interestingly, the results from a recent study have also revealed that the re-activation of p53 pathway in putative colon cancer stem cells by using ellipticine improved the cytotoxic effects induced by chemotherapeutic drug, 5-fluorouracil [149]. In the same way, a restoration of microRNA (miRNA), miR-34 in human gastric cancer cells, which acts a downstream signaling element in the p53 pathway by modulating its potential targets Bcl-2, Notch, and HMGA2, has also been shown to chemosensitize cancer cells and inhibit tumor formation and growth [150]. In fact, it has been suggested that miR-34 tumor suppressor can reduce the self-renewal ability and survival of gastric cancer stem cells [150].

In addition, numerous therapeutic approaches have also been developed for a selective delivery of cytotoxic or anti-angiogenic agents and mAbs in cancer cells including cancer stem/progenitor cells. These strategies comprise the use of genetically-modified stem cell-based delivery and nanoparticle- and liposome-encapsulated drug delivery (Figs. 1 and 2) [97,151–154]. For instance, it has been shown that human neural stem cells (NSCs) endowed with intrinsic tumor-tropic properties and genetically-engineered to secrete anti-HER2 immunoglobulin molecules can be used to selectively deliver the therapeutic antibodies to breast cancer cells *in vitro* and *in vivo*, and thereby inhibit their growth [152]. In the same way, the use of genetically-modified endothelial progenitor cells (EPCs) or mesenchymal stem cells (MSCs), which are actively recruited to tumor stroma and participate to tumor growth, vascularization and/or metastases also may constitute the potential vehicles for a selective delivery of anti-carcinogenic agents within tumors [153,155–157]. In this matter, we are reporting, in a more detailed manner, recent advancements in the establishment of deregulated signaling elements that can contribute to the malignant behavior and treatment resistance of prostate and pancreatic cancer stem/progenitor cells and their progenies and the potential therapeutic targets for improving current cancer treatments.

PROSTATE CANCER STEM/PROGENITOR CELLS AND NOVEL TARGETED THERAPIES

Numerous factors may influence the risk of developing a prostate cancer. The precise etiological causes responsible for prostate cancer initiation and progression to androgen-independent (AI) and metastatic stages, treatment resistance and disease relapse remain not well established. In regard with this, recent studies have revealed that the accumulation of genetic and/or epigenetic alterations occurring in prostate-resident adult stem/progenitor cells during the lifespan, combined with the changes in their local microenvironment, may result in their malignant transformation into tumorigenic prostate cancer stem/progenitor cells, also designated as prostate cancer-initiating cells [16,17,46,48,115,158–171]. Moreover, the acquisition of a more malignant behavior, including a migratory ability during the EMT program by tumorigenic prostate cancer stem/progenitor cells, may lead to their invasion and dissemination through circulation and metastasis at distant tissues, including bone marrow, brain, lung and liver (Fig. 1) [17,45,46,78,158,162,172–174].

This model of prostate carcinogenesis is well supported by the identification of a rare subpopulation of human prostate cancer stem/progenitor cells comprising about 0.1–3% of the total tumor cell mass in malignant prostatic adenocarcinomas and metastatic neoplasms [16,163,164,168,175–177]. More specifically, the results from immunohistochemical analyses performed on malignant primary prostate adenocarcinoma specimens from patients have revealed that a small subpopulation of basal or intermediate androgen receptor (AR⁺) prostate cancer cells expressed the CD133 stem cell-like marker [48,164,168,175,178]. In contrast, it has been noticed that the luminal AR⁺ prostate cancer cells detected in same tissue samples did not express the CD133 protein [48,164,168,175,178]. Moreover, the data from the microarray studies carried out with clinical prostate cancer tissue specimens have also revealed that the transforming growth factor- β receptor 3 (TGFBR3) down-regulation, which is an important step in prostate tumorigenesis, was accompanied by an increase in the CD133 expression level [181]. Similarly, the CD44 stem cell-like surface marker has also been detected in basal cells in normal prostate tissues and only in a minority of prostate cancer cells in certain cases of low- and high-grade prostate adenocarcinoma tissues [161,179,180].

Importantly, the prostate cancer stem/progenitor cells expressing different prostate stem cell-like markers such as CD133, CD44, $\alpha_2\beta_1$ ^{+/high}-integrin, cytokeratin (CK5/14), CK18,

CXCR4 and/or multidrug transporters have also been isolated from primary and metastatic prostate cancers and established prostate cancer cell lines [107,163,164,168,175–177,182]. These isolated AR^{-/low} prostate cancer stem/progenitor cells were able to give rise *in vitro* and *in vivo* to the bulk mass of differentiated cancer cells expressing luminal secretory androgen-responsive cell phenotypes, including high expression levels of AR [107,163,164,168,175–177,182]. These immature prostate cancer cells were also able to reconstitute the tumors *in vivo* with a histological architecture of a Gleason grade comparable to the patient's original tumors. For instance, CD133/ $\alpha_2\beta_1$ -integrin^{+/high} prostate cancer stem cells isolated from primary prostate neoplasm (P4E6), when grafted orthotopically in a matrigel plug containing human prostate stroma, formed multiple intraprostatic tumors in nude mice *in vivo*, showing a histology like the original Gleason 4 grade prostate cancer [16]. Moreover, it has been shown that certain established human prostate cancer cell lines may represent a heterogeneous population of prostate cancer cells, and the presence of a small subpopulation of tumorigenic prostate cancer stem/progenitor cells expressing stem cell-like markers may be responsible for their capacity to form tumors and metastasize in animal models *in vivo* with a high occurrence [64–66,169,183].

Thus, on the basis of these observations, it appears likely that only a small number of tumorigenic prostate cancer stem/progenitor cells and/or their early progenies with stem cell-like properties might be necessary to drive tumor growth in the prostate gland and metastases at distant tissues by giving rise to the total tumor cell mass [16,17,45,46,⁴⁸,^{162–170},175,176,184–186]. Moreover, the occurrence of distinct genetic and/or epigenetic alterations leading to a deregulated expression and/or activity of different gene products in prostate cancer stem/progenitor cells and their differentiated progenies, and more particularly during EMT process, may culminate in more aggressive prostate cancer subtypes, treatment resistance and disease relapse.

Particularly, the aberrant expression and/or activity of diverse hormones, growth factors, cytokines and/or their receptors may lead to the stimulation of tumorigenic signaling elements that contribute to the sustained growth, survival and invasion of tumor cells during prostate cancer etiology and progression (Figs. 1 and 2) [48,118,158,161,166,187–198]. Among them, EGFR, sonic hedgehog, Wnt/ β -catenin, HA/CD44 and/or SDF-1/CXCR4 cascades appear to provide critical roles for the malignant transformation of prostate cancer stem/progenitor cells and their progenies, during disease progression as well as for the resistance to current clinical therapies and disease relapse [48,118,158,161,187,189,193–197]. Moreover, the high expression and/or activity of telomerase, ABC multidrug transporters including ABCG2 and MDR-1 encoding P-gp, NF- κ B and PI₃K/Akt/mTOR signaling elements also may contribute to the malignant behavior, survival and chemoresistance of prostate cancer- and metastasis-initiating cells and their progenies [48,106,115,182,199].

CRITICAL FUNCTIONS OF PROSTATE CANCER- AND METASTASIS-INITIATING CELLS IN TREATMENT RESISTANCE

Prostate cancer is the most common malignancy and the second leading cause of cancer-related deaths in men in the United States [48,158,166,190–192,200–206]. Although progress in developing early detection tests has led to improved clinical treatments of patients diagnosed with low-grade and organ-confined prostate cancers by radical prostatectomy, radiotherapy and/or anti-hormonal therapy, the progression to locally advanced, invasive and metastatic hormone-refractory prostate cancers (HRPCs) usually leads to disease relapse [48,158,166,190–¹⁹²,200–203,205–207]. In fact, despite the patients diagnosed with localized prostate cancer initially respond to androgen deprivation, AI lesions may eventually develop and progress despite low levels of circulating androgens.

This hormone-refractory disease is resistant to conventional treatments by anti-hormonal therapy, radiotherapy and chemotherapy [48,158,166,190–^{192,201},202,204,205,208].

More specifically, the first-line systemic docetaxel-based chemotherapies used as care for patients with high-risk or metastatic HRPCs are only palliative and typically culminate in the death of patients after about 12 to 19 months [48,158,191,192,^{201–203},205,208,209]. Moreover, at present time, there are no proven targeting approaches and effective therapeutic regimens for treating high-risk localized, metastatic and relapsed HRPCs. Therefore, a better understanding of the etiological causes responsible for prostate cancer etiopathogenesis and progression to aggressive and AI disease states and resistance to current androgen ablation and chemotherapeutic treatments is highly needed.

Importantly, a growing body of experimental evidence has revealed that highly tumorigenic and/or migrating prostate cancer stem/progenitor cells endowed with high self-renewal ability and aberrant differentiation potential can provide critical functions in disease progression, metastases at distant tissues, resistance to current therapies and cancer relapse [16,17,162,165–^{170,176},184–186,210,211]. Especially, the intrinsic properties of prostate cancer stem/progenitor cells as well as their acquisition of a more malignant phenotype during disease progression may give to them the survival advantages, and thereby contribute to their resistance to current therapies [45,46,158,212]. Consequently, most patients who undergo potential curative treatments for locally advanced prostate cancers and/or disseminated disease stages may subsequently relapse due to the persistence of AR⁺ prostate cancer stem/progenitor cells and/or their early AI progenies in primary neoplasms and/or micrometastases at distant sites [14,17,26,30–^{32,36,39,40,45,46,98,99,130–132},158,163,212–218]. Thus, it appears that the molecular targeting of highly tumorigenic prostate cancer- and metastasis-initiating cells may represent a promising strategy for improving current therapies and prevent disease relapse.

MOLECULAR TARGETING OF PROSTATE CANCER- AND METASTASIS-INITIATING CELLS AND THEIR PROGENIES

Recent studies have led to the identification of diverse signaling elements in the prostate cancer stem/progenitor cells and their progenies that could be targeted to eradicate the total tumor cell mass. Thereby, these novel potential molecular therapeutic targets could be exploited to reverse treatment resistance and improve the efficacy of current anti-androgenic treatments and docetaxel-based therapies against locally advanced, AI and metastatic prostate cancers.

As a matter of fact, it has been observed that the treatment of human parental CWR22RV1 and PC3 prostate cancer cell line-derived xenografts with an inhibitor of smoothened (SMO) co-receptor of hedgehog cascade, cyclopamine resulted in a complete regression of the *in vivo* tumor growth without sign of disease recurrence after 58 and 148 days of treatment cessation, respectively [118]. These data suggest that the prostate cancer-initiating cells may be eliminated by this treatment type. It has also been noticed that the normal prostate epithelial cells were insensitive to the cytotoxic effects of cyclopamine [118].

Of clinical interest, we have also observed that a combination of cyclopamine, EGFR tyrosine kinase activity inhibitor, gefitinib and chemotherapeutic drug, mitoxantrone, alone or in combination, induced an arrest of growth and cytotoxic effects on parental metastatic DU145 and PC3 cells and CD44^{+/high} and CD44^{−/low} fractions isolated from these prostate cancer cell lines [180]. Importantly, the results from our recent works have also revealed that an increase of the expression levels of EGFR and sonic hedgehog signaling elements occurred in a small subpopulation of CD133⁺ prostate cancer stem/progenitor cells and the

bulk mass of CD133⁺ cells in certain cases of patients relative to normal prostate tissues [115]. We have shown that these tumorigenic cascades may contribute to the malignant behavior of CD133⁺ and CD133⁺ cancer cell fractions from invasive WPE1-NB26 and metastatic PC3 cell lines [115]. Of therapeutic interest, we have also observed that the CD133⁺ SP cells endowed with stem cell-like properties isolated from the WPE1-NB26 cell line were insensitive to a treatment with 2 nM docetaxel [115]. This docetaxel concentration, however, induced significant anti-proliferative and apoptotic effects on the CD133⁺ non-SP cell fraction [115]. In contrast, cyclopamine and gefitinib were effective to induce the anti-proliferative and cytotoxic effects on both the isolated CD133⁺ SP cells and CD133⁺ non-SP cell fractions [115]. Interestingly, a combination of low concentrations of docetaxel plus cyclopamine and gefitinib also induced greater anti-proliferative and apoptotic effects on CD133⁺ SP and CD133⁺ non-SP cell fractions than individual drugs or two drug combinations [115]. Hence, these data suggest that the targeting hedgehog and/or EGFR may constitute a potential strategy for reversing the resistance of prostate cancer cells with stem cell-like properties to current chemotherapeutic drug, docetaxel.

In addition, it has also been reported that the parthenolide (PTL) a sesquiterpene lactone from the plant feverfew, induced the cytotoxic effects on parental and CD44^{high} and CD44^{low} cancer cell fractions isolated from prostate cancer cell lines, DU145, PC3, VCAP and LAPC4, as well as primary prostate cancer cells from patient samples *in vitro* through an inhibition of src-related signaling components [219]. PTL was also effective to inhibit the tumor growth of CD44^{high} DU145 cell xenograft models *in vivo* [219]. Furthermore, PTL, which can act on different pathways including NF- κ B, also induced the cytotoxic effects on the CD133⁺ primary prostate tumor cells while the CD133⁺ normal cells from benign prostate hyperplasia, BPH were insensitive to this treatment type *in vitro* [106].

On the other hand, it has been shown that the activation of PI₃K/Akt/FoxO3a signaling cascade may contribute to the prostasphere formation and maintenance of PTEN-positive DU145 and PTEN-negative PC3 cells [220]. The treatment of these prostate cancer cell lines with PI₃K inhibitor, LY294002 or dual PI₃K/mTOR inhibitor NVP-BEZ235 was accompanied by a growth inhibition and cytotoxic effects on bulk mass and CD133⁺/CD44⁺ cell fraction detected in these prostate cancer cells by cytometric analyses [220]. Additional investigations are however necessary to validate these potential therapeutic targets on distinct prostate cancer stem/progenitor cell models *in vivo* and in clinical trials.

PANCREATIC CANCER STEM/PROGENITOR CELLS AND NOVEL CANCER THERAPIES

Although the precise causes responsible for pancreatic cancer development are not well known, a growing body of experimental evidence suggests that the pancreas carcinogenesis could derive from the malignant transformation of pancreatic stem/progenitor cells and/or their early progenies with stem cell-like properties into pancreatic cancer stem/progenitor cells [12,49,116,221–224]. In fact, the formation of pancreatic intraepithelial neoplasias (PanINs) has been associated with the occurrence of specific genetic mutations and/or sustained activation of growth factor cascades in a small subpopulation of pancreatic stem/progenitor cells with a ductal and/or acinar cell-like phenotype localized within the exocrine compartment of adult pancreas [49,116,221,225].

In addition, this model of pancreas carcinogenesis also implicates that the PanIN formation and cancer progression into locally invasive and metastatic disease stages, may represent a continuum resulting from the accumulation of different genetic and/or epigenetic alterations in pancreatic cancer stem/progenitor cells and their progenies. These oncogenic events may lead to their acquisition of more malignant phenotypes, including a migratory ability (Fig. 1)

[12,49]. Consistent with the major implication of highly tumorigenic and migrating pancreatic cancer stem/progenitor cells in tumor growth and metastases at distant tissues, a small subpopulation of immature cells with stem cell-like properties has been identified and isolated from primary and metastatic pancreatic cancer tissue specimens and established cell lines [12,17,223,226–228]. The isolated pancreatic cancer stem/progenitor cells, also designated as pancreatic cancer- and metastasis-initiating cells, expressed several stem cell-like markers, such as CD133, CD44, CD24, ALDH, ABCG2 transporter and/or CXCR4 [12,17,49,223,226–228]. The highly tumorigenic pancreatic cancer stem/progenitor cells endowed with a high self-renewal potential and aberrant differentiation potential were able to give rise to the bulk mass of differentiated pancreatic cancer cells *in vitro* and to reconstitute the patient's original tumors in animal model *in vivo* [12,49,223,227]. Of particular clinical interest, recent accumulating lines of evidence have also revealed that highly tumorigenic and migrating pancreatic cancer stem/progenitor cells may be more resistant to current chemotherapies, and thereby provide critical functions in tumor re-growth, metastases at distant tissues after treatment initiation and disease relapse [12,129,228].

CRITICAL FUNCTIONS OF PANCREATIC CANCER- AND METASTASIS-INITIATING CELLS IN TREATMENT RESISTANCE

Pancreatic cancer is a highly aggressive and lethal malignancy with a poor prognosis and a long-term overall five-year survival rate less than 5% for patients diagnosed with locally advanced and metastatic disease stages [49,204,224,229–233]. Although the gemcitabine-based chemotherapeutic treatments represent the standard of care for treating the patients with locally advanced and metastatic pancreatic cancers in the clinics, these regimens are only palliative and generally result in drug resistance, disease relapse and the death of cancer patients [49,224,229–232]. This inefficacy of current chemotherapies for treating patients diagnosed with pancreatic cancers underlines the critical importance to delineate the molecular mechanisms that may contribute to chemoresistance of pancreatic cancer cells and validate new molecular therapeutic targets.

Importantly, the intrinsic properties of pancreatic cancer stem/progenitor cells as well as their acquisition of more malignant phenotypes during disease progression may notably provide them with survival advantages, and thereby contribute to their resistance to current therapies and disease relapse [12,49,129,228,233–235]. In support with this assumption, it has been observed that the treatment of pancreatic cancer cells with current chemotherapeutic drug, gemcitabine led to an enrichment in the number of cancer cells with the stem cell-like properties *in vitro* and *in vivo* [12,129,228,233–235]. More specifically, it has been shown that a small subpopulation of CD133⁺ pancreatic cancer cells with stem cell-like properties isolated from patient's malignant primary neoplasm was more resistance to gemcitabine than the CD133[−] pancreatic cancer cell fraction [12]. Moreover, it has also been observed that the CD133⁺ L3.6pl cells were more resistance to gemcitabine treatment as compared to the CD133[−] L3.6pl cells *in vitro* and an enrichment of CD133⁺ L3.6pl cell fraction in total tumor cell mass also occurred after gemcitabine treatment relative to the control in mice *in vivo* [12]. In the same way, the gemcitabine treatment of pancreatic tumor xenografts *in vivo* also resulted in an enrichment in a subpopulation of pancreatic cells expressing a high level of stem cell-like markers such as ALDH and CD24 [129].

Thus, based on together these observations, it is likely that the patients with locally advanced pancreatic cancers and/or disseminated disease stages who undergo gemcitabine-based chemotherapies may subsequently relapse due to the persistence of chemoresistant pancreatic cancer stem/progenitor cells in primary neoplasms and/or micrometastases at distant tissues. Hence, these studies underlines the clinical interest to also considering the

molecular targeting of pancreatic cancer stem/progenitor cells for overcoming treatment resistance, and thereby prevent tumor re-growth and disease relapse.

MOLECULAR TARGETING OF PANCREATIC CANCER- AND METASTASIS-INITIATING CELLS AND THEIR PROGENIES

Recent investigations have led to the identification of potential molecular targets for eradicating the pancreatic cancer- and metastasis-initiating cells and improving the efficacy of current chemotherapeutic drug, gemcitabine. For instance, it has been observed that the treatment of human E3LZ10.7 pancreatic cancer cell line with SMO inhibitor, cyclopamine resulted in a reduction in the number of putative ALDH-expressing progenitor cells *in vitro* as well as an inhibition of metastases of E3LZ10.7 cells in an orthotopic xenograft model *in vivo* [116]. In the same way, the treatment of E3LZ10.7 pancreatic cancer cell line with another SMO inhibitor type, orally bioavailable small-molecule IPI-269609, also reduced the cancer cell fraction with high ALDH activity detected in primary tumors established from orthotopic implantation *in vivo* [117]. The IPI-269609 treatment also inhibited the systemic metastases of E3LZ10.7 cells in an orthotopic xenograft model *in vivo* [117]. Moreover, it has been reported that a combination of cyclopamine and gemcitabine induced a tumor regression and decrease in cancer stem cell-like marker expression such as CD24 and ALDH detected in an *in vivo* xenograft model established from human Panc185 pancreatic cells [129].

Importantly, the restoration of miR-34 expression in p53-mutant human pancreatic cancer cell lines, MiaPaCa-2 and BxPC3, by either transfection of miR-34 mimics or infection with lentiviral miR-34-MIF has also been observed to result in a down-regulation of its targets, Bcl-2 and Notch1/2 [236]. The enhanced miR-34 expression was accompanied by an inhibition of the clonogenic cell growth and invasion, induction of apoptosis and enhanced sensitivity of pancreatic cancer cells to radiation and chemotherapeutic treatment [236]. Of therapeutic interest, the miR-34 restoration in CD44⁺/CD133⁺ MiaPaCa2 cell fraction expressing high levels of Notch/Bcl-2, and loss of miR-34 also led to an 87% reduction in the number of tumor-initiating cell population and a significant inhibition of their tumorsphere growth *in vitro* and tumor formation *in vivo* [236].

Numerous studies have also indicated that the enhanced expression and activation of different oncogenic products in pancreatic cancer-initiating cells and their differentiated progenies during EMT process may confer to them more malignant phenotypes and survival advantages, including a migratory ability and gemcitabine-resistant phenotype along cancer progression. Therefore, the molecular targeting of these altered gene products induced in pancreatic cancer cells, including tumor-initiating cells during EMT process is of immense clinical interest to prevent the metastases and disease relapse (Fig. 1). In this regard, our recent studies have indicated that the MUC4 mucin is expressed in CD133⁺ pancreatic cancer cells and the CD133⁻ cells constituting the bulk tumor mass during disease progression [235]. Of therapeutic interest, we have observed that the MUC4 down-regulation reversed gemcitabine resistance of parental metastatic CD18/HPAC pancreatic cancer cells as well as the CD133⁺/CD44^{high} SP and CD133⁻/CD44^{low} non-SP cell fractions detected in total CD18/HPAC cancer cells [235]. In this context, it has also been reported that the treatment of HPAC and CFPAC-1 pancreatic cancer cells with high-dose of gemcitabine resulted in an enrichment of gemcitabine-resistant CD44⁺ cells showing greater tumorigenic ability *in vitro* and *in vivo*, and sphere-forming activity than parental cancer cell lines [233]. The expression levels of ABC transporters, including ABCB1 (MDR-1) was also enhanced in the pancreatic cell lines made resistant to gemcitabine [233]. Interestingly, it has been shown that the ABC transporter inhibitor, verapamil re-sensitized these

pancreatic cancer cells to gemcitabine treatment, and CD44 interference RNA inhibited their clonogenic activity [233].

Other therapeutic strategies to counteract the invasion and metastatic spread of pancreatic stem/progenitor cells also include the inhibition of EMT-associated signaling elements. In this regard, it has been reported that the treatment of the highly tumorigenic SP subpopulation and the non-SP cell fraction from PANC-1 pancreatic cancer cell line with a specific inhibitor of PI₃K, LY294002 or mTOR, rapamycin was accompanied by a decrease of their survival *in vitro* [237]. Moreover, the treatment of the SP cell fraction from pancreatic cancer cell lines with TGF- β has also been observed to induce the expression of EMT-associated gene such as snail and matrix metalloproteinase-2 (MMP-2) while E-cadherin expression was reduced [238]. The SP cell subpopulation also exhibited a higher invasive ability in response to TGF- β treatment, while non-SP cells did not respond to TGF- β -mediated invasion [238]. These data suggest that the treatment of pancreatic cancer cells with anti-TGF- β mAb could constitute a potential therapeutic strategy to counteract the EMT process and invasion of pancreatic cancer cells with stem cell-like properties. In this way, it has also been reported that the Notch signaling pathway may contribute to the EMT process and its down-regulation by siRNA approach led to a partial reversal of the EMT phenotype including a decreased expression of vimentin, ZEB1, snail, slug, sail, and NF- κ B [239]. The Notch inhibition was also associated with a decrease of the invasive behavior of gemcitabine-resistant pancreatic cancer cells [239]. Of particular interest, it has also been reported that the inhibition of SDF/CXCR4 axis by using anti-CXCR4 mAb reduced the metastatic capacity of highly metastatic human pancreatic cancer cell line L3.6 pl harboring a CD133⁺/CXCR4⁺ cancer cell subpopulation orthotopically implanted in the pancreas of nude mice *in vivo* [12].

CONCLUSIONS

Recent advances in basic and clinical oncology have revealed that the tumorigenic and migrating cancer stem/progenitor cells can provide critical functions in tumor formation, metastases at distant sites, treatment resistance and disease relapse. Several deregulated gene products have been identified in cancer stem/progenitor cells and their progenies as well as in their local tumor microenvironment during epithelial cancer progression. These altered gene products include different tumor suppressor gene products, oncogenes and drug resistance-associated molecules that are involved in the regulation of the self-renewal, differentiation, survival and/or treatment resistance of tumor- and metastasis-initiating cells. Importantly, it has also been shown that the molecular targeting of these deregulated gene products may constitute the potential strategies to design novel cancer treatments for reversing the MDR phenotype, improving the current cancer therapies and preventing disease recurrence. These new promising therapeutic targets may now be exploited to develop new clinical combination therapies against aggressive, metastatic, recurrent and lethal human cancers, including the most epithelial cancers.

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ABBREVIATIONS

ABC ATP-binding cassette

AI	Androgen independent
ALDH	Aldehyde dehydrogenase
AR	Androgen receptor
CXCR4	CXC chemokine receptor 4
EGFR	Epidermal growth factor receptor
EMT	Epithelial-mesenchymal transition
EPCs	Endothelial progenitor cells
Fzd	Frizzled receptor
KIT	Stem cell factor receptor
HA	Hyaluronan
HRPCs	Hormone-refractory prostate cancers
mAb	Monoclonal antibody
MAPKs	Mitogen-activated protein kinases
MDR	Multidrug resistance
miRNA	microRNA
NF-κB	Nuclear factor-kappa; B
NSCs	Neural stem cells
PanINs	Pancreatic intraepithelial neoplasias
P-gp	P-glycoprotein
PI₃K	Phosphoinositide 3'-kinase
PTEN	Phosphatase and tensin homolog deleted on chromosome 10
PTL	Parthenolide
Rb	Retinoblastoma
RTK	Receptor tyrosine kinase
SCF	Stem cell factor
SDF-1	Stromal cell-derived factor-1
SMO	Smoothened co-receptor
SP	Side population
TERT	Telomerase reverse transcriptase
Wnt	Wingless ligand

References

1. Hope KJ, Jin L, Dick JE. Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. *Nat Immunol* 2004;5(7):738–743. [PubMed: 15170211]
2. Matsui W, Wang Q, Barber JP, Brennan S, Smith BD, Borrello I, McNiece I, Lin L, Ambinder RF, Peacock C, Watkins DN, Huff CA, Jones RJ. Clonogenic multiple myeloma progenitors, stem cell properties, and drug resistance. *Cancer Res* 2008;68(1):190–197. [PubMed: 18172311]

3. Fang D, Nguyen TK, Leishear K, Finko R, Kulp AN, Hotz S, Van Belle PA, Xu X, Elder DE, Herlyn MA. Tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res* 2005;65(20):9328–9337. [PubMed: 16230395]
4. Galli R, Binda E, Orfanelli U, Cipelletti B, Gritti A, De Vitis S, Fiocco R, Foroni C, Dimeco F, Vescovi A. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. *Cancer Res* 2004;64(19):7011–7021. [PubMed: 15466194]
5. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. *Nature* 2004;432(7015):396–401. [PubMed: 15549107]
6. Al-Hajj M, Wicha MS, Ito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 2003;100(7):3983–3988. [PubMed: 12629218]
7. Kim CF, Jackson EL, Woolfenden AE, Lawrence S, Babar I, Vogel S, Crowley D, Bronson RT, Jacks T. Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* 2005;121(6):823–835. [PubMed: 15960971]
8. Eramo A, Lotti F, Sette G, Pilozzi E, Biffoni M, Di Virgilio A, Conticello C, Ruco L, Peschle C, De Maria R. Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell Death Differ* 2008;15(3):504–514. [PubMed: 18049477]
9. Wright MH, Calcagno AM, Salcido CD, Carlson MD, Ambudkar SV, Varticovski L. Brca1 breast tumors contain distinct CD44+/CD24– and CD133+ cells with cancer stem cell characteristics. *Breast Cancer Res* 2008;10(1):R10. [PubMed: 18241344]
10. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu S, Schott A, Hayes D, Bimbaum D, Wicha MS, Dontu G. ALDH1 Is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell* 2007;1(5):555–567. [PubMed: 18371393]
11. Yang ZF, Ho DW, Ng MN, Lau CK, Yu WC, Ngai P, Chu PW, Lam CT, Poon RT, Fan ST. Significance of CD90(+) cancer stem cells in human liver cancer. *Cancer Cell* 2008;13(2):153–166. [PubMed: 18242515]
12. Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, Bruns CJ, Heeschen C. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell* 2007;1(3):313–323. [PubMed: 18371365]
13. Zhang S, Balch C, Chan MW, Lai HC, Matei D, Schilder JM, Yan PS, Huang TH, Nephew KP. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. *Cancer Res* 2008;68(11):4311–4320. [PubMed: 18519691]
14. Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, Lu L, Irvin D, Black KL, Yu JS. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. *Mol Cancer* 2006;5:67. [PubMed: 17140455]
15. Schatton T, Murphy GF, Frank NY, Yamaura K, Waaga-Gasser AM, Gasser M, Zhan Q, Jordan S, Duncan LM, Weishaupt C, Fuhlbrigge RC, Kupper TS, Sayegh MH, Frank MH. Identification of cells initiating human melanomas. *Nature* 2008;451(7176):345–349. [PubMed: 18202660]
16. Maitland NJ, Bryce SD, Stower MJ, Collins AT. Prostate cancer stem cells: a target for new therapies. *Ernst Schering Found Symp Proc* 2006;5:155–179. [PubMed: 17939301]
17. Mimeault M, Hauke R, Mehta PP, Batra SK. Recent advances on cancer stem/progenitor cell research: therapeutic implications for overcoming resistance to the most aggressive cancers. *J Mol Cell Med* 2007;11(5):981–1011.
18. Shi GM, Xu Y, Fan J, Zhou J, Yang XR, Qiu SJ, Liao Y, Wu WZ, Ji Y, Ke AW, Ding ZB, He YZ, Wu B, Yang GH, Qin WZ, Zhang W, Zhu J, Min ZH, Wu ZQ. Identification of side population cells in human hepatocellular carcinoma cell lines with stepwise metastatic potentials. *J Cancer Res Clin Oncol* 2008;134(11):1155–1163. [PubMed: 18470535]
19. Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, De Maria R. Identification and expansion of human colon-cancer-initiating cells. *Nature* 2007;445(7123):111–115. [PubMed: 17122771]

20. Huang D, Gao Q, Guo L, Zhang C, Wei J, Li H, Jing WJ, Han X, Shi Y, Shih HL. Isolation and identification of cancer stem-like cells in esophageal carcinoma cell lines. *Stem Cells Dev* 2009;18(3):465–473. [PubMed: 18680391]
21. Huang Q, Zhang QB, Dong J, Wu YY, Shen YT, Zhao YD, Zhu YD, Diao Y, Wang AD, Lan Q. Glioma stem cells are more aggressive in recurrent tumors with malignant progression than in the primary tumor, and both can be maintained long-term *in vitro*. *BMC Cancer* 2008;8:304. [PubMed: 18940013]
22. Alvero AB, Chen R, Fu HH, Montagna M, Schwartz PE, Rutherford T, Silasi DA, Steffensen KD, Waldstrom M, Visintin I, Mor G. Molecular phenotyping of human ovarian cancer stem cells unravels the mechanisms for repair and chemoresistance. *Cell Cycle* 2009;8(1):158–166. [PubMed: 19158483]
23. Baba T, Convery PA, Matsumura N, Whitaker RS, Kondoh E, Perry T, Huang Z, Bentley RC, Mori S, Fujii S, Marks JR, Berchuck A, Murphy SK. Epigenetic regulation of CD133 and tumorigenicity of CD133+ ovarian cancer cells. *Oncogene* 2009;28(2):209–218. [PubMed: 18836486]
24. Todaro M, Alea MP, Di Stefano AB, Cammareri P, Vermeulen L, Iovino F, Tripodo C, Russo A, Gulotta G, Medema J-P, Stassi G. Colon cancer stem cells dictate tumor growth and resist cell death by production of interleukin-4. *Cell Stem Cell* 2007;1(4):389–402. [PubMed: 18371377]
25. Haraguchi N, Utsunomiya T, Inoue H, Tanaka F, Mimori K, Barnard GF, Mori M. Characterization of a side population of cancer cells from human gastrointestinal system. *Stem Cells* 2006;24(3):506–513. [PubMed: 16239320]
26. Hirschmann-Jax C, Foster AE, Wulf GG, Nuchtern JG, Jax TW, Gobel U, Goodell MA, Brenner MK. A distinct “side population” of cells with high drug efflux capacity in human tumor cells. *Proc Natl Acad Sci USA* 2004;101(39):14228–14233. [PubMed: 15381773]
27. Wang J, Guo LP, Chen LZ, Zeng YX, Lu SH. Identification of cancer stem cell-like side population cells in human nasopharyngeal carcinoma cell line. *Cancer Res* 2007;67(8):3716–3724. [PubMed: 17440084]
28. Bapat SA, Mali AM, Koppikar CB, Kurrey NK. Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. *Cancer Res* 2005;65(8):3025–3029. [PubMed: 15833827]
29. Chiba T, Miyagi S, Saraya A, Aoki R, Seki A, Morita Y, Yonemitsu Y, Yokosuka O, Taniguchi H, Nakauchi H, Iwama A. The polycomb gene product BMI1 contributes to the maintenance of tumor-initiating side population cells in hepatocellular carcinoma. *Cancer Res* 2008;68(19):7742–7749. [PubMed: 18829528]
30. Nicolis SK. Cancer stem cells and “stemness” genes in neurooncology. *Neurobiol Dis* 2007;25(2):217–229. [PubMed: 17141509]
31. Sato M, Shames DS, Gazdar AF, Minna JD. A translational view of the molecular pathogenesis of lung cancer. *J Thorac Oncol* 2007;2(4):327–343. [PubMed: 17409807]
32. Gray-Schopfer V, Wellbrock C, Marais R. Melanoma biology and new targeted therapy. *Nature* 2007;445(7130):851–857. [PubMed: 17314971]
33. Ma S, Lee TK, Zheng BJ, Chan KW, Guan XY. CD133+ HCC cancer stem cells confer chemoresistance by preferential expression of the Akt/PKB survival pathway. *Oncogene* 2008;27(12):1749–1758. [PubMed: 17891174]
34. Rich JN. Cancer stem cells in radiation resistance. *Cancer Res* 2007;67(19):8980–8984. [PubMed: 17908997]
35. Brabletz T, Jung A, Spaderna S, Hlubek F, Kirchner T. Opinion: migrating cancer stem cells - an integrated concept of malignant tumour progression. *Nat Rev Cancer* 2005;5(9):744–749. [PubMed: 16148886]
36. Tso CL, Shintaku P, Chen J, Liu Q, Liu J, Chen Z, Yoshimoto K, Mischel PS, Cloughesy TF, Liao LM, Nelson SF. Primary glioblastomas express mesenchymal stem-like properties. *Mol Cancer Res* 2006;4(9):607–619. [PubMed: 16966431]
37. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 2006;444(7120):756–760. [PubMed: 17051156]

38. Sengupta A, Banerjee D, Chandra S, Banerji SK, Ghosh R, Roy R, Banerjee S. Deregulation and cross talk among Sonic hedgehog, Wnt, Hox and Notch signaling in chronic myeloid leukemia progression. *Leukemia* 2007;21:949–955. [PubMed: 17361218]
39. Liu S, Dontu G, Mantle ID, Patel S, Ahn NS, Jackson KW, Suri P, Wicha MS. Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. *Cancer Res* 2006;66(12):6063–6071. [PubMed: 16778178]
40. Li L, Neaves WB. Normal stem cells and cancer stem cells: the niche matters. *Cancer Res* 2006;66(9):4553–4557. [PubMed: 16651403]
41. Katoh M. Networking of WNT, FGF, Notch, BMP, and Hedgehog signaling pathways during carcinogenesis. *Stem Cell Rev* 2007;3(1):30–38. [PubMed: 17873379]
42. Mimeault M, Batra SK. Recent insights into the molecular mechanisms involved in aging and the malignant transformation of adult stem/progenitor cells and their therapeutic implications. *Ageing Res Rev* 2009;8(2):94–112. [PubMed: 19114129]
43. Onoue T, Uchida D, Begum NM, Tomizuka Y, Yoshida H, Sato M. Epithelial-mesenchymal transition induced by the stromal cell-derived factor-1/CXCR4 system in oral squamous cell carcinoma cells. *Int J Oncol* 2006;29(5):1133–1138. [PubMed: 17016644]
44. Glinsky GV. “Stemness” genomics law governs clinical behavior of human cancer: implications for decision making in disease management. *J Clin Oncol* 2008;26(17):2846–2853. [PubMed: 18539963]
45. Mimeault M, Batra SK. Interplay of distinct growth factors during epithelial-mesenchymal transition of cancer progenitor cells and molecular targeting as novel cancer therapies. *Ann Oncol* 2007;18(10):1605–1619. [PubMed: 17355951]
46. Mimeault M, Batra SK. Functions of tumorigenic and migrating cancer progenitor cells in cancer progression and metastasis and their therapeutic implications. *Cancer Metastasis Rev* 2007;26(1):203–214. [PubMed: 17273942]
47. Mimeault M, Batra SK. Targeting of cancer stem/progenitor cells plus stem cell-based therapies: the ultimate hope for treating and curing aggressive and recurrent cancers. *Panminerva Med* 2008;50(1):3–18. [PubMed: 18427384]
48. Mimeault M, Mehta PP, Hauke R, Batra SK. Functions of normal and malignant prostatic stem/progenitor cells in tissue regeneration and cancer progression and novel targeting therapies. *Endocr Rev* 2008;29(2):234–252. [PubMed: 18292464]
49. Mimeault M, Batra SK. Recent progress on normal and malignant pancreatic stem/progenitor cell research: therapeutic implications for the treatment of type 1 or 2 diabetes mellitus and aggressive pancreatic cancer. *Gut* 2008;57(10):1456–1468. [PubMed: 18791122]
50. Moustakas A, Heldin CH. Dynamic control of TGF-beta signaling and its links to the cytoskeleton. *FEBS Lett* 2008;582(14):2051–2065. [PubMed: 18375206]
51. Katoh M, Katoh M. Integrative genomic analyses of CXCR4: transcriptional regulation of CXCR4 based on TGFbeta, Nodal, Activin signaling and POU5F1, FOXA2, FOXC2, FOXH1, SOX17, and GFI1 transcription factors. *Int J Oncol* 2010;36(2):415–420. [PubMed: 20043076]
52. Das B, Tsuchida R, Malkin D, Koren G, Baruchel S, Yeger H. Hypoxia enhances tumor stemness by increasing the invasive and tumorigenic side population fraction. *Stem Cells* 2008;26(7):1818–1830. [PubMed: 18467664]
53. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. *Nat Rev Mol Cell Biol* 2006;7(2):131–142. [PubMed: 16493418]
54. Zhou BP, Hung MC. Wnt, hedgehog and snail: sister pathways that control by GSK-3beta and beta-Trcp in the regulation of metastasis. *Cell Cycle* 2006;4(6):772–776. [PubMed: 15917668]
55. Turley EA, Veiseh M, Radisky DC, Bissell MJ. Mechanisms of disease: epithelial-mesenchymal transition--does cellular plasticity fuel neoplastic progression? *Nat Clin Pract Oncol* 2008;5(5):280–290. [PubMed: 18349857]
56. Wang X, Belguise K, Kersual N, Kirsch KH, Mineva ND, Galtier F, Chalbos D, Sonenshein GE. Oestrogen signalling inhibits invasive phenotype by repressing RelB and its target BCL2. *Nat Cell Biol* 2007;9(4):470–478. [PubMed: 17369819]

57. Spaderna S, Schmalhofer O, Hlubek F, Jung A, Kirchner T, Brabletz T. Epithelial-mesenchymal and mesenchymal-epithelial transitions during cancer progression. *Verh Dtsch Ges Pathol* 2007;91:21–28. [PubMed: 18314592]
58. Shipitsin M, Campbell LL, Argani P, Weremowicz S, Bloushtain-Qimron N, Yao J, Nikolskaya T, Serebryiskaya T, Beroukhi R, Hu M, Halushka MK, Sukumar S, Parker LM, Anderson KS, Harris LN, Garber JE, Richardson AL, Schnitt SJ, Nikolsky Y, Gelman RS, Polyak K. Molecular definition of breast tumor heterogeneity. *Cancer Cell* 2007;11(3):259–273. [PubMed: 17349583]
59. Storci G, Sansone P, Trete D, Tavolari S, Taffurelli M, Ceccarelli C, Guarnieri T, Paterini P, Pariali M, Montanaro L, Santini D, Chieco P, Bonafe M. The basal-like breast carcinoma phenotype is regulated by SLUG gene expression. *J Pathol* 2008;214(1):25–37. [PubMed: 17973239]
60. Shah AN, Summy JM, Zhang J, Park SI, Parikh NU, Gallick GE. Development and characterization of gemcitabine-resistant pancreatic tumor cells. *Ann Surg Oncol* 2007;14(12):3629–3637. [PubMed: 17909916]
61. Moustakas A, Heldin CH. Signaling networks guiding epithelial-mesenchymal transitions during embryogenesis and cancer progression. *Cancer Sci* 2007;98(10):1512–1520. [PubMed: 17645776]
62. Sarkar FH, Li Y, Wang Z, Kong D. NF-kappaB signaling pathway and its therapeutic implications in human diseases. *Int Rev Immunol* 2008;27(5):293–319. [PubMed: 18853341]
63. Ponti D, Costa A, Zaffaroni N, Pratesi G, Petrangolini G, Coradini D, Pilotti S, Pierotti MA, Daidone MG. Isolation and *in vitro* propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. *Cancer Res* 2005;65(13):5506–5511. [PubMed: 15994920]
64. Patrawala L, Calhoun T, Schneider-Broussard R, Li H, Bhatia B, Tang S, Reilly JG, Chandra D, Zhou J, Claypool K, Coghlan L, Tang DG. Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. *Oncogene* 2006;25(12):1696–1708. [PubMed: 16449977]
65. Patrawala L, Calhoun-Davis T, Schneider-Broussard R, Tang DG. Hierarchical organization of prostate cancer cells in xenograft tumors: The CD44+ α 2 β 1+ cell population is enriched in tumor-initiating cells. *Cancer Res* 2007;67(14):6796–6805. [PubMed: 17638891]
66. Wei C, Guomin W, Yujun L, Ruizhe Q. Cancer stem-like cells in human prostate carcinoma cells DU145: The seeds of the cell line? *Cancer Biol Ther* 2007;6(5):763–768. [PubMed: 17592251]
67. Yang ZF, Ngai P, Ho DW, Yu WC, Ng MN, Lau CK, Li ML, Tam KH, Lam CT, Poon RT, Fan ST. Identification of local and circulating cancer stem cells in human liver cancer. *Hepatology* 2008;47(3):919–928. [PubMed: 18275073]
68. Fillmore CM, Kuperwasser C. Human breast cancer cell lines contain stem-like cells that self-renew, give rise to phenotypically diverse progeny and survive chemotherapy. *Breast Cancer Res* 2008;10(2):R25. [PubMed: 18366788]
69. Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. Efficient tumour formation by single human melanoma cells. *Nature* 2008;456(7222):593–598. [PubMed: 19052619]
70. Shmelkov SV, Butler JM, Hooper AT, Hormigo A, Kushner J, Milde T, St Clair R, Baljevic M, White I, Jin DK, Chadburn A, Murphy AJ, Valenzuela DM, Gale NW, Thurston G, Yancopoulos GD, D'Angelica M, Kemeny N, Lyden D, Rafii S. CD133 expression is not restricted to stem cells, and both CD133+ and CD133– metastatic colon cancer cells initiate tumors. *J Clin Invest* 2008;118(6):2111–2120. [PubMed: 18497886]
71. Aktas B, Tewes M, Fehm T, Hauch S, Kimmig R, Kasimir-Bauer S. Stem cell and epithelial-mesenchymal transition markers are frequently overexpressed in circulating tumor cells of metastatic breast cancer patients. *Breast Cancer Res* 2009;11(4):R46. [PubMed: 19589136]
72. Balic M, Lin H, Young L, Hawes D, Giuliano A, McNamara G, Datar RH, Cote RJ. Most early disseminated cancer cells detected in bone marrow of breast cancer patients have a putative breast cancer stem cell phenotype. *Clin Cancer Res* 2006;12(19):5615–5621. [PubMed: 17020963]
73. Liu R, Wang X, Chen GY, Dalerba P, Gurney A, Hoey T, Sherlock G, Lewicki J, Shedden K, Clarke MF. The prognostic role of a gene signature from tumorigenic breast-cancer cells. *N Engl J Med* 2007;356(3):217–226. [PubMed: 17229949]

74. Zhang X, Komaki R, Wang L, Fang B, Chang JY. Treatment of radioresistant stem-like esophageal cancer cells by an apoptotic gene-armed, telomerase-specific oncolytic adenovirus. *Clin Cancer Res* 2008;14(9):2813–2823. [PubMed: 18451249]
75. Sung JM, Cho HJ, Yi H, Lee CH, Kim HS, Kim DK, El-Aty AM, Kim JS, Landowski CP, Hediger MA, Shin HC. Characterization of a stem cell population in lung cancer A549 cells. *Biochem Biophys Res Commun* 2008;371(1):163–167. [PubMed: 18423378]
76. Friel AM, Sargent PA, Patnaude C, Szotek PP, Oliva E, Scadden DT, Seiden MV, Foster R, Rueda BR. Functional analyses of the cancer stem cell-like properties of human endometrial tumor initiating cells. *Cell Cycle* 2008;7(2):242–249. [PubMed: 18256549]
77. Loebinger MR, Giangreco A, Groot KR, Prichard L, Allen K, Simpson C, Bazley L, Navani N, Tibbrell S, Davies D, Janes SM. Squamous cell cancers contain a side population of stem-like cells that are made chemosensitive by ABC transporter blockade. *Br J Cancer* 2008;98(2):380–387. [PubMed: 18219291]
78. Mimeault M, Hauke R, Batra SK. Recent advances on the molecular mechanisms involved in the drug resistance of cancer cells and novel targeting therapies. *Clin Pharmacol Ther* 2008;83(5):673–691. [PubMed: 17786164]
79. Levina V, Marrangoni AM, DeMarco R, Gorelik E, Lokshin AE. Drug-selected human lung cancer stem cells: cytokine network, tumorigenic and metastatic properties. *PLoS ONE* 2008;3(8):e3077. [PubMed: 18728788]
80. Phillips TM, McBride WH, Pajonk F. The response of CD24(–/low)/CD44+ breast cancer-initiating cells to radiation. *J Natl Cancer Inst* 2006;98(24):1777–1785. [PubMed: 17179479]
81. Johannessen TC, Bjerkvig R, Tysnes BB. DNA repair and cancer stem-like cells - Potential partners in glioma drug resistance? *Cancer Treat Rev* 2008;34(6):558–567. [PubMed: 18501520]
82. Chen YC, Hsu HS, Chen YW, Tsai TH, How CK, Wang CY, Hung SC, Chang YL, Tsai ML, Lee YY, Ku HH, Chiou SH. Oct-4 expression maintained cancer stem-like properties in lung cancer-derived CD133-positive cells. *PLoS ONE* 2008;3(7):e2637. [PubMed: 18612434]
83. Dylla SJ, Bevilacqua L, Park IK, Chartier C, Raval J, Ngan L, Pickell K, Aguilar J, Lazetic S, Smith-Berdan S, Clarke MF, Hoey T, Lewicki J, Gurney AL. Colorectal cancer stem cells are enriched in xenogeneic tumors following chemotherapy. *PLoS ONE* 2008;3(6):e2428. [PubMed: 18560594]
84. Fukuda K, Saikawa Y, Ohashi M, Kumagai K, Kitajima M, Okano H, Matsuzaki Y, Kitagawa Y. Tumor initiating potential of side population cells in human gastric cancer. *Int J Oncol* 2009;34(5):1201–1207. [PubMed: 19360333]
85. Vaish M. Mismatch repair deficiencies transforming stem cells into cancer stem cells and therapeutic implications. *Mol Cancer* 2007;6:26. [PubMed: 17407576]
86. Catassi A, Servent D, Paleari L, Cesario A, Russo P. Multiple roles of nicotine on cell proliferation and inhibition of apoptosis: implications on lung carcinogenesis. *Mutat Res* 2008;659(3):221–231. [PubMed: 18495523]
87. Nijhof JG, van Pelt C, Mulder AA, Mitchell DL, Mullenders LH, de Gruijl FR. Epidermal stem and progenitor cells in murine epidermis accumulate UV damage despite NER proficiency. *Carcinogenesis* 2007;28(4):792–800. [PubMed: 17127714]
88. Aubert G, Lansdorp PM. Telomeres and aging. *Physiol Rev* 2008;88(2):557–579. [PubMed: 18391173]
89. Widera D, Kaus A, Kaltschmidt C, Kaltschmidt B. Neural stem cells, inflammation and NF-kappaB: basic principle of maintenance and repair or origin of brain tumours? *J Cell Mol Med* 2008;12(2):459–470. [PubMed: 18182066]
90. Gumucio DL, Fagoonee S, Qiao XT, Liebert M, Merchant JL, Altruda F, Rizzetto M, Pellicano R. Tissue stem cells and cancer stem cells: potential implications for gastric cancer. *Panminerva Med* 2008;50(1):65–71. [PubMed: 18427389]
91. Friedman SL. Mechanisms of hepatic fibrogenesis. *Gastroenterology* 2008;134(6):1655–1669. [PubMed: 18471545]
92. Sharpless NE, DePinho RA. How stem cells age and why this makes us grow old. *Nat Rev Mol Cell Biol* 2007;8(9):703–713. [PubMed: 17717515]
93. Griffero F, Daga A, Marubbi D, Capra MC, Melotti A, Pattarozzi A, Gatti M, Bajetto A, Porcile C, Barbieri F, Favoni RE, Lo CM, Zona G, Spaziante R, Florio T, Corte G. Different response of

- human glioma tumor-initiating cells to EGFR kinase inhibitors. *J Biol Chem* 2009;284(11):7138–7148. [PubMed: 19147502]
94. Gonda TA, Tu S, Wang TC. Chronic inflammation, the tumor microenvironment and carcinogenesis. *Cell Cycle* 2009;8(13):2005–2013. [PubMed: 19550141]
95. Riethdorf S, Wikman H, Pantel K. Review: Biological relevance of disseminated tumor cells in cancer patients. *Int J Cancer* 2008;123(9):1991–2006. [PubMed: 18712708]
96. Zhou J, Wulfschlegel J, Zhang H, Gu P, Yang Y, Deng J, Margolick JB, Liotta LA, Petricoin E III, Zhang Y. Activation of the PTEN/mTOR/STAT3 pathway in breast cancer stem-like cells is required for viability and maintenance. *Proc Natl Acad Sci USA* 2007;104(41):16158–16163. [PubMed: 17911267]
97. Mimeault M, Batra SK. New promising drug targets in cancer- and metastasis-initiating cells. *Drug Discov Today*. 2010 in press.
98. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. *Nat Rev Cancer* 2005;5(4):275–284. [PubMed: 15803154]
99. Ho MM, Ng AV, Lam S, Hung JY. Side population in human lung cancer cell lines and tumors is enriched with stem-like cancer cells. *Cancer Res* 2007;67(10):4827–4833. [PubMed: 17510412]
100. Salmaggi A, Boiardi A, Gelati M, Russo A, Calatozzolo C, Ciusani E, Sciacca FL, Ottolina A, Parati EA, La Porta C, Alessandri G, Marras C, Croci D, De Rossi M. Glioblastoma-derived tumorspheres identify a population of tumor stem-like cells with angiogenic potential and enhanced multidrug resistance phenotype. *Glia* 2006;54(8):850–860. [PubMed: 16981197]
101. Frank NY, Margaryan A, Huang Y, Schatton T, Waaga-Gasser AM, Gasser M, Sayegh MH, Sadee W, Frank MH. ABCB5-mediated doxorubicin transport and chemoresistance in human malignant melanoma. *Cancer Res* 2005;65(10):4320–4333. [PubMed: 15899824]
102. Kang MK, Kang SK. Tumorigenesis of chemotherapeutic drug-resistant cancer stem-like cells in brain glioma. *Stem Cells Dev* 2007;16(5):837–847. [PubMed: 17999604]
103. Mimeault M, Batra SK. Recent advances on the development of novel anti-cancer drugs targeting cancer stem/progenitor cells. *Drug Dev Res* 2008;69(7):415–430.
104. Shervington A, Lu C. Expression of multidrug resistance genes in normal and cancer stem cells. *Cancer Invest* 2008;26(5):535–542. [PubMed: 18568776]
105. Xu JX, Morii E, Liu Y, Nakamichi N, Ikeda J, Kimura H, Aozasa K. High tolerance to apoptotic stimuli induced by serum depletion and ceramide in side-population cells: high expression of CD55 as a novel character for side-population. *Exp Cell Res* 2007;313(9):1877–1885. [PubMed: 17428472]
106. Birnie R, Bryce SD, Roome C, Dussupt V, Droop A, Lang SH, Berry PA, Hyde CF, Lewis JL, Stower MJ, Maitland NJ, Collins AT. Gene expression profiling of human prostate cancer stem cells reveals a pro-inflammatory phenotype and the importance of extracellular matrix interactions. *Genome Biol* 2008;9(5):R83. [PubMed: 18492237]
107. Li T, Su Y, Mei Y, Leng Q, Leng B, Liu Z, Stass SA, Jiang F. ALDH1A1 is a marker for malignant prostate stem cells and predictor of prostate cancer patients' outcome. *Lab Invest* 2010;90(2):234–244. [PubMed: 20010854]
108. Rappa G, Fodstad O, Lorico A. The stem cell-associated antigen CD133 (Prominin-1) is a molecular therapeutic target for metastatic melanoma. *Stem Cells* 2008;26(12):3008–3017. [PubMed: 18802032]
109. Yao J, Zhang T, Ren J, Yu M, Wu G. Effect of CD133/prominin-1 antisense oligodeoxynucleotide on *in vitro* growth characteristics of Huh-7 human hepatocarcinoma cells and U251 human glioma cells. *Oncol Rep* 2009;22(4):781–787. [PubMed: 19724856]
110. Xu M, Yuan Y, Xia Y, Achilefu S. Monoclonal antibody CC188 binds a carbohydrate epitope expressed on the surface of both colorectal cancer stem cells and their differentiated progeny. *Clin Cancer Res* 2008;14(22):7461–7469. [PubMed: 19010863]
111. Misra S, Toole BP, Ghatak S. Hyaluronan constitutively regulates activation of multiple receptor tyrosine kinases in epithelial and carcinoma cells. *J Biol Chem* 2006;281(46):34936–34941. [PubMed: 16959784]

112. Ghatak S, Misra S, Toole BP. Hyaluronan oligosaccharides inhibit anchorage-independent growth of tumor cells by suppressing the phosphoinositide 3-kinase/Akt cell survival pathway. *J Biol Chem* 2002;277(41):38013–38020. [PubMed: 12145277]
113. Bourguignon LY, Peyrollier K, Xia W, Gilad E. Hyaluronan-CD44 interaction activates stem cell marker Nanog, Stat-3-mediated MDR1 gene expression, and ankyrin-regulated multidrug efflux in breast and ovarian tumor cells. *J Biol Chem* 2008;283(25):17635–17651. [PubMed: 18441325]
114. Gilg AG, Tye SL, Tolliver LB, Wheeler WG, Visconti RP, Duncan JD, Kostova FV, Bolds LN, Toole BP, Maria BL. Targeting hyaluronan interactions in malignant gliomas and their drug-resistant multipotent progenitors. *Clin Cancer Res* 2008;14(6):1804–1813. [PubMed: 18347183]
115. Mimeault M, Johansson SL, Henichart JP, Depreux P, Batra SK. Cytotoxic effects induced by docetaxel, gefitinib and cyclopamine on side population and non-side population cell fractions from human invasive prostate cancer cells. *Mol Cancer Ther*. 2010 in press.
116. Feldmann G, Dhara S, Fendrich V, Bedja D, Beaty R, Mullendore M, Karikari C, Alvarez H, Iacobuzio-Donahue C, Jimeno A, Gabrielson KL, Matsui W, Maitra A. Blockade of hedgehog signaling inhibits pancreatic cancer invasion and metastases: a new paradigm for combination therapy in solid cancers. *Cancer Res* 2007;67(5):2187–2196. [PubMed: 17332349]
117. Feldmann G, Fendrich V, McGovern K, Bedja D, Bisht S, Alvarez H, Koorstra JB, Habbe N, Karikari C, Mullendore M, Gabrielson KL, Sharma R, Matsui W, Maitra A. An orally bioavailable small-molecule inhibitor of Hedgehog signaling inhibits tumor initiation and metastasis in pancreatic cancer. *Mol Cancer Ther* 2008;7(9):2725–2735. [PubMed: 18790753]
118. Karhadkar SS, Bova GS, Abdallah N, Dhara S, Gardner D, Maitra A, Isaacs JT, Berman DM, Beachy PA. Hedgehog signalling in prostate regeneration, neoplasia and metastasis. *Nature* 2004;431(7009):707–712. [PubMed: 15361885]
119. Villanueva A, Newell P, Chiang DY, Friedman SL, Llovet JM. Genomics and signaling pathways in hepatocellular carcinoma. *Semin Liver Dis* 2007;27(1):55–76. [PubMed: 17295177]
120. Medicherla S, Li L, Ma JY, Kapoun AM, Gaspar NJ, Liu YW, Mangadu R, O'Young G, Protter AA, Schreiner GF, Wong DH, Higgins LS. Antitumor activity of TGF-beta inhibitor is dependent on the microenvironment. *Anticancer Res* 2007;27(6B):4149–4157. [PubMed: 18229422]
121. Rosa DD, Ismael G, Lago LD, Awada A. Molecular-targeted therapies: lessons from years of clinical development. *Cancer Treat Rev* 2008;34(1):61–80. [PubMed: 17826917]
122. Schmidt C. Lapatinib study supports cancer stem cell hypothesis, encourages industry research. *J Natl Cancer Inst* 2008;100(10):694–695. [PubMed: 18477795]
123. Agelopoulos K, Buerger H, Brandt B. Allelic imbalances of the egfr gene as key events in breast cancer progression--the concept of committed progenitor cells. *Curr Cancer Drug Targets* 2008;8(5):431–445. [PubMed: 18690849]
124. Yamashita T, Ji J, Budhu A, Forgues M, Yang W, Wang HY, Jia H, Ye Q, Qin LX, Wauthier E, Reid LM, Minato H, Honda M, Kaneko S, Tang ZY, Wang XW. EpCAM-Positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. *Gastroenterology* 2008;136(3):1012–1024. [PubMed: 19150350]
125. Magnifico A, Albano L, Campaner S, Delia D, Castiglioni F, Gasparini P, Sozzi G, Fontanella E, Menard S, Tagliabue E. Tumor-initiating cells of HER2-positive carcinoma cell lines express the highest oncoprotein levels and are sensitive to trastuzumab. *Clin Cancer Res* 2009;15(6):2010–2021. [PubMed: 19276287]
126. Tanaka H, Nakamura M, Kameda C, Kubo M, Sato N, Kuroki S, Tanaka M, Katano M. The Hedgehog signaling pathway plays an essential role in maintaining the CD44+CD24-/low sub-population and the side population of breast cancer cells. *Anticancer Res* 2009;29(6):2147–2157. [PubMed: 19528475]
127. Rubin LL, de Sauvage FJ. Targeting the Hedgehog pathway in cancer. *Nat Rev Drug Discov* 2006;5(12):1026–1033. [PubMed: 17139287]
128. Clement V, Sanchez P, de Tribolet N, Radovanovic I, Altaba A. HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. *Curr Biol* 2007;17(2):165–172. [PubMed: 17196391]

129. Jimeno A, Feldmann G, Suarez-Gauthier A, Rasheed Z, Solomon A, Zou GM, Rubio-Viqueira B, Garcia-Garcia E, Lopez-Rios F, Matsui W, Maitra A, Hidalgo M. A direct pancreatic cancer xenograft model as a platform for cancer stem cell therapeutic development. *Mol Cancer Ther* 2009;8(2):310–314. [PubMed: 19174553]
130. Mimeault M, Hauke R, Batra SK. Stem cells -- A revolution in therapeutics--Recent advances on the stem cell biology and their therapeutic applications in regenerative medicine and cancer therapies. *Clin Pharmacol Ther* 2007;82(3):252–264. [PubMed: 17671448]
131. Mimeault, M.; Batra, SK. Stem Cell Applications in Disease Research: Recent Advances on Stem Cell and Cancer Stem Cell Biology and their Therapeutic Implications. In: Faraday, AV.; Dyer, JT., editors. *Progress in Stem Cell Applications*. NOVA Publisher; New York: 2008. p. 57-98.
132. Mimeault M, Batra SK. Recent advances on the significance of stem cells in tissue regeneration and cancer therapies. *Stem Cells* 2006;24(11):2319–2345. [PubMed: 16794264]
133. Strizzi L, Bianco C, Normanno N, Salomon D. Cripto-1: a multifunctional modulator during embryogenesis and oncogenesis. *Oncogene* 2005;24(37):5731–5741. [PubMed: 16123806]
134. Hu XF, Xing PX. Cripto as a target for cancer immunotherapy. *Expert Opin Ther Targets* 2005;9(2):383–394. [PubMed: 15934922]
135. Ugel S, Scarselli E, Iezzi M, Mennuni C, Pannellini T, Calvaruso F, Cipriani B, De PR, Ricci-Vitiani L, Peranzoni E, Musiani P, Zanovello P, Bronte V. Autoimmune B cell lymphopenia following successful adoptive therapy with telomerase-specific T lymphocytes. *Blood*. 2009 Epub ahead of Print.
136. Sabisz M, Skladanowski A. Cancer stem cells and escape from drug-induced premature senescence in human lung tumor cells: implications for drug resistance and *in vitro* drug screening models. *Cell Cycle* 2009;8(19):3208–3217. [PubMed: 19738435]
137. Chen JS, Pardo FS, Wang-Rodriguez J, Chu TS, Lopez JP, Aguilera J, Altuna X, Weisman RA, Ongkeko WM. EGFR regulates the side population in head and neck squamous cell carcinoma. *Laryngoscope* 2006;116(3):401–406. [PubMed: 16540898]
138. Hu C, Li H, Li J, Zhu Z, Yin S, Hao X, Yao M, Zheng S, Gu J. Analysis of ABCG2 expression and side population identifies intrinsic drug efflux in the HCC cell line MHCC-97L and its modulation by Akt signaling. *Carcinogenesis* 2008;29(12):2289–2297. [PubMed: 18820285]
139. Sims-Mourtada J, Izzo JG, Ajani J, Chao KS. Sonic Hedgehog promotes multiple drug resistance by regulation of drug transport. *Oncogene* 2007;26:5674–5679. [PubMed: 17353904]
140. Zhou J, Zhang H, Gu P, Bai J, Margolick JB, Zhang Y. NF-kappaB pathway inhibitors preferentially inhibit breast cancer stem-like cells. *Breast Cancer Res Treat* 2007;29:909–912.
141. Li YM, Song ST, Jiang ZF, Zhang Q, Su CQ, Liao GQ, Qu YM, Xie GQ, Li MY, Ge FJ, Qian QJ. Telomerase-specific oncolytic virotherapy for human hepatocellular carcinoma. *World J Gastroenterol* 2008;14(8):1274–1279. [PubMed: 18300357]
142. Filatenkov A, Muller AM, Tseng WW, jbachsh-Jones S, Winer D, Luong R, Shizuru JA, Engleman EG, Strober S. Ineffective vaccination against solid tumors can be enhanced by hematopoietic cell transplantation. *J Immunol* 2009;183(11):7196–7203. [PubMed: 19890041]
143. Mine T, Matsueda S, Li Y, Tokumitsu H, Gao H, Danes C, Wong KK, Wang X, Ferrone S, Ioannides CG. Breast cancer cells expressing stem cell markers CD44+ CD24 lo are eliminated by Numb-1 peptide-activated T cells. *Cancer Immunol Immunother* 2009;58(8):1185–1194. [PubMed: 19048252]
144. Todaro M, D'Asaro M, Caccamo N, Iovino F, Francipane MG, Meraviglia S, Orlando V, La Mendola C, Gulotta G, Salerno A, Dieli F, Stassi G. Efficient killing of human colon cancer stem cells by gammadelta T lymphocytes. *J Immunol* 2009;182(11):7287–7296. [PubMed: 19454726]
145. Sell S. Stem cell origin of cancer and differentiation therapy. *Crit Rev Oncol Hematol* 2004;51(1):1–28. [PubMed: 15207251]
146. Massard C, Deutsch E, Soria JC. Tumour stem cell-targeted treatment: elimination or differentiation. *Ann Oncol* 2006;17(11):1620–1624. [PubMed: 16600978]
147. Perabo FG, Muller SC. New agents for treatment of advanced transitional cell carcinoma. *Ann Oncol* 2006;18(5):835–843. [PubMed: 17018703]
148. Moserle L, Indraccolo S, Ghisi M, Frasson C, Fortunato E, Canevari S, Miotti S, Tosello V, Zamarchi R, Corradin A, Minuzzo S, Rossi E, Basso G, Amadori A. The side population of

ovarian cancer cells is a primary target of IFN- α antitumor effects. *Cancer Res* 2008;68(14):5658–5668. [PubMed: 18632618]

149. Huang C, Zhang XM, Tavaluc RT, Hart LS, Dicker DT, Wang W, El-Deiry WS. The combination of 5-Fluorouracil plus p53 pathway restoration is associated with depletion of p53-deficient or mutant p53-expressing putative colon cancer stem cells. *Cancer Biol Ther* 2009;8(22):2186–2193. [PubMed: 19923910]
150. Ji Q, Hao X, Meng Y, Zhang M, Desano J, Fan D, Xu L. Restoration of tumor suppressor miR-34 inhibits human p53-mutant gastric cancer tumorspheres. *BMC Cancer* 2008;8:266. [PubMed: 18803879]
151. Yang W, Yan HX, Chen L, Liu Q, He YQ, Yu LX, Zhang SH, Huang DD, Tang L, Kong XN, Chen C, Liu SQ, Wu MC, Wang HY. Wnt/beta-catenin signaling contributes to activation of normal and tumorigenic liver progenitor cells. *Cancer Res* 2008;68(11):4287–4295. [PubMed: 18519688]
152. Frank RT, Edmiston M, Kendall SE, Najbauer J, Cheung CW, Kassa T, Metz MZ, Kim SU, Glackin CA, Wu AM, Yazaki PJ, Aboody KS. Neural stem cells as a novel platform for tumor-specific delivery of therapeutic antibodies. *PLoS ONE* 2009;4(12):e8314. [PubMed: 20016813]
153. Zischek C, Niess H, Ischenko I, Conrad C, Huss R, Jauch KW, Nelson PJ, Bruns C. Targeting tumor stroma using engineered mesenchymal stem cells reduces the growth of pancreatic carcinoma. *Ann Surg* 2009;250(5):747–753. [PubMed: 19826249]
154. Vlashi E, Kim K, Lagadec C, Donna LD, McDonald JT, Eghbali M, Sayre JW, Stefani E, McBride W, Pajonk F. *In vivo* imaging, tracking, and targeting of cancer stem cells. *J Natl Cancer Inst* 2009;101(5):350–359. [PubMed: 19244169]
155. Rafii S, Lyden D, Benezra R, Hattori K, Heissig B. Vascular and haematopoietic stem cells: novel targets for anti-angiogenesis therapy? *Nat Rev Cancer* 2002;2(11):826–835. [PubMed: 12415253]
156. Davidoff AM, Ng CY, Brown P, Leary MA, Spurbeck WW, Zhou J, Horwitz E, Vanin EF, Nienhuis AW. Bone marrow-derived cells contribute to tumor neovasculature and, when modified to express an angiogenesis inhibitor, can restrict tumor growth in mice. *Clin Cancer Res* 2001;7(9):2870–2879. [PubMed: 11555605]
157. Loebinger MR, Eddaoudi A, Davies D, Janes SM. Mesenchymal stem cell delivery of TRAIL can eliminate metastatic cancer. *Cancer Res* 2009;69(10):4134–4142. [PubMed: 19435900]
158. Mimeault M, Batra SK. Recent advances on multiple tumorigenic cascades involved in prostatic cancer progression and targeting therapies. *Carcinogenesis* 2006;27:1–22. [PubMed: 16195239]
159. De Marzo AM, DeWeese TL, Platz EA, Meeker AK, Nakayama M, Epstein JI, Isaacs WB, Nelson WG. Pathological and molecular mechanisms of prostate carcinogenesis: implications for diagnosis, detection, prevention, and treatment. *J Cell Biochem* 2004;91(3):459–477. [PubMed: 14755677]
160. Litvinov IV, Vander Griend DJ, Xu Y, Antony L, Dalrymple SL, Isaacs JT. Low-calcium serum-free defined medium selects for growth of normal prostatic epithelial stem cells. *Cancer Res* 2006;66(17):8598–8607. [PubMed: 16951173]
161. Chen BY, Liu JY, Chang HH, Chang CP, Lo WY, Kuo WH, Yang CR, Lin DP. Hedgehog is involved in prostate basal cell hyperplasia formation and its progressing towards tumorigenesis. *Biochem Biophys Res Commun* 2007;357(4):1084–1089. [PubMed: 17466949]
162. Nikitin AY, Matoso A, Roy-Burman P. Prostate stem cells and cancer. *Histol Histopathol* 2007;22(9):1043–1049. [PubMed: 17523082]
163. Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 2005;65(23):10946–10951. [PubMed: 16322242]
164. Collins AT, Maitland NJ. Prostate cancer stem cells. *Eur J Cancer* 2006;42(12):1213–1218. [PubMed: 16632344]
165. Sharifi N, Kawasaki BT, Hurt EM, Farrar WL. Stem cells in prostate cancer: Resolving the castrate-resistant conundrum and implications for hormonal therapy. *Cancer Biol Ther* 2006;5(8):901–906.

166. Kasper S, Cookson MS. Mechanisms leading to the development of hormone-resistant prostate cancer. *Urol Clin North Am* 2006;33(2):201–210. [PubMed: 16631458]
167. Lam JS, Reiter RE. Stem cells in prostate and prostate cancer development. *Urol Oncol* 2006;24(2):131–140. [PubMed: 16520277]
168. Miki J, Furusato B, Li H, Gu Y, Takahashi H, Egawa S, Sesterhenn IA, McLeod DG, Srivastava S, Rhim JS. Identification of putative stem cell markers, CD133 and CXCR4, in hTERT-immortalized primary nonmalignant and malignant tumor-derived human prostate epithelial cell lines and in prostate cancer specimens. *Cancer Res* 2007;67(7):3153–3161. [PubMed: 17409422]
169. Rowehl RA, Crawford H, Dufour A, Ju J, Botchkina GI. Genomic analysis of prostate cancer stem cells isolated from a highly metastatic cell line. *Cancer Genomics, Proteomics* 2008;5(6):301–310. [PubMed: 19287071]
170. Guzman-Ramirez N, Voller M, Wetterwald A, Germann M, Cross NA, Rentsch CA, Schalken J, Thalmann GN, Cecchini MG. *In vitro* propagation and characterization of neoplastic stem/progenitor-like cells from human prostate cancer tissue. *Prostate* 2009;69(15):1683–1693. [PubMed: 19644960]
171. Blum R, Gupta R, Burger PE, Ontiveros CS, Salm SN, Xiong X, Kamb A, Wesche H, Marshall L, Cutler G, Wang X, Zavadil J, Moscatelli D, Wilson EL. Molecular signatures of prostate stem cells reveal novel signaling pathways and provide insights into prostate cancer. *PLoS ONE* 2009;4(5):e5722. [PubMed: 19478945]
172. Cunha GR, Rieke W, Thomson A, Marker PC, Risbridger G, Hayward SW, Wang YZ, Donjacour AA, Kurita T. Hormonal, cellular, and molecular regulation of normal and neoplastic prostatic development. *J Steroid Biochem Mol Biol* 2004;92(4):221–236. [PubMed: 15663986]
173. Ao M, Franco OE, Park D, Raman D, Williams K, Hayward SW. Cross-talk between paracrine-acting cytokine and chemokine pathways promotes malignancy in benign human prostatic epithelium. *Cancer Res* 2007;67(9):4244–4253. [PubMed: 17483336]
174. Chung LW, Baseman A, Assikis V, Zhau HE. Molecular insights into prostate cancer progression: the missing link of tumor microenvironment. *J Urol* 2005;173(1):10–20. [PubMed: 15592017]
175. Rizzo S, Attard G, Hudson DL. Prostate epithelial stem cells. *Cell Prolif* 2005;38(6):363–374. [PubMed: 16300650]
176. Brown MD, Gilmore PE, Hart CA, Samuel JD, Ramani VA, George NJ, Clarke NW. Characterization of benign and malignant prostate epithelial Hoechst 33342 side populations. *Prostate* 2007;67(13):1384–1396. [PubMed: 17639507]
177. Guzman-Ramirez N, Voller M, Wetterwald A, Germann M, Cross NA, Rentsch CA, Schalken J, Thalmann GN, Cecchini MG. *In vitro* propagation and characterization of neoplastic stem/progenitor-like cells from human prostate cancer tissue. *Prostate* 2009;69(15):1683–1693. [PubMed: 19644960]
178. Heer R, Robson CN, Shenton BK, Leung HY. The role of androgen in determining differentiation and regulation of androgen receptor expression in the human prostatic epithelium transient amplifying population. *J Cell Physiol* 2007;212(3):572–578. [PubMed: 17541959]
179. Simon RA, di Sant'agnese PA, Huang LS, Xu H, Yao JL, Yang Q, Liang S, Liu J, Yu R, Cheng L, Oh WK, Palapattu GS, Wei J, Huang J. CD44 expression is a feature of prostatic small cell carcinoma and distinguishes it from its mimickers. *Hum Pathol* 2009;40(2):252–258. [PubMed: 18835619]
180. Alam TN, O'Hare MJ, Laczko I, Freeman A, Al-Beidh F, Masters JR, Hudson DL. Differential expression of CD44 during human prostate epithelial cell differentiation. *J Histochem Cytochem* 2004;52(8):1083–1090. [PubMed: 15258184]
181. Sharifi N, Hurt EM, Kawasaki BT, Farrar WL. TGFBR3 loss and consequences in prostate cancer. *Prostate* 2007;67(3):301–311. [PubMed: 17192875]
182. Mathew G, Timm EA, JrSotomayor P, Godoy A, Montecinos VP, Smith GJ, Huss WJ. ABCG2-mediated DyeCycle Violet efflux defined side population in benign and malignant prostate. *Cell Cycle* 2009;8(7):1053–1061. [PubMed: 19270533]

183. Tang DG, Patrawala L, Calhoun T, Bhatia B, Choy G, Schneider-Broussard R, Jeter C. Prostate cancer stem/progenitor cells: Identification, characterization, and implications. *Mol Carcinog* 2007;46(1):1–14. [PubMed: 16921491]
184. Wang S, Garcia AJ, Wu M, Lawson DA, Witte ON, Wu H. Pten deletion leads to the expansion of a prostatic stem/progenitor cell subpopulation and tumor initiation. *Proc Natl Acad Sci USA* 2006;103(5):1480–1485. [PubMed: 16432235]
185. Zhou Z, Flesken-Nikitin A, Nikitin AY. Prostate cancer associated with p53 and Rb deficiency arises from the stem/progenitor cell-enriched proximal region of prostatic ducts. *Cancer Res* 2007;67(12):5683–5690. [PubMed: 17553900]
186. Man YG, Gardner WA. Focal degeneration of basal cells and the resultant auto-immunoreactions: A novel mechanism for prostate tumor progression and invasion. *Med Hypotheses* 2007;70(2):387–408. [PubMed: 17658698]
187. Mimeault M, Pommery N, Henichart JP. New advances on prostate carcinogenesis and therapies: involvement of EGF-EGFR transduction system. *Growth Factors* 2003;21(1):1–14. [PubMed: 12795332]
188. Zhou W, Grandis JR, Wells A. STAT3 is required but not sufficient for EGF receptor-mediated migration and invasion of human prostate carcinoma cell lines. *Br J Cancer* 2006;95(2):164–171. [PubMed: 16804520]
189. Shah RB, Ghosh D, Elder JT. Epidermal growth factor receptor (ErbB1) expression in prostate cancer progression: correlation with androgen independence. *Prostate* 2006;66(13):1437–1444. [PubMed: 16741920]
190. Rau KM, Kang HY, Cha TL, Miller SA, Hung MC. The mechanisms and managements of hormone-therapy resistance in breast and prostate cancers. *Endocr Relat Cancer* 2005;12(3):511–532. [PubMed: 16172190]
191. Di Lorenzo G, De Placido S. Hormone refractory prostate cancer (HRPC): present and future approaches of therapy. *Int J Immunopathol Pharmacol* 2006;19(1):11–34. [PubMed: 16569343]
192. Winkvist E, Waldron T, Berry S, Ernst DS, Hotte S, Lukka H. Non-hormonal systemic therapy in men with hormone-refractory prostate cancer and metastases: a systematic review from the Cancer Care Ontario Program in Evidence-based Care's Genitourinary Cancer Disease Site Group. *BMC Cancer* 2006;6(1):112. [PubMed: 16670021]
193. van Leenders GJ, Aalders TW, Hulsbergenvan de Kaa CA, Ruiter DJ, Schalken JA. Expression of basal cell keratins in human prostate cancer metastases and cell lines. *J Pathol* 2001;195(5):563–570. [PubMed: 11745692]
194. Sheng T, Li C, Zhang X, Chi S, He N, Chen K, McCormick F, Gatalica Z, Xie J. Activation of the hedgehog pathway in advanced prostate cancer. *Mol Cancer* 2004;3(1):29. [PubMed: 15482598]
195. Stecca B, Mas C, Altaba AR. Interference with HH-GLI signaling inhibits prostate cancer. *Trends Mol Med* 2005;11(5):199–203. [PubMed: 15882606]
196. Datta S, Datta MW. Sonic Hedgehog signaling in advanced prostate cancer. *Cell Mol Life Sci* 2006;63(4):435–448. [PubMed: 16389455]
197. Chen BY, Lin DP, Liu JY, Chang H, Huang PH, Chen YL, Chang HH. A mouse prostate cancer model induced by Hedgehog overexpression. *J Biomed Sci* 2006;13(3):373–384. [PubMed: 16374524]
198. Bisson I, Prowse DM. WNT signaling regulates self-renewal and differentiation of prostate cancer cells with stem cell characteristics. *Cell Res* 2009;19(6):683–697. [PubMed: 19365403]
199. Mimeault M, Batra SK. Characterization of non-malignant and malignant prostatic stem/progenitor cells by Hoechst side population method. *Methods Mol Biol* 2009;568:139–149. [PubMed: 19582424]
200. Berry DL, Moinpour CM, Jiang CS, Ankerst DP, Petrylak DP, Vinson LV, Lara PN, Jones S, Taplin ME, Burch PA, Hussain MH, Crawford ED. Quality of life and pain in advanced stage prostate cancer: results of a Southwest Oncology Group randomized trial comparing docetaxel and estramustine to mitoxantrone and prednisone. *J Clin Oncol* 2006;24(18):2828–2835. [PubMed: 16782921]

201. Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, Oudard S, Theodore C, James ND, Turesson I, Rosenthal MA, Eisenberger MA. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N Engl J Med* 2004;351(15):1502–1512. [PubMed: 15470213]
202. Calabro F, Sternberg CN. Current indications for chemotherapy in prostate cancer patients. *Eur Urol* 2007;51(1):17–26. [PubMed: 17007996]
203. Gignac GA, Morris MJ, Hussain M. Castration resistant, taxane naive metastatic prostate cancer: Current clinical approaches and future directions. *J Urol* 2007;178(3 Pt 2):S30–S35. [PubMed: 17644121]
204. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. *CA Cancer J Clin* 2008;58(2):71–96. [PubMed: 18287387]
205. Ye XC, Choueiri M, Tu SM, Lin SH. Biology and clinical management of prostate cancer bone metastasis. *Front Biosci* 2007;12:3273–3286. [PubMed: 17485298]
206. Grubb RL III, Kibel AS. Prostate cancer: screening, diagnosis and management in 2007. *Mol Med* 2007;104(5):408–413.
207. Yuan X, Balk SP. Mechanisms mediating androgen receptor reactivation after castration. *Urol Oncol* 2009;27(1):36–41. [PubMed: 19111796]
208. Petrylak DP, Tangen CM, Hussain MH, Lara PN, Jr Jones JA, Taplin ME, Burch PA, Berry D, Moinpour C, Kohli M, Benson MC, Small EJ, Raghavan D, Crawford ED. Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. *N Engl J Med* 2004;351(15):1513–1520. [PubMed: 15470214]
209. Clarke NW. Management of the spectrum of hormone refractory prostate cancer. *Eur Urol* 2006;50(3):428–438. [PubMed: 16797118]
210. Zenzmaier C, Untergasser G, Berger P. Aging of the prostate epithelial stem/progenitor cell. *Exp Gerontol* 2008;43(11):981–985. [PubMed: 18639623]
211. Gu G, Yuan J, Wills M, Kasper S. Prostate cancer cells with stem cell characteristics reconstitute the original human tumor *in vivo*. *Cancer Res* 2007;67(10):4807–4815. [PubMed: 17510410]
212. Mimeault M, Mehta PP, Hauke R, Batra SK. Functions of normal and malignant prostatic stem/progenitor cells in tissue regeneration and cancer progression and novel targeting therapies against advanced prostate cancers. *Endocr Rev* 2008;29(2):234–252. [PubMed: 18292464]
213. Kurbel S. Selective reduction of estrogen receptor (ER) positive breast cancer occurrence by estrogen receptor modulators supports etiological distinction between ER positive and ER negative breast cancers. *Med Hypotheses* 2005;64(6):1182–1187. [PubMed: 15823713]
214. Rizo A, Vellenga E, de Haan G, Schuringa JJ. Signaling pathways in self-renewing hematopoietic and leukemic stem cells: do all stem cells need a niche? *Hum Mol Genet* 2006;15(Spec No 2):R210–R219. [PubMed: 16987886]
215. de Jonge-Peeters SD, Kuipers F, de Vries EG, Vellenga E. ABC transporter expression in hematopoietic stem cells and the role in AML drug resistance. *Crit Rev Oncol Hematol* 2007;62(3):214–226. [PubMed: 17368038]
216. Beachy PA, Karhadkar SS, Berman DM. Tissue repair and stem cell renewal in carcinogenesis. *Nature* 2004;432(7015):324–331. [PubMed: 15549094]
217. Mimeault M, Hauke R, Batra SK. Recent advances on the molecular mechanisms involved in drug-resistance of cancer cells and novel targeting therapies. *Clin Pharmacol Ther* 2008;83(5):673–691. [PubMed: 17786164]
218. Mimeault M, Batra SK. Recent progress on tissue-resident adult stem cell biology and their therapeutic implications. *Stem Cell Rev* 2008;4(1):27–49. [PubMed: 18288619]
219. Kawasaki BT, Hurt EM, Kalathur M, Duhagon MA, Milner JA, Kim YS, Farrar WL. Effects of the sesquiterpene lactone parthenolide on prostate tumor-initiating cells: An integrated molecular profiling approach. *Prostate* 2009;69(8):827–837. [PubMed: 19204913]
220. Dubrovskaya A, Kim S, Salamone RJ, Walker JR, Maira SM, Garcia-Echeverria C, Schultz PG, Reddy VA. The role of PTEN/Akt/PI3K signaling in the maintenance and viability of prostate cancer stem-like cell populations. *Proc Natl Acad Sci USA* 2009;106(1):268–273. [PubMed: 19116269]

221. Carriere C, Seeley ES, Goetze T, Longnecker DS, Korc M. The Nestin progenitor lineage is the compartment of origin for pancreatic intraepithelial neoplasia. *Proc Natl Acad Sci U S A* 2007;104(11):4437–4442.
222. Pasca di MM, Sekine S, Ermilov A, Ferris J, Dlugosz AA, Hebrok M. Hedgehog/Ras interactions regulate early stages of pancreatic cancer. *Genes Dev* 2006;20(22):3161–3173. [PubMed: 17114586]
223. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, Wicha M, Clarke MF, Simeone DM. Identification of pancreatic cancer stem cells. *Cancer Res* 2007;67(3):1030–1037. [PubMed: 17283135]
224. Brand RE, Lerch MM, Rubinstein WS, Neoptolemos JP, Whitcomb DC, Hruban RH, Brentnall TA, Lynch HT, Canto MI. Advances in counselling and surveillance of patients at risk for pancreatic cancer. *Gut* 2007;56(10):1460–1469. [PubMed: 17872573]
225. Pasca di MM, Sekine S, Ermilov A, Ferris J, Dlugosz AA, Hebrok M. Hedgehog/Ras interactions regulate early stages of pancreatic cancer. *Genes Dev* 2006;20(22):3161–3173. [PubMed: 17114586]
226. Olempska M, Eisenach PA, Ammerpohl O, Ungefroren H, Fandrich F, Kalthoff H. Detection of tumor stem cell markers in pancreatic carcinoma cell lines. *Hepatobiliary Pancreat Dis Int* 2007;6(1):92–97. [PubMed: 17287174]
227. Gou S, Liu T, Wang C, Yin T, Li K, Yang M, Zhou J. Establishment of clonal colony-forming assay for propagation of pancreatic cancer cells with stem cell properties. *Pancreas* 2007;34(4):429–435. [PubMed: 17446842]
228. Zhou J, Wang CY, Liu T, Wu B, Zhou F, Xiong JX, Wu HS, Tao J, Zhao G, Yang M, Gou SM. Persistence of side population cells with high drug efflux capacity in pancreatic cancer. *World J Gastroenterol* 2008;14(6):925–930. [PubMed: 18240351]
229. Mimeault M, Brand RE, Sasson AA, Batra SK. Recent advances on the molecular mechanisms involved in pancreatic cancer progression and therapies. *Pancreas* 2005;31(4):301–316. [PubMed: 16258363]
230. Rivera F, Lopez-Tarruella S, Vega-Villegas MA, Salcedo M. Treatment of advanced pancreatic cancer: From gemcitabine single agent to combinations and targeted therapy. *Cancer Treat Rev* 2009;35(4):335–339. [PubMed: 19131170]
231. El Maalouf G, Le TC, Batty GN, Faivre S, Raymond E. Markers involved in resistance to cytotoxics and targeted therapeutics in pancreatic cancer. *Cancer Treat Rev* 2009;35(2):167–174. [PubMed: 19027240]
232. Bai J, Sata N, Nagai H. Gene expression analysis for predicting gemcitabine sensitivity in pancreatic cancer patients. *HPB (Oxford)* 2007;9(2):150–155. [PubMed: 18333132]
233. Hong SP, Wen J, Bang S, Park S, Song SY. CD44-positive cells are responsible for gemcitabine resistance in pancreatic cancer cells. *Int J Cancer* 2009;125(10):2323–2331. [PubMed: 19598259]
234. Wang YH, Li F, Luo B, Wang XH, Sun HC, Liu S, Cui YQ, Xu XX. A side population of cells from a human pancreatic carcinoma cell line harbors cancer stem cell characteristics. *Neoplasma* 2009;56(5):371–378. [PubMed: 19580337]
235. Mimeault M, Johansson SL, Senapati S, Momi N, Chakraborty S, Batra SK. MUC4 down-regulation reverse chemoresistance of pancreatic cancer stem/progenitor cells and their progenies. *Cancer Lett.* 2010 in press.
236. Ji Q, Hao X, Zhang M, Tang W, Yang M, Li L, Xiang D, Desano JT, Bommer GT, Fan D, Fearon ER, Lawrence TS, Xu L. MicroRNA miR-34 inhibits human pancreatic cancer tumor-initiating cells. *PLoS ONE* 2009;4(8):e6816. [PubMed: 19714243]
237. Zhou J, Wang CY, Liu T, Wu HS, Zhou F, Xiong JX, Zhao G, Yang M, Yin T, Zhou MT. Phosphatidylinositol 3-kinase/mammalian target of rapamycin pathway is critical for survival and proliferation of pancreatic cancer stem-like side population cells. *Zhonghua Yi Xue Za Zhi* 2008;88(42):2994–2998. [PubMed: 19080079]
238. Kabashima A, Higuchi H, Takaishi H, Matsuzaki Y, Suzuki S, Izumiya M, Iizuka H, Sakai G, Hozawa S, Azuma T, Hibi T. Side population of pancreatic cancer cells predominates in TGF-

- beta-mediated epithelial to mesenchymal transition and invasion. *Int J Cancer* 2009;124(12):2771–2779. [PubMed: 19296540]
239. Wang Z, Li Y, Kong D, Banerjee S, Ahmad A, Azmi AS, Ali S, Abbruzzese JL, Gallick GE, Sarkar FH. Acquisition of epithelial-mesenchymal transition phenotype of gemcitabine-resistant pancreatic cancer cells is linked with activation of the notch signaling pathway. *Cancer Res* 2009;69(6):2400–2407. [PubMed: 19276344]
240. Levina V, Marrangoni A, Wang T, Parikh S, Su Y, Herberman R, Lokshin A, Gorelik E. Elimination of human lung cancer stem cells through targeting of the stem cell factor-c-kit autocrine signaling loop. *Cancer Res* 2009;70(1):338–346. [PubMed: 20028869]
241. Calabrese C, Poppleton H, Kocak M, Hogg TL, Fuller C, Hamner B, Oh EY, Gaber MW, Finklestein D, Allen M, Frank A, Bayazitov IT, Zakharenko SS, Gajjar A, Davidoff A, Gilbertson RJ. A perivascular niche for brain tumor stem cells. *Cancer Cell* 2007;11(1):69–82. [PubMed: 17222791]

Fig. 1.

Scheme showing the molecular events associated with the epithelial cancer initiation and progression and potential therapeutic strategies against locally invasive and metastatic epithelial cancers. The molecular events associated with the malignant transformation of tissue-resident adult stem cells into tumorigenic cancer stem/progenitor cells, also termed as tumor-initiating cells, induced through the accumulation of genetic and/or epigenetic alterations during epithelial cancer initiation is shown. Furthermore, the acquisition of a migratory phenotype by tumorigenic stem/progenitor cells, which may be induced by the sustained activation of distinct growth factor signaling pathways during the epithelial-mesenchymal transition (EMT) program, is also indicated. The possible invasion of certain tumorigenic and migrating cancer stem/progenitor cells, also designated as metastasis-initiating cells, in the activated stroma, which may lead to their dissemination through the peripheral circulation and metastases at distant tissues and organs, is also illustrated. In addition, the therapeutic strategies consisting of targeted therapy of tumor- and metastasis-initiating cells, immunotherapy, selective delivery of anti-tumoral drugs by using genetically-modified stem cells and anti-angiogenic therapy by targeting the circulating endothelial progenitor cells (EPCs) and intratumoral vascular endothelial cells are also illustrated.

Fig. 2.

Novel therapeutic strategies against aggressive and recurrent epithelial cancers by molecular targeting distinct signaling pathways and multidrug resistance-associated molecules involved in aggressive behavior and resistance phenotype of tumorigenic and migrating cancer stem/progenitor cells. The anti-carcinogenic effects induced by a monoclonal antibody (mAb) directed against CD133 or CD44 stem cell-like marker or Wingless (Wnt) ligand of Wnt/ β -catenin tumorigenic pathway are shown. Moreover, the cytotoxic effects induced by diverse pharmacological agents such as the selective inhibitors of receptor tyrosine kinase (RTK) activity “gefitinib or erlotinib”, smoothened (SMO) hedgehog signaling element “cyclopamine or IPI-269609”, CXC chemokine receptor 4 (CXCR4) “AMD3100” on cancer stem/progenitor cell behavior are indicated. Particularly, the anti-proliferative, anti-invasive, anti-metastatic and/or apoptotic effects induced by these pharmacological agents in cancer stem/progenitor cells through down-regulation of expression levels of numerous gene products are indicated. In addition, the potent inhibitory effect mediated by a mAb or specific inhibitor of ATP-binding cassette (ABC) multidrug transporter on drug efflux and aldehyde dehydrogenase (ALDH) “diethylaminobenzaldehyde, DEAB” on drug metabolism concomitant with intracellular drug accumulation and decreased drug resistance is also illustrated.

Table 1

Potential Therapeutic Molecular Targets in Tumor- and Metastasis-Initiating Cells and their Progenies

Targeted Element	Name of Inhibitory Agent	Cancer Type	Ref.
CD133 stem cell-like surface marker	Anti-CD133 mAbs directed against epitope 1 (AC133/W6B3C1) or epitope 2 (AC141)	Melanoma	[108]
CD133/prominin-1	Antisense oligodeoxynucleotide	Brain and liver	[109]
CC188	Anti-CC188 mAb	Colorectal cancer	[110]
TERT	TERT-based ACT	Colon cancer	[135]
Oncogenic Signaling Element			
EGFR	TKI, Gefitinib or erlotinib	Brain cancer	[93]
ErbB2	Trastuzumab	Breast cancer	[125]
EGFR (HER-1)/ErbB2 (HER2)	Lapitinib plus anti-ErbB2 mAb, trastuzumab	Breast cancer	[122]
Hedgehog	SMO inhibitor (cyclopamine) alone or with temozolomide	Brain cancer	[128]
	Cyclopamine, gefitinib plus docetaxel	Prostate cancer	[115]
	Cyclopamine alone or with gemcitabine	Pancreatic cancer	[116,129]
	Orally bioavailable small-molecule hedgehog inhibitor, IPI-269609	Pancreatic cancer	[117]
	si-RNA against Gli-1	Breast cancer	[126]
Wnt/ β -catenin	Lentivirus-delivered stable expression micro-RNA against β -catenin plus cisplatin	Liver cancer	[151]
HA/CD44 axis	Small HA oligosaccharides	Brain cancer	[114]
	Anti-CD44 mAb	Liver cancer	[11]
SCF/KIT	Anti-SCF antibody or imatinib plus cisplatin	Lung cancer	[240]
IL4/IL-4R	IL-4 receptor α antagonist	Colon cancer	[24]
	Anti-IL-4 neutralizing antibody		
SDF/CXCR4 axis	Anti-CXCR4 mAb	Pancreatic cancer	[12]

ACT, adoptive cell therapy; CXCR4, CXC chemokine receptor 4; EGFR; epidermal growth factor receptor; HA, hyaluronan; IL-4, interleukin-4; mAb, monoclonal antibody; SDF-1, stromal cell-derived factor-1; siRNA, small interference RNA; SMO, smoothened; TERT, telomerase reverse transcriptase; TKI, tyrosine kinase activity inhibitor.

Table 2

Potential Therapeutic Molecular Targets Involved in Survival and Drug Resistance of Tumor- and Metastasis-Initiating Cells and Tumor Angiogenesis

Targeted Element	Name of Inhibitory Agent	Cancer Type	Ref.
ABC transporters	Verapamil plus mitoxantrone	Aerodigestive SCC	[77]
	Gefitinib	Head and neck SCC	[137]
	ABCG2-specific inhibitor, 4-FTC or PI ₃ K/Akt/mTOR inhibitor, LY294002 or rapamycin plus doxorubicin	Liver cancer	[138]
	Anti-ABCB5 mAb	Melanoma	[15]
ALDH	ALDH1-specific inhibitor, DEAB or ALDH1A-targeted shRNA plus bioactive metabolite (4-HC) of cyclophosphamide (CPA)	Colorectal cancer	[83]
PI ₃ K/Akt/mTOR	LY294002 specific inhibitor of PI ₃ K or dual PI ₃ K/mTOR inhibitor, NVP-BEZ235	Prostate cancer	[220]
	LY94002 or specific inhibitor of mTOR, rapamycin	Pancreatic cancer	[236]
NF-κB	Parthenolide, PDTC and its analog DETC	Breast cancer	[140]
	Parthenolide	Prostate cancer	[106]
Tumor Angiogenic Signaling			
VEGF	Anti-VEGF mAb, bevacizumab	Brain cancer	[241]

ALDH, aldehyde dehydrogenase; CPA, cyclophosphamide; DEAB, diethylaminobenzaldehyde; DETC, diethyldithiocarbamate; EGFR, epidermal growth factor receptor; 4-FTC, 4-fumitremorgin C; 4-HC, 4-hydroxycyclophosphamide; PDTC, pyrrolidinethiocarbamate; PI₃K, phosphatidylinositol-3' kinase; SCC, squamous cell carcinoma; shRNA, small hairpin RNA; VEGF, vascular endothelial growth factor.