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Cancer stemness and metastasis: Therapeutic consequences and perspectives

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ABSTRACT

The transient and dynamic nature of cancer cells that underlie metastasis cannot only be explained by the progressive accumulation of irreversible genetic changes occurring in the primary tumour. The capacity of cells to switch between different cellular identities, as in epithelial to mesenchymal (EMT) and mesenchymal to epithelial transitions (MET), allows them to respond to different cellular environments, thus efficiently disseminating from the primary mass to eventually colonise distant organs. These more dynamic stem-like cancer cells are earmarked by the ability to self-renew and de-/re-differentiate, and eventually recapitulate the heterogeneous composition of the primary tumour in a distant organ site. This dynamic concept of metastasis has profound consequences and implications for cancer diagnostics, prognostics and therapy. Many of the characteristics that define stem cells, like dormancy, active DNA repair, the expression of several drug transporters, and resistance to apoptosis may underlie the capacity of migrating cancer cells to survive conventional therapeutic protocols based on genotoxic agents targeting active proliferating cells. Accordingly, signal transduction pathways that regulate the balance between self-renewal and differentiation are likely to represent future targets in the development of tailor-made intervention strategies. Also, specific stem cell features, such as the capacity to migrate to diseased areas (pathotropism), open novel avenues towards the development of cell vehicles capable of tracking and delivering anti-cancer compounds to disseminated metastatic lesions.

1. Reversible vs. irreversible changes in cancer stemness and metastasis

Primary malignancies are responsible for a mere 10% of deaths from cancer. In the vast majority of cancer cases the main cause of morbidity and mortality is metastases formed by cancer cells that have detached from the primary tumours and, after a long journey through blood and lymphatic vessels, have colonised distant sites. It is often said that an accumulation of genetic changes accompanies malignant transformation at the primary sites and that tumour cells capable of metastasising to distant sites have acquired specific 'hits' that enable them to adapt to different tissue environments.[1] However, the rate-limiting steps that confer this adaptability to different tissue environments cannot solely be explained by irreversible genetic alterations, possibly indicating the existence of a more dynamic component to human tumour progression towards dissemination and metastasis.[2]

Accumulating evidence in a wide range of malignancies suggests that a subpopulation of tumour cells with distinct stem-like properties is responsible for tumour initiation, invasive growth and possibly dissemination to distant organ sites. According to this hypothesis, tumours are characterised by the same hierarchical organisation observed among stem cell

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niches in the developing embryo and in the adult organism where primordial stem cells divide asymmetrically to give rise to more committed progenitors that will eventually differentiate into more specialised cell types. These so-called cancer stem cells (CSCs), or tumour-initiating cells, are able to self-renew and differentiate into the multiple lineages of the heterogeneous tumour mass.[3] However, the concept of cancer stem cells has been a rather controversial one, mainly because of its operational definition based on the limiting dilution transplantation assay in immune-deficient, i.e. NOD/SCID, mice. By employing more highly immune-compromised recipient animals (NOD-scid IL2Rγnull), Quintana and collaborators[4] showed that the relative frequency of melanoma-initiating cells dramatically increased by several orders of magnitude. Moreover, the suggestion that mammary CSCs could arise from more differentiated tumour cells by EMT[5] also cast some doubts on the hierarchical organisation of malignancies.

Nonetheless, several signal transduction pathways known to regulate self renewal and homeostasis in adult stem cell niches, are also deregulated and play key roles in the malignant transformation in the same tissues.[6] The canonical Wnt pathway, known to be a critical regulator of self-renewal in the intestinal, epidermal and haematopoietic stem cell niches, is constitutively activated in colon, skin and haematopoietic cancers.[7] Similarly, alterations in the Hedgehog (Hh) and Notch signal transduction pathways, essential for regulating cell fate and numbers in a multitude of embryonic and adult tissues, are common in human basal carcinomas of the skin, T-cell leukaemia and in pancreatic, gastric, prostate, cervical and breast carcinomas.[8,9] Notably, in agreement with the close relationship among stemness, self-renewal and cancer, gene expression signatures characteristic of embryonic stem (ES) cells are present in poorly differentiated glioblastomas, and in bladder and breast carcinomas.[10]

Although specific gene mutations have been reported to deregulate the above signalling pathways in tumour onset, the adaptive plasticity of a metastasising cancer cell cannot be exclusively explained by irreversible genetic defects. Paradoxically, one could envisage that reversible epigenetic changes are likely to be preferred to accommodate the variable environments that the disseminated tumour cell encounter during their journey from the primary lesion to the organ site of metastasis. Indeed, in both embryonic and adult stem cells, differentiation is mainly controlled by epigenetic mechanisms. ES cells rely on Polycomb group proteins to reversibly repress genes required for differentiation. Notably, the Polycomb group targets found in ES cells are more likely to undergo DNA hypermethylation in cancer than non-targets,[10,11] thus supporting a scenario where reversible gene repression is exploited by the cancer cell, not only to sustain the growth of the primary tumour by deregulating the balance between self-renewal and differentiation, but possibly also in its dissemination into surrounding tissues and in the formation of distant metastases.

2. EMT, tumour dissemination and metastasis

Epithelial to mesenchymal transition (EMT), i.e. the developmental process through which epithelial cells acquire a mesenchymal identity, is now recognised as an essential feature of tumour invasion.[12,13] Throughout development, the capacity of cells to switch between these two cellular states plays a fundamental role in the generation of complex body patterns. Also, in the adult organism, EMT is engaged during wound healing, tissue regeneration and organ fibrosis. In cancer, EMT is thought to confer to tumour cells the capacity to detach from the primary mass by losing cell adhesive properties and acquiring more motile features, thus enabling local invasion, intravasation into blood or lymph vessels, extravasation and the recapitulation of the primary mass at distant sites through the reverse process of mesenchymal to epithelial transition (MET).[12] In colon cancer, nuclear accumulation of β-catenin, the hallmark of Wnt signalling activation and cancer stemness, is preferentially found at the invasive front of the primary lesion where it earmarks cells undergoing EMT and detaching into the tumour microenvironment.[2,14] These observations have led to the concept of migrating cancer stem cells (MCSCs) where EMT-competent cells have stem cell features that confer them the plasticity to adapt to different environments and efficiently form metastatic colonies in distant tissues.[2] This model is further supported by the expression of EMT markers in CSCs from mammary carcinomas and, vice versa, by the activation of stem cell markers in EMT-induced mammary epithelial cells.[5] However, as EMT cells are capable of degrading the surrounding matrix, they also enable the detachment and dissemination of non-EMT tumour cells.[15] This implies that disseminated tumour cells (DTCs) may reflect the heterogeneous composition of the primary tumour, encompassing both differentiated and more stem-like cellular types. The question then arises whether only the EMT-competent MCSCs are capable of forming distant metastases or, when exposed to specific environmental cues, more differentiated tumour cells can also trigger colonisation at distant sites. In fact, alternative models have been proposed according to which EMT cells alone cannot form metastasis, but actively cooperate with non-EMT cells to complete metastasis. In this scenario, EMT at the primary tumour allows matrix degradation and facilitates local and distant invasion of both EMT- and non-EMT tumour cells with an unaltered adhesive phenotype.[16,17] From this perspective, the fact that metastatic lesions often recapitulate the heterogeneous composition of the primary tumour may be explained both by individual migrating CSCs capable of recapitulating the disease at distant organ sites or, alternatively, by collective migration and colonisation of cooperating EMT and non-EMT tumour cells.

Disseminating and circulating tumour cells (DTCs and CTCs) present in the bone marrow and peripheral blood of cancer patients can be detected and analysed at the single-cell level and are thought to have diagnostic and possibly also therapeutic relevance in the prevention and eradication of metastatic disease.[18] In view of the above-mentioned discussion on the relative role of differentiated and stem-like tumour cells in metastasis formation, DTCs and CTCs are likely to encompass both cell types and to recapitulate the heterogeneous composition of the primary lesion. However, the relative representation of individual cellular types in DTC/CTC preparations may be biased by the isolation methods currently employed, as these protocols often rely on the

expression of epithelial markers (e.g. cytokeratins, EpCAM), possibly leading to an over-representation of more differentiated tumour cells. Nevertheless, a striking 70% enrichment of $CD44^+/CD24^{low}$ cells, previously shown to represent mammary CSCs,[19] was reported in DTCs isolated from bone marrow samples of early breast cancer patients compared with the corresponding primary lesions where these tumour-initiating cells constitute less than 10% of the bulk cells.[20] Accordingly, $CK19^+/Muc1^-$ cells, previously shown to have stem cell-like properties in human breast,[21] were detected among BM-derived DTCs in breast cancer patients.[22] Notably, TWIST1, an EMT-inducing transcription factor, was also described to be an integral part of the gene expression signature of disseminated breast cancer cells present in bone marrow after chemotherapy.[23] The relative abundance of cancer stem cells among DTCs may also be explained by the emerging role of EpCAM, the epithelial antigen most frequently employed to isolate disseminating and circulating tumour cells, in stem cell signalling.[24,25]

Compared with DTCs from bone marrow, less experimental evidence is available on the prognostic significance of tumour cells circulating in peripheral blood.[18] A recent study on a relatively large cohort of blood samples revealed that a major proportion of CTCs from metastatic breast cancer patients show EMT and tumour stem cell characteristics.[26] However, whether these cells have prognostic value and/or may serve as an indicator of response to therapy is yet to be determined.

A different issue in the detection and prognostic relevance of CTCs is the anatomical site of venous blood sampling. Although peripheral blood offers clear advantages in terms of ease of sampling, it is plausible to think that, on their way from the primary malignancy to peripheral blood circulation, CTCs are progressively filtered when passing through organs like, in the case of cancer of the colon and pancreas, the liver. Hence, the composition of CTCs from the peripheral blood may differ from that of CTCs isolated from anatomical locations physically closer to the primary mass, e.g. the portal vein. Indeed, in colorectal cancer the detection rate of circulating cells in venous blood drawn from more proximal sites (i.e. mesenteric and portal vein) is significantly higher compared with peripheral blood samples.[27–29]

3. Migrating cancer stem cells: therapeutic consequences and perspectives

The presence of a distinct population with stem cell characteristics among disseminated and circulating cancer cells may be of clinical relevance, not only for their putative role in metastasis formation and recurrence, but also for their role in resistance to conventional therapy. CSCs are likely to share many of the properties of normal stem cells including dormancy (quiescence), active DNA repair machinery, the expression of several ABC drug transporters and an intrinsic resistance to apoptosis, which may underlie their capacity to survive therapeutic protocols based on genotoxic agents targeting actively proliferating cells.[30] Alterations in the apoptotic machinery have also been related to chemo-resistance in several tumour types.[31] In thyroid cancer, resistance to therapy-induced cell death results from the elevated expression of anti-apoptotic proteins and has been associated with the autocrine production of interleukin-4 (IL-4).[32] IL-4 was previously shown to induce apoptosis resistance in chronic lymphocytic leukaemia B cells and to enhance the expression of anti-apoptotic proteins in normal and transformed lymphocytes, as well as in prostate, breast and bladder cancer cell lines.[33,34] In pancreatic cancer cells, IL-4 enhances proliferation, and its blockage has significant growth inhibitory effects.[35] More recently, a IL-4 antagonist was shown to strongly enhance the anti-tumour efficacy of conventional chemotherapeutic drugs through selective sensitisation of colon cancer stem cells earmarked by $CD133^+$ expression.[36] Hence, the efficacy of conventional chemotherapy regimens may be significantly enhanced when combined with anti-IL4 adjuvant treatment.[37]

Resistance to radiotherapy has been associated with reactive oxygen species (ROS), critical mediators of radiation-induced cell killing. As stem and early progenitor cells from the different tissues contain lower levels of ROS than their differentiated progeny, Diehn et al.[38] hypothesised that the same feature may be shared by cancer stem cells. Indeed, human and mouse mammary CSCs contain lower ROS levels than more differentiated tumour cells and accumulate less DNA damage upon irradiation. Lower ROS levels in CSCs results from increased expression of free radical scavenging systems. Accordingly, pharmacological depletion of ROS scavengers significantly increases radio-sensitization.[38]

The intrinsic similarities between normal and cancer stem cells pose a challenge for the development of targeted therapies, as most tailor-made treatments will be likely to have cytotoxic effects on normal tissues. During recent years, the progressively increasing availability of cell surface markers to allow prospective CSCs isolation from the bulk of tumour masses has allowed the establishment of CSC-specific expression profiles and comparison with their normal counterparts.[3] However, these cancer stem cell signatures are obtained from enriched (and never pure) CSC fractions, where the frequency of tumor-initiating cells is 1/100 in the best cases. Nevertheless (and somewhat surprisingly), significant differences between bulk tumor cells and CSCs indicate that specific signal transduction pathways are differentially activated in the latter, thus representing attractive therapeutic targets.

In agreement with the central role played by Notch signalling in the control of stem cell homeostasis, γ-secretase inhibitors (GSIs) capable of blocking Notch receptor cleavage and pathway activation have been under clinical evaluation for the treatment of malignancies such as T-cell acute lymphoblastic leukaemia (T-ALL).[39] Likewise, inhibition of Hedgehog (Hh) signalling affects tumour initiation and systemic metastasis in orthotopic xenografts from human pancreatic cancer cell lines. Notably, Hh signalling inhibition had a minimal effect on primary tumour volume, but led to significant reduction of CSCs isolated by aldehyde dehydrogenase expression, previously shown as a stem cell marker.[40] Additional examples of promising clinical applications of CSCs depletion by Hh signalling inhibition come from medulloblastoma[41] and glioblastoma.[42] The PTEN/Akt/mTOR pathways have also been described to confer resistance to conventional

therapies[43] and play a central role in the viability and maintenance of CSCs in leukaemia,[44] breast,[45] prostate[46] and pancreatic cancer. Accordingly, combinatorial therapy aimed at Shh and mTOR signalling inhibition, together with standard chemotherapy, proved to be capable of selectively eliminating CSCs.[47]

Recently, high-throughput screening for selective CSC inhibitors was successfully carried out on EMT-induced breast epithelial cells as an *in vitro* model of mammary CSCs.[48] This screen led to the identification of a compound, salinomycin, that specifically inhibits proliferation of mammary CSCs and significantly reduces their relative proportion by more than 100-fold relative to paclitaxel, the commonly employed chemotherapeutic drug for breast cancer. Salinomycin is a potassium ionophore and, although the precise mechanisms of its action are as yet unclear, it appears to induce terminal epithelial differentiation accompanied by cycle arrest rather than trigger toxicity. Also, *in vivo*, salinomycin administration to mouse models for mammary carcinogenesis inhibits tumour growth and induces increased epithelial differentiation of tumour cells. In addition, global gene expression analyses show that salinomycin treatment results in the loss of expression of breast CSC genes.[48] Although mainly based on a rather artificial model of cancer stemness, this study provides for the first time proof of the feasibility of identifying cancer therapeutic agents with specific toxicity for epithelial CSCs.[48]

As observed for salinomycin, the ability of specific agents to trigger terminal differentiation in cancer cells is an attractive concept in the development of CSC-targeted therapies.[49] Indeed, differentiation-inducing agents, such as retinoids, have been proven to be effective against malignancies like acute promyelocytic leukaemia.[50] The role played by dietary fibres and their breakdown product sodium butyrate in colorectal cancer prevention may also be explained by their effects on CSC differentiation.[51] Notably, butyrate is a histone deacetylase (HDAC) inhibitor and possibly exerts its differentiation-inducing effects by triggering epigenetic changes, i.e. chromatin modifications leading to gene expression reprogramming.[52]

As mentioned above, genes involved in self-renewal and differentiation are often regulated by reversible epigenetic mechanisms. Accordingly, epigenetic modifications, such as CpG island methylation and histone acetylation, potentially underlie the aberrant expression of many of these developmentally regulated genes in cancer.[53] Because of their reversible nature, cancer-specific epigenetic changes represent attractive targets for therapeutic intervention. The expression of genes silenced by CpG island hypermethylation or histone acetylation can be restored by DNA-demethylating agents or HDAC inhibitors. Examples of the former group include 5-aza-CR (azacitidine) and 5-aza-CdR (decitabine), two demethylating drugs now FDA-approved for use in the treatment of myelodysplastic syndromes (MDS), acute myeloid leukaemia (AML) and chronic myeloid leukaemia (CML).[54] Zebularine, a stable DNA cytosine methylation inhibitor, exhibits greater growth inhibition in cancer compared with normal cells and as such holds clinical promise as an anti-cancer therapy.[55,56] Reduced cytotoxic effects were reported for SAHA (suberoylanilide hydroxamic acid), an HDAC inhibitor recently approved for use in the treatment of cutaneous T cell lymphoma.[57] Although at present it is not known whether DNA-demethylating agents and HDAC inhibitors exert their effects on CSCs, the general idea of intervening in the reversible epigenetic alterations that characterise stem-like tumour cells is an appealing and promising one.[49]

An attractive option in exploiting stem cells in the development of novel anti-cancer therapies is the possibility of employing them to deliver and release cytotoxic drugs to the sites of metastasis. Pathotropism, i.e. the ability of progenitor and stem cells to specifically migrate to pathological sites, such as areas of inflammation or neoplastic growths, has mainly been considered for the replacement of damaged tissues in degenerative pathologies. More recently, it was shown that the pathotropism of stem cells is of potential interest as a therapeutic method in patients with disseminated metastatic cancer. In particular, neural stem cells (NSCs) are able to migrate to sites of neoplasia in response to chemotactic signals emanating from cancer cells.[58,59] By engineering pathotropic NSCs to express an anti-cancer pro-drug converting enzyme, Aboody et al. demonstrated successful eradication of whole-body disseminated metastases in a mouse model of neuroblastoma.[60] In this approach, murine NSCs were transduced with the gene encoding for a secreted form of rabbit carboxylesterase (rCE), an enzyme capable of activating the anti-cancer pro-drug CPT-11. Mice bearing disseminated neuroblastoma metastases underwent intravenous administration of the modified NSCs and were systemically treated with CPT-11. The rCE-secreting stem cells administered *in vivo* selectively migrate to disseminated metastases where the enzyme activates the CPT-11 pro-drug, thus increasing anti-tumour activity *in situ*. Long-term monitoring of the treated animals showed complete tumour-free survival compared with control groups. A similar strategy based on a different enzyme/pro-drug combination (cytosine deaminase 1/5-fluorocytosine) was also successfully employed by the same authors in a mouse model of disseminated melanoma metastases in the brain.[61] Notwithstanding the built-in safety modality of this approach (the carrier stem cells will also be selectively killed by the activated pro-drug), the use of stem cells as tools for cancer treatment raises some obvious concerns and safety issues relative to the potential tumorigenicity of the delivery vehicle. Ideally, stem cells employed as delivery vehicles should retain the capacity to differentiate *in vivo* in a predictable and controlled way and, at the same time, maintain replication potential to allow propagation and expansion *in vitro*. In contrast, cell immortalisation for *in vitro* propagation is normally achieved through oncogene transformation, which raises additional concerns about the safety of the delivery vehicle.[62] More recently, mesenchymal stem cells (MSCs) obtained from post-natal bone marrow showed tremendous potential for cell-mediated gene therapy in several disease processes including cancer. The evidence that sites of active tumorigenesis favour the homing of exogenous MSCs has provided support for their development into a genetically engineered tool to track malignant tissues (minimal residual disease) and for the delivery of anti-cancer agents within the tumour microenvironment.[63]

In conclusion, the introduction of the concept of cancer stemness and the potential role of migrating CSCs in tumour dissemination and in the formation of distant metastases

have opened a broad spectrum of novel diagnostic and therapeutic avenues aimed at their detection and selective targeting. Although it is early days to anticipate the successful clinical translation of any of these CSC-targeted strategies, one is tempted to be optimistic for the future in view of the multitude of prognostic and therapeutic options offered by our progressively increasing understanding of stem cell biology in homeostasis and cancer.

Conflict of interest statement

None declared.

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REFERENCES

1. Gupta GP, Massague J. Cancer metastasis: building a framework. *Cell* 2006;**127**(4):679–95.
2. 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):744–9.
3. Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nat Rev Cancer* 2008;**8**(10):755–68.
4. Quintana E, Shackleton M, Sabel MS, et al. Efficient tumour formation by single human melanoma cells. *Nature* 2008;**456**(7222):593–8.
5. Mani SA, Guo W, Liao MJ, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008;**133**(4):704–15.
6. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001;**414**(6859):105–11.
7. Reya T, Clevers H. Wnt signalling in stem cells and cancer. *Nature* 2005;**434**(7035):843–50.
8. Jiang J, Hui CC. Hedgehog signaling in development and cancer. *Dev Cell* 2008;**15**(6):801–12.
9. Koch U, Radtke F. Notch and cancer: a double-edged sword. *Cell Mol Life Sci* 2007;**64**(21):2746–62.
10. Ben-Porath I, Thomson MW, Carey VJ, et al. An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. *Nat Genet* 2008;**40**(5):499–507.
11. Widschwendter M, Fiegl H, Egle D, et al. Epigenetic stem cell signature in cancer. *Nat Genet* 2007;**39**(2):157–8.
12. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. *Nat Rev Cancer* 2002;**2**(6):442–54.
13. Friedl P, Wolf K. Tumour-cell invasion and migration: diversity and escape mechanisms. *Nat Rev Cancer* 2003;**3**(5):362–74.
14. Fodde R, Brabletz T. Wnt/beta-catenin signaling in cancer stemness and malignant behavior. *Curr Opin Cell Biol* 2007;**19**(2):150–8.
15. Wicki A, Lehembre F, Wick N, et al. Tumor invasion in the absence of epithelial-mesenchymal transition: podoplanin-mediated remodeling of the actin cytoskeleton. *Cancer Cell* 2006;**9**(4):261–72.
16. Tsuji T, Ibaragi S, Hu GF. Epithelial-mesenchymal transition and cell cooperativity in metastasis. *Cancer Res* 2009;**69**(18):7135–9.
17. Tsuji T, Ibaragi S, Shima K, et al. Epithelial-mesenchymal transition induced by growth suppressor p12CDK2-AP1 promotes tumor cell local invasion but suppresses distant colony growth. *Cancer Res* 2008;**68**(24):10377–86.
18. Pantel K, Brakenhoff RH, Brandt B. Detection, clinical relevance and specific biological properties of disseminating tumour cells. *Nat Rev Cancer* 2008;**8**(5):329–40.
19. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 2003;**100**(7):3983–8.
20. Balic M, Lin H, Young L, et al. 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):5615–21.
21. Gudjonsson T, Villadsen R, Nielsen HL, et al. Isolation, immortalization, and characterization of a human breast epithelial cell line with stem cell properties. *Genes Dev* 2002;**16**(6):693–706.
22. Alix-Panabieres C, Vendrell JP, Pelle O, et al. Detection and characterization of putative metastatic precursor cells in cancer patients. *Clin Chem* 2007;**53**(3):537–9.
23. Watson MA, Ylagan LR, Trinkaus KM, et al. Isolation and molecular profiling of bone marrow micrometastases identifies TWIST1 as a marker of early tumor relapse in breast cancer patients. *Clin Cancer Res* 2007;**13**(17):5001–9.
24. Munz M, Baeuerle PA, Gires O. The emerging role of EpCAM in cancer and stem cell signaling. *Cancer Res* 2009;**69**(14):5627–9.
25. Gires O, Klein CA, Baeuerle PA. On the abundance of EpCAM on cancer stem cells. *Nat Rev Cancer* 2009;**9**(2):143. author reply 143.
26. Aktas B, Tewes M, Fehm T, et al. 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.
27. Taniguchi T, Makino M, Suzuki K, Kaibara N. Prognostic significance of reverse transcriptase-polymerase chain reaction measurement of carcinoembryonic antigen mRNA levels in tumor drainage blood and peripheral blood of patients with colorectal carcinoma. *Cancer* 2000;**89**(5):970–6.
28. Wind J, Tuynman JB, Tibbe AG, et al. Circulating tumour cells during laparoscopic and open surgery for primary colonic cancer in portal and peripheral blood. *Eur J Surg Oncol* 2009;**35**(9):942–50.
29. Koch M, Weitz J, Kienle P, et al. Comparative analysis of tumor cell dissemination in mesenteric, central, and peripheral venous blood in patients with colorectal cancer. *Arch Surg* 2001;**136**(1):85–9.
30. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. *Nat Rev Cancer* 2005;**5**(4):275–84.
31. Johnstone RW, Ruefli AA, Lowe SW. Apoptosis: a link between cancer genetics and chemotherapy. *Cell* 2002;**108**(2):153–64.
32. Stassi G, Todaro M, Zerilli M, et al. Thyroid cancer resistance to chemotherapeutic drugs via autocrine production of interleukin-4 and interleukin-10. *Cancer res* 2003;**63**(20):6784–90.
33. Dancescu M, Rubio-Trujillo M, Biron G, et al. Interleukin 4 protects chronic lymphocytic leukemic B cells from death by apoptosis and upregulates Bcl-2 expression. *J Exp Med* 1992;**176**(5):1319–26.
34. Conticello C, Pedini F, Zeuner A, et al. IL-4 protects tumor cells from anti-CD95 and chemotherapeutic agents via up-

regulation of antiapoptotic proteins. *J Immunol* 2004;**172**(9):5467–77.
35. Prokopchuk O, Liu Y, Henne-Bruns D, Kornmann M. Interleukin-4 enhances proliferation of human pancreatic cancer cells: evidence for autocrine and paracrine actions. *Br J Cancer* 2005;**92**(5):921–8.
36. Todaro M, Alea MP, Di Stefano AB, et al. Colon cancer stem cells dictate tumor growth and resist cell death by production of interleukin-4. *Cell Stem Cell* 2007;**1**(4):389–402.
37. Rich JN, Bao S. Chemotherapy and cancer stem cells. *Cell Stem Cell* 2007;**1**(4):353–5.
38. Diehn M, Cho RW, Lobo NA, et al. Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature* 2009;**458**(7239):780–3.
39. Tammam J, Ware C, Efferson C, et al. Down-regulation of the Notch pathway mediated by a gamma-secretase inhibitor induces anti-tumour effects in mouse models of T-cell leukaemia. *Br J Pharmacol* 2009;**158**(5):1183–95.
40. Feldmann G, Fendrich V, McGovern K, et al. An orally bioavailable small-molecule inhibitor of Hedgehog signaling inhibits tumor initiation and metastasis in pancreatic cancer. *Mol Cancer Ther* 2008;**7**(9):2725–35.
41. Rudin CM, Hann CL, Laterra J, et al. Treatment of medulloblastoma with hedgehog pathway inhibitor GDC-0449. *N Engl J Med* 2009;**361**(12):1173–8.
42. Bar EE, Chaudhry A, Lin A, et al. Cyclopamine-mediated hedgehog pathway inhibition depletes stem-like cancer cells in glioblastoma. *Stem Cells* 2007;**25**(10):2524–33.
43. Jiang BH, Liu LZ. Role of mTOR in anticancer drug resistance: perspectives for improved drug treatment. *Drug Resist Updat* 2008;**11**(3):63–76.
44. Yilmaz OH, Valdez R, Theisen BK, et al. Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. *Nature* 2006;**441**(7092):475–82.
45. Zhou J, Wulfkuhle J, Zhang H, et al. 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):16158–63.
46. Dubrovska A, Kim S, Salamone RJ, et al. 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):268–73.
47. Mueller MT, Hermann PC, Witthauer J, et al. Combined targeted treatment to eliminate tumorigenic cancer stem cells in human pancreatic cancer. *Gastroenterology* 2009;**137**(3):1102–13.
48. Gupta PB, Onder TT, Jiang G, et al. Identification of selective inhibitors of cancer stem cells by high-throughput screening. *Cell* 2009;**138**(4):645–59.
49. Beug H. Breast cancer stem cells: eradication by differentiation therapy? *Cell* 2009;**138**(4):623–5.
50. Sell S. Stem cell origin of cancer and differentiation therapy. *Crit Rev Oncol Hematol* 2004;**51**(1):1–28.
51. Hague A, Butt AJ, Paraskeva C. The role of butyrate in human colonic epithelial cells: an energy source or inducer of differentiation and apoptosis? *Proc Nutr Soc* 1996;**55**(3):937–43.
52. Spira AI, Carducci MA. Differentiation therapy. *Curr Opin Pharmacol* 2003;**3**(4):338–43.
53. Esteller M. Epigenetics in cancer. *N Engl J Med* 2008;**358**(11):1148–59.
54. Plimack ER, Kantarjian HM, Issa JP. Decitabine and its role in the treatment of hematopoietic malignancies. *Leuk Lymphoma* 2007;**48**(8):1472–81.
55. Cheng JC, Yoo CB, Weisenberger DJ, et al. Preferential response of cancer cells to zebularine. *Cancer cell* 2004;**6**(2):151–8.
56. Marquez VE, Kelley JA, Agbaria R, et al. Zebularine: a unique molecule for an epigenetically based strategy in cancer chemotherapy. *Ann NY Acad Sci* 2005;**1058**:246–54.
57. Duvic M, Vu J. Vorinostat: a new oral histone deacetylase inhibitor approved for cutaneous T-cell lymphoma. *Expert Opin Investig Drugs* 2007;**16**(7):1111–20.
58. Benedetti S, Pirola B, Pollo B, et al. Gene therapy of experimental brain tumors using neural progenitor cells. *Nat Med* 2000;**6**(4):447–50.
59. Aboody KS, Brown A, Rainov NG, et al. Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. *Proc Natl Acad Sci USA* 2000;**97**(23):12846–51.
60. Aboody KS, Bush RA, Garcia E, et al. Development of a tumor-selective approach to treat metastatic cancer. *PLoS One* 2006;**1**:e23.
61. Aboody KS, Najbauer J, Schmidt NO, et al. Targeting of melanoma brain metastases using engineered neural stem/progenitor cells. *Neurooncology* 2006;**8**(2):119–26.
62. Fodde R. Stem cells and metastatic cancer: fatal attraction? *PLoS Med* 2006;**3**(12):e482.
63. Fritz V, Jorgensen C. Mesenchymal stem cells: an emerging tool for cancer targeting and therapy. *Curr Stem Cell Res Ther* 2008;**3**(1):32–42.