Cancer stem cells*

Minireview

A. SOLTYSOVA, V. ALTANEROVA, C. ALTANER**

Cancer Research Institute, e-mail: exonalt@savba.sk, Slovak Academy of Sciences, Bratislava, Slovak Republic

Received March 15, 2005

There is an increasing evidence supporting the cancer stem cell hypothesis. Normal stem cells in the adult organism are responsible for tissue renewal and repair of aged or damaged tissue. A substantial characteristic of stem cells is their ability for self-renewal without loss of proliferation capacity with each cell division. The stem cells are immortal, and rather resistant to action of drugs. They are able to differentiate and form specific types of tissue due to the influence of microenvironmental and some other factors. Stem cells divide asymmetrically producing two daughter cells – one is a new stem cell and the second is progenitor cell, which has the ability for differentiation and proliferation, but not the capability for self-renewal. Cancer stem cells are in many aspects similar to the stem cells. It has been proven that tumor cells are heterogeneous comprising rare tumor initiating cells and abundant non-tumor initiating cells. Tumor initiating cells – cancer stem cells have the ability of self-renewal and proliferation, are resistant to drugs, and express typical markers of stem cells. It is not clear whether cancer stem cells originate from normal stem cells in consequence of genetic and epigenetic changes and/or by redifferentiation from somatic tumor cells to the stem-like cells. Probably both mechanisms are involved in the origin of cancer stem cells. Dysregulation of stem cell self-renewal is a likely requirement for the development of cancer. Isolation and identification of cancer stem cells in human tumors and in tumor cell lines has been successful. To date, the existence of cancer stem cells has been proven in acute and chronic myeloid leukemia, in breast cancer, in brain tumors, in lung cancer and gastrointestinal tumors.

Cancer stem cell model is also consistent with some clinical observations. Although standard chemotherapy kills most cells in a tumor, cancer stem cells remain viable. Despite the small number of such cells, they might be the cause of tumor reccurrence, sometimes many years after the "successful" treatment of primary tumor. Growth of metastases in distinct areas of body and their cellular heterogeneity might be consequence of cancer stem cell differentiation and/or dedifferentiation and asymmetric division of cancer stem cells. Further characterization of cancer stem cells is needed in order to find ways to destroy them, which might contribute significantly to the therapeutic management of malignant tumors.

Key words: stem cells, progenitors, mutation, cancer stem cells, differentiation, redifferentiation, resistance, therapy

Normal stem cells

Stem cells can be divided into three categories: embryonic, germinal, and progenitor somatic stem cells. Embryonic stem (ES) cells are derived from the inner cell mass of the blastocyst. ES cells are omnipotent and are precursors of all cells of the organism. ES cells can be propagated indefinitely in an undifferentiated state *in vitro*. Embryonic stem cells have an indefinite replicative life span, which is attributable to expression of telomerase, like majority of cancer cells.

ES cells differentiate to all cell lineages *in vivo* and can differentiate into many cell types *in vitro*. Embryonic stem cells give rise to cells that form various organs by the process of determination. The omnipotency of the embryonic cells during embryo development is gradually restricted to cells, which are able to differentiate into tissue specific cells with very different function. Although ES cells have been isolated from humans [40], their use in research as well as therapeutics is hampered by ethical considerations [10].

*This work was partially supported by the VEGA grant 2/5077/25, by grant from the League against Cancer of the Slovak Republic, from the state program "Use of Cancer Genomics to Improve the Human Population Health", and from the Centre of excellence of the SAS "Molecular medicine".

**Corresponding author

Normal stem cells also exist for most mature tissues, including haematopoietic, neural [12], gastrointestinal [31], hepatic [2] and mesenchymal tissues [30]. Compared with ES cells, tissue-specific adult stem cells reside in adult tissues, they have less capacity for self-renewal and, although they differentiate into multiple lineages, they are not omnipotent. Progenitor somatic stem cells in adult organisms are responsible for normal tissue renewal and potentially for repair of small damages of tissues. The appropriate differentiation into tissue specialized cells is induced by factors of the local milieu in the body, by cell fusion, and some other so far unknown molecular mechanisms.

Germinal stem cells in the adult are responsible for production of eggs and sperm.

Somatic stem cells are immortal what is necessary to fulfill their biological function over the lifespan of the organism. They have tightly regulated process of self-renewal, and possess high resistance to exogenous and endogenous agents. Various types of somatic stem cells differ in proliferation as well as in the differentiation potential. During early embryonic growth, stem cells divide symmetrically, whereby each daughter cell retains the properties of the parental cell. The adult stem cells divide asymmetrically, whereby one daughter cell retains the properties of the parental stem cell, whereas the other daughter cell begins the process of tissue determination [34]. Mesenchymal progenitor stem cells are the major contributors to normal tissue renewal. Stem cells are present in the bone marrow and are able to differentiate terminally. Bone marrow contains hematopoietic stem cells and other primitive progenitor cells often referred to as a mesenchymal stem cell (MSC) and multipotent adult progenitor cells (MAPCs) [22, 23]. MSC are rare pluripotent cells supporting hematopoietic cell lineages. In addition they can differentiate into the mesenchymal lineage, including bone, cartilage, fat, muscle, tendon and marrow stroma. MAPCs co-purify with MSC and are able to differentiate at the single cell level, not only into mesenchymal cells, but also to the haematopoietic lineage, and to the epithelial cells of liver, lung and gut [15]. Adult stem cells vary in their morphology and characteristics depending on the tissue of origin. In general, they are situated in a spatially distinct site within a tissue, termed a niche that is created by supporting cells and extracellular microenvironment [11].

A major characteristic of stem cells is their ability for self-renewal without loss of proliferation capacity with each cell division. In addition stem cells have the capacity to divide for long periods of time in an environment where most of the cells are quiescent. Recently it was reported that microRNAs expression is required for stem cells to bypass the normal G1/S checkpoint [16]. While the ability of adult human stem cells exist throughout the life of the organism is attributed to telomerase activity, the level of telomerase activity measured in most adult stem cells does not appear to be sufficient to prevent telomere loss. Most of the normal somatic cells do not have an indefinite replicative life span due to progressive shortening of telomeres upon each cell division. Embryonic stem cells maintain their telomere length at 8–12 kb and thus are capable of unlimited self-renewal. In comparison to embryonic stem cells, the ability of some adult stem cells to maintain their telomeres seems to be limited and is not sufficient to prevent their senescence. Consequently adult stem cells are at higher risk of malignant transformation. It was observed that overexpression of telomerase activity in normal human mesenchymal stem cells correlated with the accumulation of mutations over time in culture, was connected with their tumorigenicity, when transplanted into mice [33].

Origin of cancer stem cells

It is generally accepted that transformation of normal cell to tumor cell is a multistep process caused by accumulation of mutations and epigenetic changes. It is very likely that cancer stem cells originate in similar manner by transformation of the normal stem cells in the organism. Cancer stem cells continue to divide asymmetrically creating at least at the beginning two different cell populations. One population retains the self-renewing properties of the parental cancer stem cell and the other population is tumor cell with ability to differentiate but without the ability to initiate tumor growth. This presumption is supported by the observation that not all the cells within a tumor can maintain tumor growth, and that large numbers of tumor cells are needed to transplant a tumor in immunologically competent animals. Moreover, most tumors consist of heterogeneous cell population; they are but not all clonal despite that originate from a single cell. This is consistent with the behavior of stem cells to form various stem cell lineages. Further support for mutated stem cells origin of tumors is observation by pathologists that some tumor cells resemble morphologically the organ of origin, and they retain at least some biological function. Many tumors appear dedifferentiated and may even resemble embryonic cells, for instance neuroblastomas [28].

There are several possibilities to explain the involvement of stem cells in cancer development. It has been hypothesized that some tumors may originate from cancer stem cells, which may be reactivated in specific sites to recreate the original tissue phenotype. Cancer stem cells may also originate from severely genetically damaged somatic cells by redifferentiation to a progenitor-like state thus acquiring self-renewal capacities *de novo*. It is likely that both processes are involved. Additional mutations acquired by cancer stem cell together with its self-renewal capacity might result in malignant tumor development. Origin of cancer stem cell and implementation to tumor therapy is schematically summarized in Figure 1.

Cancer stem cells might be derived from self-renewing normal stem cells due to altered proliferative pathways or from progenitor cells that have acquired the ability to self-renew as a result of oncogenic mutations. In both cases changes

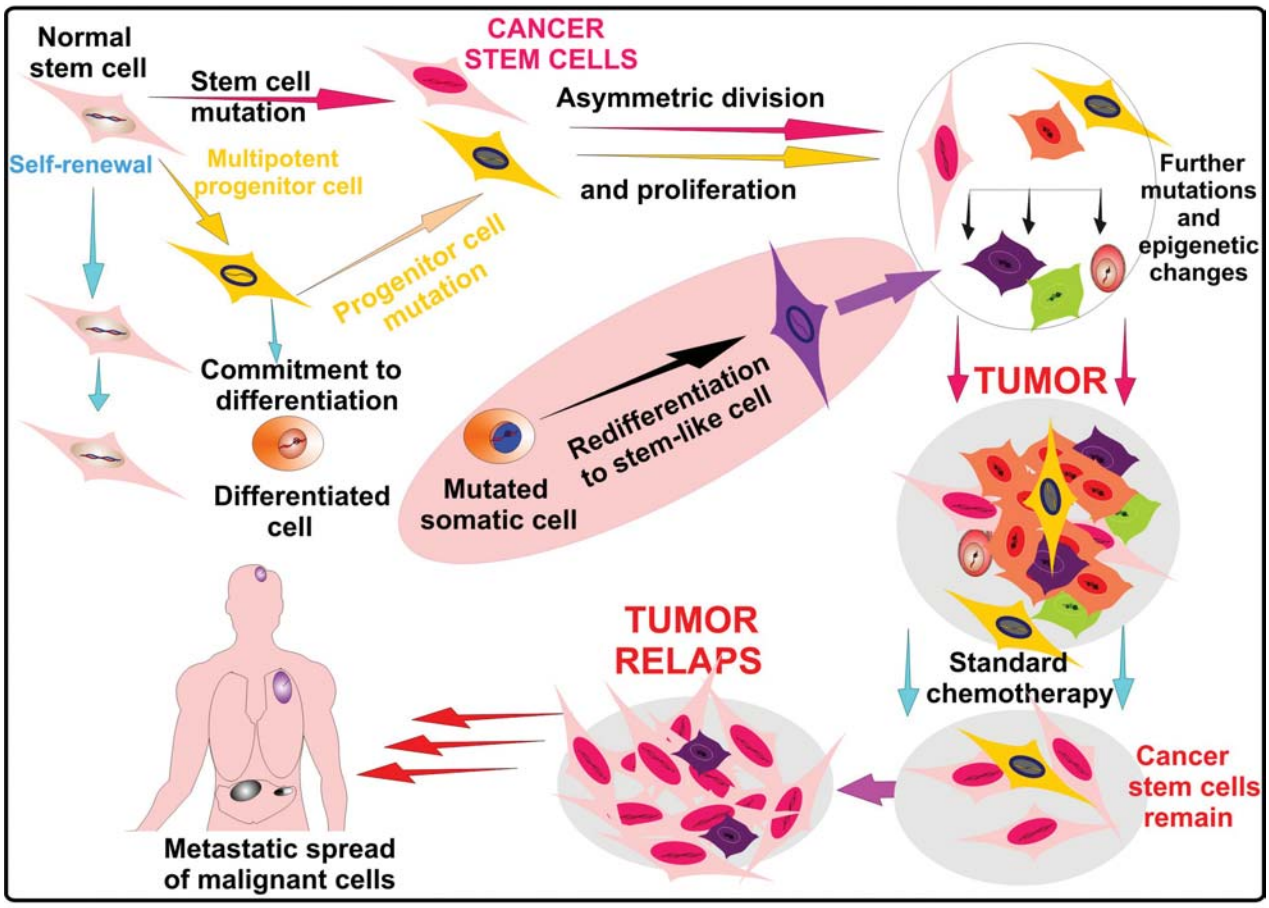

Figure 1. Origin of cancer stem cell and implementation to tumor therapy. Normal stem cell divides asymmetrically producing two daughter cells – one is a new stem cell and the second is progenitor cell, which differentiate and proliferate into mature cells. Cancer stem cells originate from normal stem cell and/or from progenitor cells by mutation and epigenetic changes. Further mutations of these cells lead to formation of heterogeneous tumor containing different clones of tumor cells. When this tumor is treated by chemotherapy, the most cells are killed, but cancer stem cells remain because of their resistance. These stem cells can initiate malignant tumor growth sometimes after long period of time. They can be source of metastatic spread of tumor cells in different organs of the body. Cancer stem cell can originate from somatic cell, which by mutation regains stem-like properties, mainly the capacity of renewal and propagation.

in regulation of self-renewal in stem cells is a key event in tumorigenesis. It was found that the Bmi-1, Notch, Wnt and Sonic hedgehog pathways, tumor suppressor genes and oncogenes are involved in regulation of self-renewal of both normal and cancer stem cells. [4, 5, 36, 38, 42]. Additional studies implicate the Wnt beta-catenin pathway in the maintenance of stem-cell self-renewal in other tissues as well [3, 21]. Epigenetic events play an important role, such as modification of chromatin, histone deacetylation, etc. Several studies suggest that epigenetic reprogramming is responsible for the loss of the tumor cells' capacity to form tumors [27, 37]. The stem cell origin will dictate the tumor type, with contributions by the genetic background of the individual and microenvironmental influences.

The cancer stem cell model has been supported by demonstration that cancer stem cells can be isolated and have been characterized in some details. The hematopoetic cancer stem cells were described first. BONNET et al [7] identified a common immunophenotype of leukemic stem cells with self-renewal potential. Cells of each type of AML have been isolated and divided into two subtypes with phenotypes $CD34^+$, $CD38^+$ and $CD34^+$, $CD38^-$. These two types of cells were injected to NOD/SCID mouse. A fraction of $CD34^+$, $CD38^-$ cells transferred human AML to mouse. It was concluded that the neoplastic cells in leukemia are composed of several clonal populations, which are heterogeneous with respect to proliferation and differentiation [for a review see 32]. Rare stem cells within the leukemic population possess extensive proliferation and self-renewal capacity that is not found in the majority of the leukemic cells. It was determined that leukemic stem cells are necessary and sufficient to maintain the leukemia [26].

It was reported that acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity [19]. They were able to demonstrate heterogeneity in leukemia-initiating cells of leukemia induced on SCID mice. The data suggest that in AML, the initial target cell is within the hematopoietic stem cell compartment. The leukemic stem cells emerge with subsequent alterations of progenitor cells. In chronic myeloid leukemia hematopoietic stem cells may not be involved in disease development. The origin of leukemia cells is rather from a progenitor cell that re-acquired self-renewal capacities.

Additional modifications necessary for full transformation was proven in experiments on transgenic mice that utilized expression of the *bcr/abl* fusion gene targeted exclusively to myeloid progenitors and their myelomonocytic progeny. These mice developed a myeloid disease sharing many characteristics with human CML, despite absence of *bcr/abl* expression in hematopoietic stem cells. When these mice were crossed with *bcl-2* transgenic mice, blast crisis was induced in these animals. These results are in agreement with the clinical finding that the majority of myeloid blast crisis patients have leukemic cells with high expression of bcl-2 gene [20].

A study by GUAN et al [14] demonstrated that most AML stem cells are quiescent. This finding means that these cells will survive standard chemotherapy directed to dividing cells. Isolation of highly quiescent leukemic stem cells in chronic myeloid leukemia was reported as well [18]. Recently it was found that treatment of CML patients with imatinib mesylate (Gleevec), which is otherwise highly effective in induction of remission, only suppressed the disease. The drug is not able to destroy the leukemic stem cells, which are the root cause of the CML [6, 13]. These observations strongly support the stem cell origin of acute and chronic myeloid leukemia.

There are several examples of cancer stem cell identification in solid tumors. AL-HAJJ et al [1] transplanted human patients' breast cancer cells into mice and identified a subpopulation ($CD44^{+}$, $CD24^{-/low}$) that was uniquely capable of generating heterogeneous primary and secondary tumors similar to the original patient specimens. These results suggested that the tumorigenic cells can both initiate self-renew and growth as tumor cells and form also non-tumorigenic cancer cells. Several other papers that promulgate the existence of breast cancer stem cells analyzed the origin of tumor-initiating cells and examined the clinical relevance of cancer stem cells in bone marrow of patients with metastatic breast cancer [8, 9, 29].

Another example of existence of cancer stem cells in solid tumors came from study of pediatric brain tumors. HEMMATI et al. [17] was able to isolate tumorigenic cells with characteristics similar to neural stem cells. These cells were shown to be multipotent, self-renewing and are able to produce proliferating neurospheres, which retain the ability to differentiate into neurons and glia. The gene expression of these tumor-derived neurospheres did not differ from normal neurospheres. However, unlike neural stem cell-derived neurospheres, these cells were longer alive and gave rise to abnormal dual-phenotype cells.

SINGH et al [35] reported the identification and purification of cancer stem cells from human brain tumors of different phenotypes that possess a marked capacity for proliferation, self-renewal, and differentiation. The increased self-renewal capacity of the brain tumor stem cell was highest from the most aggressive surgical samples of medulloblastoma compared with low-grade gliomas. The brain tumor stem cells were exclusively isolated with the cell fraction expressing the neural stem cell surface marker CD133. These CD133+ cells could differentiate in tissue culture into tumor cells that phenotypically resembled the tumor from the patient.

Identification of bronchioalveolar stem cells in normal human lung and lung tumor has also been reported. The data support the hypothesis that these cells are a stem population, which after transformation give rise to lung adenocarcinoma [24].It was reported that in canine bronchial carcinogenesis alveolar pluripotential stem cells are involved [39].

Cancer stem cells are also able to keep their self-renewal potential during many passages of *in vitro* cultivation. KONDO et al [25] reported presence of side cell populations (SP) in the following cell lines, which are known to be kept in culture for decades: C6 (rat glioma), BI04 (rat neuroblastoma), MCF-7 (human breast cancer), and HeLa (human adenocarcinoma). They confirm an asymmetric way of cell replication of the C6 cell line. They found that sorted SP cells could produce both new SP and non-SP cells in culture. The cells can produce neurons and glia in culture and form tumors containing both glia and neurons in nude mice. Supportive environments were found to be important for *in vitro* maintenance of SP cells; they required presence of platelet-derived factor (PDGF) and basic fibroblast growth factors (bFGF) for their cultivation in absence of serum. These results support the conclusion that SP cells in C6 cell line behave as cancer stem cells.

Implications for tumor therapy

Isolation and identification of cancer stem cells in leukemia and in some solid tumors has important implications for the study and treatment of malignancies. The bulk human tumor mass is composed of different tumor cell clones that differ with respect to proliferation, differentiation, and ability to initiate tumor. In human leukemia, rare leukemic stem cells are responsible for maintaining the tumor clone, generating leukemic cells that possess extensive proliferative and self-renewal potential. In a wide variety of stem cells, the ABC transporters are expressed causing drug resistance [43]. Cytotoxic chemotherapy kills most cells in a tumor, but cancer stem cells survive due to their relative high resistance to drugs and because of their silent replication. Despite the small number of such cells, their property of being immortal is expected to be sufficient to allow tumor reccurrence. The

relapse can occur many years after initial treatment by chemotherapy or radiotherapy. It is hypothesized that if tumors are derived from an early stem cell or its progenitor cells, the metastases are formed readily and are phenotypically more heterogeneous. Metastases derived from a later stem cell are more homogenous and have more restricted metastatic potential [41]. Cellular heterogeneity of metastases, their growth in distinct areas of body under different environment might be consequence of cancer stem cell differentiation and/or dedifferentiation. Further characterization of cancer stem cells is needed in order to induce their terminal differentiation, which might be a therapeutic approach. Therapies whose target is expressed on or by the cancer stem cells initiating tumors are more likely to be successful. The efforts to elucidate molecular differences in normal and cancer stem cells are expected to produce promising approach to targeted causal therapy of malignant tumors.

We would like to thank Dr. K. BORIS-LAWRIE for comments and language corrections.

References

[1] 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: 3983–3988.

[2] ALISON M, SARRAF C. Hepatic Stem Cell*s*. J Hepatol 1998; 29: 676–682.

[3] ANDL T, REDDY ST, GADDAPARA T, MILLAR SE. WNT signals are required for the initiation of hair follicle development. Dev Cell 2002; 2: 643–653.

[4] AUSTIN TW, SOLAR GP, ZIEGLER FC, LIEM L, MATTHEWS W. A role for the Wnt gene family in hematopoiesis: expansion of multilineage progenitor cells. Blood 1997; 89: 3624–3635.

[5] BHARDWAJ G, MURDOCH B, WU D, BAKER DP, WILLIAMS KP et al. Sonic hedgehog induces the proliferation of primitive human hematopoietic cells via BMP regulation. Nat Immunol 2001; 2: 172–180.

[6] BHATIA R, HOLTZ M, NIU N, GRAY R, SNYDER DS et al. Persistence of malignant hematopoietic progenitors in chronic myelogenous leukemia patients in complete cytogenetic remission following imatinib mesylate treatment. Blood 2003; 101: 4701–4707.

[7] BONNET D, DICK JE. Human acute myeloid leukemia is organizedas a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997; 3: 730–737.

[8] DEMIRKAZIK A, KESSINGER A, LYNCH J, REED E, TARANTOLO S, SHARP JG. Effect of prior therapy and bone marrow metastases on progenitor cell content of blood stem cell harvests in breast cancer patients. Biol Blood Marrow Transplant 2002; 8: 268–272.

[9] DICK JE. Breast cancer stem cells revealed. Proc Natl Acad Sci USA 2003; 100: 3547–3549.

[10] FRANKEL MS. In search of stem cell policy. Science 2000; 287: 1397.

[11] FUCHS E., TUMBAR T, GUASCH G. Socializing with the neighbors: Stem cells and their niche. Cell 2004; 116: 769–778.

[12] GAGE FH. Mammalian neural stem cells. Science 2000; 287: 1433–1438.

[13] GRAHAM SM, JORGENSEN HG, ALLAN E, PEARSON C, ALCORN MJ et al. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. Blood 2002; 99: 319–325.

[14] GUAN Y, GERHARD B, HOGGE DE. Detection, isolation, and stimulation of quiescent primitive leukemic progenitor cells from patients with acute myeloid leukemia (AML). Blood 2003; 101: 3142–3149.

[15] GUDJONSSON T, VILLADSEN R, NIELSEN HL, RONNOV-JESSEN L, BISSEL MJ, PETERSEN OW. Isolation, immortalization, and characterization of a human breast epithelial cell line with stem cell properties. Genes Dev 2002; 16: 693–706.

[16] HATFIELD SD, SHCHERBATA HR, FISCHER KA, NAKAHARA K, CARTHEW RW, RUOHOLA-BAKER H. Stem cell division is regulated by the microRNA pathway. Nature 2005; 435: 974–978.

[17] HEMMATI HD, NAKANO I, LAZAREFF JA, MASTERMAN-SMITH M, GESCHWIND DH et al. Cancerous stem cells can arise from pediatric brain tumors. Proc Natl Acad Sci USA 2003; 100: 15178–15183.

[18] HOLYOAKE T, JIANG X, EAVES C, EAVES A. Isolation of a highlyquiescent subpopulation of primitive leukemic cells in chronicmyeloid leukemia. Blood 1999; 94: 2056–2064.

[19] 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: 738–743.

[20] JAISWAL S, TRAVER D, MIYAMOTO T, AKASHI K, LAGASSE E, WEISSMAN IL. Expression of BCR/ABL and BCL-2 in myeloid progenitors leads to myeloid leukemias. Proc Natl Acad Sci USA 2003; 100: 10002–10007.

[21] JAMORA C, DASGUPTA R, KOCIENIEWSKI P, FUCHS E. Links between signal transduction, transcription and adhesion in epithelial bud development. Nature 2003; 422: 317–322.

[22] JIANG Y, HENDERSON D, BLACKSTAD M, CHEN A, MILLER RF, VERFAILLE CM. Neuroectodermal differentiation from mouse multipotent adult progenitor cells. Proc Natl Acad Sci USA 2003; 100: 11854–11860.

[23] JIANG Y, JAHAGIRDAR BN, REINHARDT RL, SCHWARTZ RE, KEENE CD, ORTIZ-GONZALEZ XR et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002; 418: 41–49.

[24] KIM CF, JACKSON EL, WOOLFENDEN AE, LAWRENCE S, BABAR I et al. Identification of Bronchioalveolar Stem Cells in Normal Lung and Lung Cancer. Cell 2005; 121: 823–835.

[25] KONDO T, SETOGUCHI T, TAGA T. Persistence of a small subpopulation of cancer stem-like cells in the C6 glioma cell line. Proc Natl Acad Sci USA 2004; 101: 781–786.

[26] LAPIDOT T, SIRARD C, VORMOOR J, MURDOCH B, HOANG T et al. A cell initiating human acute myeloid leukaemiaafter transplantation into SCID mice. Nature 1994; 367: 645–648.

[27] LI L, CONNELLY MC, WETMORE C, CURRAN T, MORGAN JI. Mouse embryos cloned from brain tumors. Cancer Res 2003; 63: 2733–2736.

[28] NAKAGAWARA A, OHIRA M. Comprehensive genomics link-

ing between neural development and cancer: neuroblastoma as a model. Cancer Lett 2004; 204: 213–224.

[29] PECORA AL, LAZARUS HM, JENNIS AA, PRETI RA, GOLDBERG SL et al. Breast cancer cell contamination of blood stem cell products in patients with metastatic breast cancer: predictors and clinical relevance. Biol Blood Marrow Transplant 2002; 8: 536–543.

[30] PITTENGER MF, MACKAY AM, BECK SC, JAISWAL RK, DOUGLAS R et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284: 143–147.

[31] POTTEN CS. Stem cells in gastrointestinal epithelium: numbers, characteristics and death. Philos Trans R Soc Lond B Biol Sci 1998; 353**:** 821–830.

[32] REYA T, MORRISON SJ, CLARKE MF, WEISSMAN IL. Stem cells, cancer, and cancer stem cells. Nature 2001; 414: 105–111.

[33] SERAKINCI N, GULDBERG P, BURNS JS, ABDALLAH B, SCHRODDER H et al. Adult human mesenchymal stem cell as a target for neoplastic transformation. Oncogene 2004; 23: 5095–5098.

[34] SHERLEY JL. Asymmetric cell kinetics genes: the key to expansion of adult stem cells in culture. Stem cells 2002; 20: 561–572.

[35] SINGH SK, CLARKE ID, TERASAKI M, BONN VE, HAWKINS C et al. Identification of a cancer stem cell in human brain tumors. Cancer Res 2003; 63: 5821–5828.

[36] SPINK KE, POLAKIS P, WEIS WI. Structural basis of the Axin-adenomatous polyposis coli interaction. EMBO J 2000; 19: 2270–2279.

[37] SURANI MA. Reprogramming of genome function through epigenetic inheritance. Nature 2001; 414: 122–128.

[38] TAIPALE J, BEACHY PA. The hedgehog and Wnt signaling pathways in cancer. Nature 2001; 411: 349–354.

[39] TEN HAVE-OPBROEK AA, BENFIELD JR, HAMMOND WG, DIJKMAN JH. Alveolar stem cells in canine bronchial carcinogenesis. Cancer Lett 1996; 101: 211–217.

[40] THOMSON JA, ITSKOVITZ-ELDOR J, SHAPIRO SS, WAKNITZ MA, SWIERGIEL JJ et al. Embryonic stem cell lines derived from human blastocysts. Science 1998; 282: 1145–1147.

[41] TU SM, LIN SH, LOGOTHETIS CJ. Stem-cell origin of metastasis and heterogeneity of solid tumours. Lancet Oncol 2002; 3: 508–513.

[42] WILLERT K, BROWN JD, DANENBERG E, DUNCAN AW, WEISSMAN IL et al. Wnt proteins are lipid-modified and can act as stem cell growth factors. Nature 2003; 423: 448–452.

[43] ZHOU S, SCHUETZ JD, BUNTING KD, COLAPIETRO AM, SAMPATH J et al. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. Nat Med 2001; 7: 1028–1034.