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Stem cells within established cancer cell lines: an impact on *in vitro* experiments

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Abstract

Cancer stem cells (CSCs) are described as cells within a tumor that are able to indefinite self-renewal, form tumors when transplanted *in vivo*, differentiate into multiple lineages, and express genes such as *OCT3/4, SOX2, KLF4, NANOG*. Although these traits of CSCs are commonly accepted, there is still a lot of controversy regarding these cells. There are very few methods which allow to obtain these cells, like separation based on surface markers, presence of side population, sphere forming assays, and aldefluor assays. This paper seeks the confirmation of CSCs presence in cancer cell lines such as: breast, prostate, pancreatic, liver, brain, and cervical. Nowadays, researchers use two models of cell culture: established cancer cell lines (ECCLs) and primary cell culture. A major problem with these models is that tumors in organism evolve and cell cultures represent only small fragment of tumor development. Since CSCs were found, there exist high hopes of revealing new therapies targeting CSCs. However, the appearance of new populations with the ability to induce tumors should pour a bucket of water to create a cure for cancer.

Introduction

The first studies concerning the connection between stem cells and neoplasms were presented in the second half of the 19th century by Virchow and Cohnheim. Virchow's conclusions were based on similarities between teratocarcinomas and embryonic tissue.[1] Cohenheim's hypothesis was that carcinoma is stem cell disorder which came from his interpretation of karyotypic chromosomal differences between epithelial and mesenchymal tumors.[2] Next, in 1937, Furth and Kahn found evidence of existence of tumor stem cells by showing that leukemia could be transferred by a single cell in a mouse model. In the 1970's it was discovered that only a small population (0.0001-1%) of tumor cells could induce a tumor *in vivo*.[3] The turning point in the study of cancer stem cells (CSCs) was Bonnet and Dick experiment in which they proved that cells isolation based on CD34 and CD38 markers allows to obtain cells which differentiate and proliferate, showing the potential for self-renewal expected from the leukemic stem cell.[4] From that moment CSCs were found in many tumors such as: prostate, skin, colon, brain.[5-8] Several of created theories, suggest that CSCs can evade current therapies and lead to recurrence of disease.[9] In order to develop effective cancer therapy, it is essential to characterize CSCs and find ways to kill them.[10] It seems obvious that new therapies should focus on targeting and killing these cells. Human cancer cell lines have been used to test the functions of oncogenes and tumor suppressor genes for many decades now. In fact, most potential anti-cancer therapies are first tested in established cell lines. However, recent researches question is the usefulness of established cell lines for studying cancer stem cells.[11] Lately tumor initiating cells (TIC) were found in many established cancer cell lines.[12-14] Nevertheless, there is still a lot of controversy regarding CSCs, *e.g.* Do stem cells really exist within established cancer cell lines? How big is a population of stem cells within cell line? Which methods of isolation are the best? What properties of normal stem cells they possess and what is the stemness of cancer stem cells?

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Cancer stem cells hypothesis

Cancer stem cells (CSCs) do not refer to the stem cell of cancer origin, they are rather defined as cancer cells capable to initiate a tumor that replicates the original tumor when transplanted.[11] However, it is still unclear how CSCs arise, although there are two controversial theories. The first is that adult stem cells in various tissues could be transformed into malignancies through multiple steps resembling carcinogenesis, during which a vast number of genes are involved. The second one is that dedifferentiation of transformed malignant cells results in the production of cancer stem cells. In fact, both hypothesis have been challenged to be firmly supported by empirical evidences. Recent reports demonstrated that the pluripotent stem cells could be generated from somatic cells by defined factors, including OCT4, SOX2, C-MYC, KLF4, NANOG, and LIN28, and that the cells with epithelial-mesenchymal transition also have the characteristics of stem cells.[15-17] But it is possible that both mechanisms described above are true and the method of CSCs development depends on the type of cancer.[15] In literature CSCs are described as cells within a tumor that are able to indefinite self-renewal, form tumors when transplanted *in vivo*, differentiate into multiple lineages, and express genes such as *OCT3/4, SOX2,* *KLF4, NANOG*.[16,17] A peculiar capacity of many normal stem cell populations is their relatively high expression of ATP-binding drug transporters (ABC) which can protect the normal stem cells from cytotoxic agents. Such a property of CSCs models would explain the persistence of resistant tumor cell populations after chemotherapy. Although these traits of CSCs are commonly accepted, there is still a lot of controversy over the molecular markers. It is well known that on the CSCs surface, markers characteristic for normal stem cells (CD133, CD44) are present. But it has been proved that cells without these specific markers (*e.g.* CD133) can also induce tumors in immunodeficient mice.[18] Such information should change thinking about CSCs as homogenous cells population which can be easily targeted in human body or within cell line. Since CSCs were found, there exist high hopes of revealing new therapies based on killing these cells. However, the appearance of new populations with the ability to induce tumors should pour a bucket of water to create a cure for cancer.

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Isolation of cancer stem cells

Since CSCs were found in Acute Myeloid Leukemia (AML) by Bonnet and Dick, many investigators tried to improve the existing method of isolation. An enormous amount of effort was put into this procedure because the isolation of cells is a crucial step in every project and there are very few methods which allow obtaining these cells, like using surface markers, side population, sphere forming assays, and aldefluor assays.

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Surface markers

Bonnet *et al*., proved that it is possible to isolate population of cells which can induce tumor by using CD44 and CD38 surface markers.[4] Other studies have also shown that this method is useful in the isolation of stem cells from cancer cell lines (Table 1).[19,20] Even if surface markers are a serviceable tool, it is necessary to remember about the disadvantages of this method. It is unclear, however, whether those expression patterns can be used for the isolation of pure cultures or just to enrich tumor stem cells. Many of the surface proteins used, are not specific, meaning that they are also expressed on normal stem cells and tumor-initiating cells of other tumor types. Additionally, identification using antibodies can lead to false-positive results due to non-specific cross-reactivities.[3]

Side population

The side population method is widely used now. The principles of this technique are based on the ability of stem cells and cancer stem cells to exclude vital dyes such as Hoechst 33342 or Rhodamine 123. These cells are capable of expressing transmembrane transporters, such as the ATP-binding cassette protein, ABC transporter ABCG2/BCRP1 (breast cancer resistance protein 1).[36] Moreover, the ABC transporters contribute to drug resistance in cancers (Table 2).[37] A number of research groups have found that some established cancer cell lines, which have been maintained in cultures for decades are able to self-renewal in culture, are resistant to anticancer drugs including Mitoxantrone, and can form tumors when transplanted *in vivo*.[10] There are two major problems concerning it. Firstly, toxicity: since it has been demonstrated that Hoechst interferes with C2C12 cells, as long as the dye is present in the nucleus, more and more researchers have found that Hoechst staining can affect cell differentiation. Secondly, instrumentation: Hoechst isolation requires the use of more specialized flow cytometer, in which the ultra-violet (UV) laser device must be installed for fluorescence activation. Thus, it is very expensive to own and operate.[16] It has to be remembered, that this protocol is not a promising tool, because the non-SP (side population) cells are also able to change in SP cells.[38]

Sphere forming assay

Another method for CSCs isolation is their capability to grow in serum-free medium which contains proper mitogens (bFGF and EGF). Although many protocols use sphere formation methods to concentrate CSCs in a culture, it is of interest to investigate exactly why CSCs as well as normal stem cells are enriched in the spheres.[10,12]

Aldefluor assay

Aldefluor assay is a new and promising method for both cancer stem cells (CSCs) and normal stem cells (SCs) isolation. This technique uses Aldehyde Dehydrogenase – 1 (ALDH1) as a marker. ALDH1 is a detoxifying enzyme that oxidizes intracellular aldehydes and thereby confers resistance to alkylating agents. In fact, the detoxification capacity of ALDH1, by protecting SCs against oxidative insult, might underlie the well-recognized longevity of stem cells. ALDH1 also converts retinol into retinoic acid, a modulator of cell proliferation, which may also modulate stem cells proliferation.[49] Recent studies showed that this method is valid for neuronal stem cells, hematopoietic stem cells and progenitor cells isolation.[50,51] It was also reported that ALDH1 activity was used to isolate cancer stem cells from head and neck squamous cancer, colon cancer, and breast cancer.[49,52,53] Cells isolated from mice brain by this technique showed signs of capability to self-renewal and the ability to generate neurospheres and neuroepithelial stem-like cells.[54] Although results were promising, it is necessary to remember about certain limitations. For example, the stem cells population identified by using the aldefluor assay is probably heterogeneous, and needs to be dissected using additional markers such as CD44 and CD133.[36]

Stem cells in breast cancer cell lines

The first breast cancer stem cells were found in solid tumor by Al Hajji *et al*., who used CD44$^+$ and CD24$^-$ phenotype and showed that hundred of these cells could form tumors in

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Table 1. Cancer stem cell markers.

Tumor	Stem cell marker
Acute myeloid leukemia	CD34$^+$/CD38$^-$/CD45$^-$low[21] CD32$^+$/CD35$^+$[22] CD44$^+$[23]
Acute lymphoblastic leukemia	CD90$^+$/CD110$^+$[24] CD9$^+$[25] CD133$^+$/CD19$^-$/CD38$^-$[26]
Breast cancer	CD44$^+$/CD24$^-$[27], ALDH$^+$[28]
Brain cancer	CD133$^+$[29]
Prostate cancer	CD133$^+$/CD44$^+$[30]
Colon cancer	CD133$^+$[31] CD44$^+$/ALDH$^+$[32]
Liver cancer	CD133$^+$/ALDH$^+$[33]
Pancreatic cancer	CD133$^+$[34]
Head and neck cancer	CD44$^+$/CD17$^+$[35]

Table 2. The most important multidrug resistance protein belonging to ABC family.

Gene	Protein	Drug transported by protein	Type of cancer where ABC pomp is present
ABCA2	ABCA2	Estramustine	Small cell lung cancer[39]
ABCB1	PGP/MDR	Doxorubicin, etoposide, vinblastine, paclitaxel	Breast cancer,[40] ovarian cancer[41]
ABCC1	MRP1	Doxorubicin, daunorubicin, vincristine, etoposide, camptothecince, methotrexate	Neuroblastoma[42]
ABCC2	MRP2	Vinblastine, cisplatine, doxorubicin, methotrexate	Non-small lung cancer[43]
ABCC3	MRP3	Methotrexate, etoposide	Lung cancer[44]
ABCC4	MRP4	6-mercaptopurine, 6-thioguanine, methotrexate and its metabolites	Breast cancer[45]
ABCC5	MRP5	6-mercaptopurine, 6-thioguanine, methotrexate and its metabolites	Non-small lung cancer[46]
ABCC11	MRP8	5-fluorouracil	Breast cancer[47]
ABCG2	MXR/BCRP	Mitoxantrone, topotecan, doxorubicin, daunorubicin, irinotecan, methotrexate, imatirub	Colorectal cancer[48]

mice, whereas tens of thousands of cells with alternate phenotypes failed to form tumors.[55] Although the analysis of clinical samples is traditionally thought to provide more relevant data, it turned out that cell lines adapt to *in vitro* culture and can potentially no longer resemble their primary counterparts. However, it has been demonstrated that, despite the ability to grow *in vitro*, breast cancer cell lines possess similar molecular and genetic signatures to the tumors from which they have been derived.[56] Recent studies showed that $CD44^+/CD24^-$ phenotype does not allow to distinct tumorigenic cells from non-tumorigenic cells. The SUM149 line which has only 5% of $CD44^+/CD24^-$, exhibits a similar tumorigenic potential as SUM159 and SUM1319 lines which have more than 90% of $CD44^+/CD24^-$. So, it seems that there is a smaller connection between $CD44^+/CD24^-$ phenotype and CSCs in breast cancer cell lines than it used to be thought. That is why additional marker should be used. Fillmore and Kuperwasser, proposed epithelial surface antigen (ESA) and they used flow cytometry to detect $CD44^+/CD24/ESA^+$ cells. The percentage of these cells ranges from 0.01-0.5% in luminal lines, such as MCF7 and SUM225, to about 2.5% in the basal and mixed cell lines SUM149, SUM159, SUM1315, and MDA.MB.231. Most importantly, these cells exhibit properties of self-renewal *in vitro*, tumors formation from very few cells, slow divisions, and selective resistance to chemotherapy.[57] A similar experiment was made by Charafe-Jauffret *et al*., who tried to isolate CSCs from thirty three breast cancer cell lines by using the aldefluor assay. They managed to isolate CSCs from twenty three out of thirty three cell lines. The aldefluor-positive population (Table 3),[58] ranged from 0.2% to nearly 100%. Moreover these cells were able to generate tumors, self-renewal, and recapitulate the phenotypic heterogenity of the initial tumor when injected to NOD/SCID mice.[58] Apart from results described above there are findings which suggest that the use of additional markers such as ESA and ALDH1 may not be sufficient to identify all cancer stem cell populations in breast cancer cell lines and that there is a need for a further study of this issue.[11]

Table 3. The percentage of aldefluor-positive cells found in each breast cell line.

Results	Breast cancer cell line
ALDEFLUOR-negative	T-47D, SUM185, ZR-75-30, MDA-MB-134, SUM190, SUM44, ZR-75-B,
ALDEFLUOR-positive 0-1%	MCF7, SUM225, MDA-MB-231, Hs578T, MCF10A, BT-20
ALDEFLUOR-positive 1-5%	SUM229, S68, MDA-MB-453, ZR-75-1, HCC1806, MDA-MB-436, MDA-MB-147, HCC1937, HCC1954, BrCa-MZ-01
ALDEFLUOR-positive 5-99%	SUM149, SUM159, 184A1, SK-BR-7, HME1,
ALDEFLUOR-positive 100%	SK-BR-3, HCC38

Stem cells in prostate cancer cell lines

Prostate cancer is the most common in the western world. The early detection of it, can be cured by surgery and radiation therapy. In advanced stages androgen ablation therapy is used, but in most cases patients die because of metastases. The presence of stem cells in prostate cancer cell lines would confirm their usefulness as a model during the development of new anticancer therapies.[59] Patrawala *et al*., studied the tumorigenicity of $ABCG2^+$ (an ATP-binding cassette transporter associated with multidrug resistance) cells. Out of six prostate cancer cell lines (LNCaP, LAPC9, Du145, PC3, PPC-1) only LAPC9 contained a small population of $ABCG2^+$ cells. A hundred of these cells were able to induce tumors in NOD/SCID mice when thousands of LAPC9 $ABCG2^-$ cells could not do so. Furthermore, $ABCG2^+$ cells possess properties such as self-renewal, expression of stemness genes, and an ability to give rise to non-side population cells. The fact that the side populations can be heterogeneous and the higher tumorigenicity of $ABCG2^+$ cells may result from combined effects of several other populations should be taken under consideration.[60] CD133 and CD44 markers were useful during CSCs isolation from tumor samples and the CD133 surface marker was used by Pfeiffer and Shalken to identify CSCs in prostate cancer cell lines (Du145, 22Rv1, LAPC-4, DuCaP, LNCaP and PC-3).[61] In five cell lines there was no detectable $CD133^+$ population, only Du145 cell line had a small subset of these cells. Surprisingly, there was no difference in colony-forming assay between $CD133^+$ and $CD133^-$ populations. It was assumed, that CD133 is not a good marker for CSCs in prostate cancer cell lines and a similar suggestion was proposed by Bisson and Prowse, who found out that CD133 selection by fluorescence-activated cell sorting (FACS) failed to enrich C4-2B cells with sphere forming ability.[62,63] However, it is not clear whether CD133 is a marker or not, because Wei *et al.* isolated the $CD44^+$ integrin$\alpha 2\beta 1^+$ cells to enrich $CD133^+$ cells from the Du145 cell line which had self-renewal capacity, formed sphere-like clones similar to brain cancer stem cells.[64] Regardless of results obtained from the CD133 molecule, investigators managed to isolate CSCs from prostate cancer cell lines by a sphere-formation assay. PC-3, Du145, LNCaP, VCap, 22RV1, LAPC-4, DuCaP cell lines contained small subset of self-renewing cells with abilities to form spheres, characteristic for stem cell growth. Furthermore, these cells express the putative stem cell markers α2-integrin and BCRP (breast cancer resistance protein). All these results showed that prostate cancer cell lines have populations of CSCs but it is necessary to reveal new markers for the CSCs isolation from these cell lines.

Stem cells in pancreatic cancer cell lines

Three pancreatic cell lines PANC-1, PSN-1, and CFPAC-1 were capable to form spheres in cancer stem cells medium. In order to further characterization, the expression of CD24, CD44, ESA, and CD133 markers were examined. However, this phenotype was not sufficient to distinguish the sphere-capable from the sphere-incapable cells and an additional marker needs to be used. Adding CD44v6, a protein marker of metastasis in pancreatic cancer, allowed to separate the *sphere-capable* from the *sphere-incapable* cells. PANC-1 cells expressed higher levels of CD24, CD44, ESA, CD133 and CD44v6 markers than cells cultured in the adherent standard cell conditions (RPMI + 10% FBS). Moreover, cells isolated from pancreatic cancer cell lines cultured in cancer stem cells medium demonstrated a self-renewal capability and multipotentiality, which strongly suggest, that these cancer cell lines contain stem cells.[34] More evidence for stem cells in pancreatic cancer cell lines were provided by Dembinski and Krauss, who worked on BxPC-3 and PANC03.27 cells.[65] To isolate slow-cycling cells they used a label retention method. Cells were labeled with the long lipophilic tracer dye (DiI). Cells obtained by this technique displayed multiple cancer stem cells properties. They were able to re-establish colonies of tumor tissue that were visually indistinguishable from the preselected cell population and possessed an increased invasive and metastatic potential. Also DiI^+ cells had an increased tumor formation ability and could form tumor in severe combined immunodeficiency (SCID) mice. Moreover, both cell lines had population which expressed CD24, CD133, and CD44 surface markers.[65] This data strongly suggest that BxPC-3 and PANC03.27 cell lines contain subset of cancer stem cells (CSCs). Results for BxPC-3 are even more plausible if we take Yao *et al*. work under consideration.[66] They used Hoechst 33342 staining and FACS analysis to isolate CSCs from BxPC-3, CFPAC-1, MIA PaCa-2, PANC-1, and SW1990 cell lines. The SP cells exhibited an increased tumorigenic ability following *in vivo* transplantation into BALB/C nude mice and an increased chemoresistance due to *in vitro*

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exposure to gemcitabine. FACS analysis showed that the SP cells contained more $CD44^+/CD24^+/CD133^+$ cells than the non-SP cells. In conclusion, these observations suggest that SP cells in the pancreatic cancer cell lines possess the properties of CSCs. Side population in the pancreatic cancer cell lines SW1990 and CFPAC-1 is enriched with cancer stem-like cells.[66]

Stem cells in liver cancer cell lines

Understanding the mechanism underlying carcinogenesis within the liver, can help to reveal new therapies against liver malignancies. Isolating CSCs from liver cancer cell lines will facilitate it. Several groups attempted to isolate CSCs from hepatocellular carcinoma (HCC) cell lines using different methods. Few researchers tried to identify CSCs by side population assay with Hoechst 33342 dye. They managed to identify CSCs in Huh7, Hep3B, PLC/PRF/5, HCCLM3, MHCC97-H, MHCC97-L using this technique. There was a significant difference in SP proportion for example: Shi *et al.*, identified 28.7% of SP cells in HCCLM3 line and only 0.9% in Hep3B (comparatively to Haraguchi *et al.*, and Chiba *et al.* findings), but all the SP showed similar self-renewal capability, clonogenicity and the ability to induce tumors in NOD/SCID mice.[67] Transplantation of as few as 1000 SP cells from Huh7 cell line induced tumors, whereas an injection of thousands of non-SP cells failed to initiate tumors in mice model. Moreover, these cell lines expressed such genes as *ABCG2, ABCB1, CEACAM6* which are responsible for chemoresistance.[67-69] Suetsugu *et al.*, examined three liver cancer cell lines (Huh7, hepatoblastoma cell line HepG2 and human fetal hepatocyte cell line Hc) for the presence of $CD133^+$ cells. Only in the Huh7 cell line a population of $CD133^+$ was found. $CD133^+$ cells were more tumorigenic than $CD133^+$ cells and exhibited mature hepatocyte markers on lower level (glutamine synthetase and cytochrome P450 3A4).[70] $CD133^+$ cells obtained by Ma *et al.* from Huh7, PLC8024 and HepG2 lines had not only the ability to form tumors *in vivo*, and high colony-forming efficiency but also could proliferate into non-hepatocyte-like, and angiomyogenic-like lineages.[71] The CD133 molecule is not the only one used to identify CSCs in HCC cell lines. Recent studies validate CD90 as a marker for CSCs in some HCC cell lines. Yang *et al.*, used this molecule to obtain tumorigenic cells from HepG2, Hep3B, PLC, Huh7, MHCC97-L, and MHCC96-H cell lines.[18] There were differences among cell lines in the tumorigenic potential but in all these cell lines $CD90^+$ cells were found. Five hundred of MHCC97-L and MHCC97-H cells were able to induce tumor in NOD/SCID mice, whereas over thousand of cells from HepG2, Hep3B, PLC, Huh7 lines were needed to get the same results.[18] The evidences of the presence of CSCs seems to be obvious but methods used to isolate them should be further studied. CD133 could not be found in all HCC cell lines and CD90 gave excellent results in the identification of tumorigenic cells but it is necessary to provide data about other characteristic traits of CSCs in these cells.

Stem cells in brain tumor cell lines

Kondo *et al.*, used Hoechst 33342 dye for SP isolation from rat glioma C6 cell line and rat neuroblastoma B104 cell line.[72] The C6 cells as well as the B104 cells contained 0.4% of SP cells. Furthermore, the SP cells have self-renew capacity, and proliferate into heterogeneous population. Also the C6 SP cells can form neurospheres and produce neurons, which have NSCs (neuronal stem cells) properties. Finally, they produce tumors in nude mice with high efficiency, whereas the non-SP C6 cells do not.[72] Zheng *et al.* and Shen *et al.*, also found cells with stem cell properties in the C6 glioma cell line but there is a huge difference between their results and results presented by Kondo *et al.*[13,72,73] They used tumor formation assay instead of the SP method and found out that C6 line is mostly composed from CSCs (Zheng *et al.* suggested 100% and Shen *et al.* suggested 80%). Moreover, it seems that Hoechst 33342 is very harmful for CSCs in C6 glioma line because after two hours of incubation with Hoechst 33342, there was only 1.8% of $CD133^+$ cells with stem cell properties instead of 88.5%.[13,73] These three works showed, that mistakes can be made very easily during researches on stem cells in established cancer cell lines, and that in fact the nature of these cells is still not known. Cruz *et al.*, provided possible explanation to such differences and controversions in experimental findings connected with the fact that all glioma cells have stem cell properties, but their phenotype varies depending on the environmental conditions.[74] The SP cells were also found in other glioma cell lines such as SK-MK-1, U87MG, U375MG, KNS42, and U251.75 The percentage of the SP cells ranged from 0.1% for the U251 cell line to 2.8% for the SK-MG-1 cell line. The SK-MG-1 cell line was chosen for further investigation. The SP cells from SK-MG-1 are capable of self-renewal, and generation of both, SP and non-SP cells, whereas few SP cells were generated from the non-SP cells. The SP cells also showed a multi-lineage differentiation potential, they could produce glial and neuronal-lineage cells simultaneously under different conditions. The SP cells formed spheres in neuron-specific medium and had a significant ability to proliferate *in vitro* as well as grow into xenografted brain tumors *in vivo*. These results suggest that SP cells from SK-MG-1 cell line possess stem cell properties, like self-renewal, multi-lineage differentiation potential, and tumorigenicity.[75]

Stem cells in cervical cancer cell lines

Cervical cancer is the second most frequent cancer occurring in women worldwide and the most common disease in Indian women.[76] The major role in the cervical cancer pathogenesis plays human papilloma virus (HPV), but this factor alone is not sufficient.[77] There are three pre-malignant stages: Cervical Intraepithelial Neoplasia (CIN) 1, 2 and 3.[78] But usually it will takes 10-15 years to change the normal cervical epithelial cell in a malignant one. Rajkumar *et al.*, have identified that genes such as: *UBE2C, CCNB1, CCNB2, CDC20, NUP210, MELK,* and *PLOD2*, could play a role in the early phase of tumorigenesis.[79] The same group has found that *AGRN, CCL18, DTXL, IL8, INDO, ISG15, ISG20, MMP1, MMP3, STAT1*, and TOP2A are upregulated in cervical cancer. Immunohistochemistry showed also overexpression of *MMP3, UBE2C*, and *p16* in cancer cells when compared to normal cervical epithelium.[79]

The first identified stem cell markers in human cervical carcinoma were: Nanog, Nucleostemin, and Musashi1.[80] To enrich the population of cancer stem-like cells, CD17 and CD44 markers were used.[81] Feng *et al.*, proved that this cellular pool possesses tumorigenic capacity and expresses embryonic and adult stemness-related genes such as: *Oct-4, Piwil2, C-myc, Stat3* and *Sox2*.[82] Bortolomai *et al.*, isolated cervical stem cells from the A431 cell line.[83] To confirm the presence and size of the stem-like cells population, after the isolation, ALDH1 enzymatic activity and Hoechst dye exclusion were used. In this work, it was demonstrated that A431 cell line contains 0.13% SP cells. It has to be mentioned that, even if the A431 sphere cells were characterized by stemness properties (self-renewal and clone forming capacity), they mostly formed differentiated colonies (paraclones) in adherent conditions. The A431 cells display high expression level of genes releted with the self-renewal (*NANOG, NESTIN*, and *OCT4*). The study of Bortolomai *et al.*, showed that Osteopontin can serve as a stem cell marker for cervical cancer.[83] Moreover, Geng *et al.* provided experimental data which showed that SP cells from A431 cell line expressed not only self-renewal genes but also had 5 up-regulated oncogenes *CoAA, ADAMTS1, AKR1C, LUM, SPP1* and one down-regulated suppressor *MGMT* gene, which made these cells a potential therapeutic target.[84]

Heterogeneous model of primary cell culture

The primary culture means cell culture established immediately after the isolation of material (explantation). This culture can relate to normal cells as well as cancer cells.

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The goal of the primary cultures is their uniqueness. Primary cultures come from certain unique individual. Thanks to the techniques of culture, propagation, freezing and storing of cells, the primary cultures offer a possibility of performing repeatable tests on the material derived from the patient. It is very difficult to find in a laboratory conditions, the model reflecting the process of stem cells or progenitor cells maturation and differentiation. It seems, that the primary cultures *in vitro* can be such a simplified model illustrating the existing dependencies between stem cells and differentiated cells.[85,86]

The isolated cells are known to lose most functional differentiation when separated and placed in cell culture.[87] The cellular identity is not lost permanently, we can make cells remember many of their original tissue specific traits by controlling the microenvironment.[88] There are now much evidences that the microenvironment regulates the specificity of tissue and significantly contributes to tumorigenesis. It has to be remembered that the role of the environment in the genes expression is very important. In glioma cells for example, the expression of gene in primary cultures of tumor cells is much closer to *in vivo* tumors than to established cancer cell lines.[89] What sounds surprisingly is that cancer cells for unknown reasons, grow much less than normal cells.[90] The tumor cells when cultured to generate a cell line, are characterized with an initially slow overall growth. Loss of a microenvironment of normal cells can lead to described above phenomena. The use of standardized growth conditions, the effect of the culture protocol, and the loss of cell-cell interactions can also evoke slowing growth ratio.[17]

The primary cultures have many disadvantages, most of all the difficulties associated with their establishment, the differences between the cell cultures derived from similar tissues coming from different organisms and the necessity of experiential assessment of cell viability.[85,86] In the *in vitro* studies there are often the established cell lines used, both transformed and cancer. The transformed and tumor cell lines, if omitting the possibility of occurring the genetic drift and contingent viral transfection, are characterized by a stable phenotype of cells. The cancer cells in established cancer cell lines do not age in the *in vitro* conditions.[91,92] It is thought that the established cell lines are burdened with an error arising from the lack of diversity in terms of cell aging and differentiation. Immortalized and tumor cell lines do not undergo the process of replicative senescence, which is an equivalent to the aging process of cells in the *in vivo* conditions.[91] The primary cultures give the opportunity to study the impact of age heterogeneous cells population on the growing colonies of cells *in vitro*. The microscopic observation of living cells shows that at the time of establishing the primary culture, colonies of epithelial cells grow only in the places with clusters of cells, which proliferate rapidly, providing the confluence on the surface of culture dish. This observation applies to many epithelial cells, such as the epithelium of human prostate, urinary bladder of rabbit and rat, human epiderma and melanocytes.[59,93-98] As a rule, setting of the primary epithelial culture is failed, when in a suspension of digested cells only a single cell are observed. The morphology of cells in the primary cultures at the time of their establishment, tends to reflect on the stem cells importance. In the clusters of cells that giving rise to the primary cultures, the progenitor cells are found.[99] They can be combined, e.g. with fragments of basement membrane and contain the other cells forming a niche of normal stem cells. These results are consistent with the assumptions in other works.[59,97,100] Stem cells give rise to epithelial cell cultures *in vitro*, whereas differentiated cells have inferior properties in this regard. Each digested and prepared for *in vitro* culture tissue must have a certain number of progenitor cells, whose potential determines an appropriate number of divisions. The proliferation of stem cells gives beginning to the colony of intensively and long-dividing cells (holoclones). The primary culture containing holoclones develops properly, and can be used for regeneration. Cells with a low ability to proliferate form colonies of paraclones. These colonies probably do not contain stem cells.[93,96,98,101]

The proper understanding of *in vitro* models, used for research purposes, should be connected with the degree of cells differentiation heterogeneity. It is considered that examining the influence of different substances on one type of cells, seemingly in the *in vitro* studies, there is in fact an influence of these substances on the heterogeneous group of cells with varying mark of differentiation and proliferative potential, different receptors expression and resistance to drugs examined. In spite of such looking, the *in vitro* cultures resemble very simplified model of tissue conditions. All the experiments on immortalized cell lines do not often give an essential results, and may even contain misleading information. Such experiments only inform us about the overall toxicity of the substance, drug or other agent to all cells. Results obtained from homogenous and heterogeneous *in vitro* culture models will be different. The example is an influence of doxazosin (α1-receptor antagonist), which induces apoptosis in the prostate epithelial cells. Doxazosin induces apoptosis in the epithelial cells and prostate stroma *in vivo* and *in vitro*.[85,86] The primary culture of the prostate epithelium is composed of stem cells and differentiated cells. In the highest concentration of doxazosin (80 μM), the percentage of apoptotic cells among the primary epithelial culture cells was 50. The same concentration of doxazosin causes that only 10% of prostate epithelium stem cells were in the phase of apoptosis. The same complex at the same concentration works in a different way on whole population of cells, on stem cells, as well as differentiated cells.[102] Stem cells and differentiated cells show different sensitivity to different cytotoxic agents, which induce apoptosis. It is easy to notice, how without a separate analysis of stem cells and differentiated cells the incorrect conclusions can be made.

Heterogeneous model of established cancer cell line: implications

The proper information from *in vitro* cytotoxic tests had been obtained only when the primary cultures were analyzed. Primary cultures show heterogeneity in the range of differentiation state of cells. It is impossible to see such differences in the analysis with the use of homogenous (from assumption) cancer cell lines or the primary cultures, not separated on stem cells and differentiated cells. There are known numerous examples of the chemical compounds exhibiting toxicity to the cells in culture, while their *in vivo* effect was subsequently irrelevant. The platinum complexes, potentially active against cancer cell lines, have not been introduced into clinical practice, probably due to the fact that they was tested on the homogenous (i.e. not separated on stem cells and differentiated cells) model of established cancer cell line. What is more, chemotherapeutic agents registered and used in the treatment of cancer are cytotoxic against tumor cell lines *in vitro*, while their action *in vivo* is incomplete, and lead to treatment failure.[103,104] The presented model of the heterogeneous primary culture of normal cells has its counterpart in the tumor cell culture. Among the cell lines derived from tumors, the populations of stem cells were found in the case of prostate, breast, skin, liver, pancreas, and other cancers. As a result, there can be assumed that in the cancer cell lines heterogeneity in terms of differentiation is also observed.[64,105,106] The cancer also have a population of stem cells that is responsible for their growth, and other tumor characteristic biological properties.[107,108] The mechanisms responsible for cell resistance against medicines, and other substances, vary between stem cells and differentiated cells.[109-112] The analysis of the results of *in vitro* cytotoxicity tests should be based on separate analysis describing the cytotoxicity for stem cells and differentiated cells. The model of the heterogeneous cancer cell line is still a simplified model and needs to be developed. This model partially explains discrepancies between results obtained from *in vitro* experiments and clinical practice. With the rapidly developing techniques of cell isola-

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tion is not just theoretical model, but it can be used in studies of the effects of substances *in vitro*, probably helping to predict real drug action *in vivo*.[113,114]

Advantages and disadvantages of primary cultures and established cancer cell lines

It is well known, that validity of a model depends on how it resembles original tumor. Nowadays, in laboratory conditions two models of cell culture are used: established cancer cell lines (ECCLs) and primary cell cultures. A major problem with these models is that tumors in organism evolve.

Both models have advantages and disadvantages (Table 4). It is easy to notice, that advantages of ECCLs are the simplicity in culturing a reproducible results and their easy access. On the other hand, there are even primary cultures of tumor cells which are not immortal but their genomic stability can be better than it is in ECCLs. Moreover, their gene pattern is very similar to original tumor. It is wisely to be aware of models pros and cons before using them in experiments. It is also important to remember about the optimalization of protocols.[17]

Table 4. Comparison of primary cell cultures and established cell lines traits.

Traits	Primary cell cultures	ECCLs
Morphology	Distinguishable[99]	Non-distinguishable[10]
ABC pomp	-/+[109-112]	+ In CSCs[36]
Oncogenic and anti-oncogenic changes	+[91,92]	+[3]
Stages of tumor development	Only one[17]	Only one[17]
Methylation of genes	+[115]	+ (Very high)[116]
Loss of differentiation potential	+[87]	+ (Very high)[87]
Immortality	+/-[91,92]	+[16,17]
Genetic stability	High[85]	Low[17]
Resemblance to tumor original cells	High[88,89]	+ (Medium)[17]
Repeatability of results	Low[86]	High[17]
Heterogeneity	High[94,95]	Low[103,104]
Phenotype	Not stable[91,92]	Stable[105,106]

Conclusions

Established cancer cell lines (ECCLs) contain a cancer stem cells (CSCs) population. However, it is necessary to remember that the percentage of CSCs in cell lines, their capability to form tumors, self-renewal potential can vary strongly even among cell lines derived from the same type of cancer. It is believed that CSCs are responsible for metastasis and we hope to reveal new drugs and therapies which will effectively kill all cancer cells but the good *in vitro* model is essential. It is still not known whether the CSCs identified in ECCLs have the same properties as the CSCs obtained from samples provided by patients. Moreover, the percentage of CSCs in ECCLs is mostly different than the percentage of CSCs in patient samples and we need to take under consideration the evolution of cancer cell lines because of thousands of passages. Despite the fact that the CSCs are in multiple ECCLs it does not mean that they can be easily isolated due to a lot of problems with protocols used to identify CSCs. There are a few methods but all of them are very laborious, time-consuming and flawed. However, without developing reliable techniques of CSCs isolation, it is almost impossible to develop new treatment strategies in experimental and clinical oncology. Heterogeneity within established cancer cell lines will change our view on experimental works *in vitro* and probably lead to reevaluation of the previous results.

References

1. Virchow R. Die multiloculäre, ulcerirende Echinokokkengeschwulst der Leber. Verhandlungen der Physicalisch-Medicinischen Gesellschaft 1855; 84-95.
2. Cohnheim J. Ueber Entzündung und Eiterung. Virchows Archiv für Pathologische Anatomie und Physiologie und für Klinische Medizin 1867;40:1-79.
3. Moltzahn FR, Volkmer JP, Rottke D, Ackermann R. "Cancer stem cells"- lessons from Hercules to fight the Hydra. Urol Oncol 2008;26:581-9.
4. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997;3:730-7.
5. Maitland NJ, Collins AT. Prostate cancer stem cells: a new target for therapy. J Clin Oncol 2008;26:2862-70.
6. Fang D, Nguyen TK, Leishear K, et al. A tumorigenic subpopulation with stem cell properties in melanomas. Cancer Res 2005;65:9328-37.
7. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 2007;445:106-10.
8. Singh SK, Clarke ID, Terasaki M, et al. Identification of a cancer stem cell in human brain tumors. Cancer Res 2003;63:5821-8.
9. Werbowetski-Ogilvie TE, Bhatia M. Pluripotent human stem cell lines: what we can learn about cancer initiation. Trends Mol Med 2008;14:323-32.
10. Kondo T. Stem cell-like cancer cells in cancer cell lines. Cancer Biomark 2007;3:245-50.
11. Hwang-Verslues WW, Kuo WH, Chang PH, et al. Multiple lineages of human breast cancer stem/progenitor cells identified by profiling with stem cell markers. PLoS One 2009;4:e8377.
12. Yu SC, Ping YF, Yi L, et al. Isolation and characterization of cancer stem cells from a human glioblastoma cell line U87. Cancer Lett 2008;265:124-34.
13. Zheng X, Shen G, Yang X, Liu W. Most C6 cells are cancer stem cells: evidence from clonal and population analyses. Cancer Res 2007;67:3691-7.
14. Yun K, Tennent B. Cancer stem cells. Drug Discov Today Dis Models 2007;4:47-52.
15. Liang Y, Zhong Z, Huang Y, et al. Stem-like cancer cells are inducible by increasing genomic instability in cancer cells. J Biol Chem 2010;285:4931-40.
16. Liu WH, Qian NS, Li R, Dou KF. Replacing Hoechst33342 with rhodamine123 in isolation of cancer stem-like cells from the MHCC97 cell line. Toxicol In Vitro 2010;24:538-45.
17. van Staveren WC, Solís DY, Hébrant A, et al. Human cancer cell lines: Experimental models for cancer cells in situ? For cancer stem cells? Biochim Biophys Acta 2009;1795:92-103.
18. Yang ZF, Ho DW, Ng MN, et al. Significance of CD90+ cancer stem cells in human liver cancer. Cancer Cell 2008;13:153-66.
19. Vassilopoulos A, Wang RH, Petrovas C, et al. Identification and characterization of cancer initiating cells from BRCA1 related mammary tumors using markers for normal mammary stem cells. Int J Biol Sci 2008;4:133-42.
20. Ma S, Chan KW, Guan XY. In search of liver cancer stem cells. Stem Cell Rev 2008;4:179-92.
21. Witte KE, Ahlers J, Schäfer I, et al. High proportion of leukemic stem cells at diag-

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nosis is correlated with unfavorable prognosis in childhood acute myeloid leukemia. Pediatr Hematol Oncol 2011;2:91-9.
22. Saito Y, Kitamura H, Tomizawa-Murasawa M, et al. Identification of therapeutic targets for quiescent, chemotherapy-resistant human leukemia stem cells. Sci Transl Med 2010;2:17ra9.
23. Quéré R, Andradottir S, Brun AC, et al. High levels of the adhesion molecule CD44 on leukemic cells generate acute myeloid leukemia relapse after withdrawal of the initial transforming event. Leukemia 2011;25:515-26.
24. Yamazaki H, Nishida H, Iwata S, et al. CD90 and CD110 correlate with cancer stem cell potentials in human T-acute lymphoblastic leukemia cells. Biochem Biophys Res Commun 2009;383:172-7.
25. Nishida H, Yamazaki H, Yamada T, et al. CD9 correlates with cancer stem cell potentials in human B-acute lymphoblastic leukemia cells. Biochem Biophys Res Commun 2009;382:57-62.
26. Cox CV, Diamanti P, Evely RS, et al. Expression of CD133 on leukemia-initiating cells in childhood ALL. Blood 2009;113:3287-96.
27. Reuben JM, Lee BN, Gao H, et al. Primary breast cancer patients with high risk clinicopathologic features have high percentages of bone marrow epithelial cells with ALDH activity and CD44(+)CD24(lo) cancer stem cell phenotype. Eur J Cancer 2011.
28. Prud'homme GJ, Glinka Y, Toulina A, et al. Breast cancer stem-like cells are inhibited by a non-toxic aryl hydrocarbon receptor agonist. PLoS One 2010;5:e13831.
29. Qin K, Jiang X, Zou Y, et al. Study on the proliferation and drug-resistance of human brain tumor stem-like cells. Cell Mol Neurobiol 2010;30:955-60.
30. Fan X, Liu S, Su F, et al. Effective enrichment of prostate cancer stem cells from spheres in a suspension culture system. Urol Oncol 2010.
31. Fabrizi E, dj Martino S, Pelacchi F, Ricci-Vitiani L. Therapeutic implications of colon cancer stem cells. World J Gastroenterol 2010;16:3871-7.
32. Todaro M, Francipane MG, Medema JP, Stassi G. Colon cancer stem cells: promise of targeted therapy. Gastroenterology 2010;138;2151-62.
33. Lingala S, Cui YY, Chen X, et al. Immunohistochemical staining of cancer stem cell markers in hepatocellular carcinoma. Exp Mol Pathol 2010;89:27-35.
34. Gaviraghi M, Tunici P, Valensin S, et al. Pancreatic cancer spheres are more than just aggregates of stem marker-positive cells. Biosci Rep 2010;31:45-55.
35. Benzion J, Kaplan MJ, Dowecki I, et al. Frequency of cells expressing CD44, a head and neck cancer stem cell marker: correlation with tumor aggressiveness. Head Neck 2011.
36. Charafe-Jauffret E, Ginestier C, Birnbaum D. Breast cancer stem cells: tools and models to rely on. BMC Cancer 2009;9:202.
37. Styczynski J, Drewa T. Leukemic stem cells: from metabolic pathways and signaling to a new concept of drug resistance targeting. Acta Biochim Pol 2007;54:717-26.
38. Fong D, Yeh A, Naftalovich R, et al. Curcumin inihibits the side population (SP) phenotype of the rat C6 glioma cell line: towards targeting of cancer stem cells with phytochemicals. Cancer Lett 2010;293:65-72.
39. Boonstra R, Timmer-Bosscha H, van Echten-Arends J, et al. Mitoxantrone resistance in a small lung cancer cell line is associated with ABCA2 upregulation. Br J Cancer 2004;90:2411-7.
40. Wang J, Wang B, Bi J. Significiant association between ABCB1 gene C3435T polymorphism and breast cancer risk. Breast Cancer Res Treat 2011;126:815-7.
41. Grimm C, Polterauer S, Zeillinger R, et al. Two multidrug-resistance (ABCB1) gene polymorphism as prognostic parameter in women with ovarian cancer. Anticancer Res 2010;30:3487-91.
42. Munoz M, Henderson M, Haber M, Norris M. Role of the MRP1/ABCC1 multidrug transporter protein in cancer. IUBMB Life 2007;59:752-7.
43. Han B, Gao G, Wu W, et al. Association of ABCC2 polymorphisms with platinum-based chemotherapy response and severe toxicity in non-small lung cancer patients. Lung Cancer 2010;72:238-43.
44. Müller PJ, Dally H, Klappenecker CN, et al. Polymorphisms in ABCG2, ABCC3 and CNT1 genes and their possible impact on chemoteherapy outcome of lung cancer patients. Int J Cancer 2009;124:1669-74.
45. Low SK, Kivotani K, Mushiroda T, et al. Association study of genetic polymorphism in ABCC4 with cyclophosphamide-induced adverse drug reactions in breast cancer patients. J Hum Genet 2009;54: 564-71.
46. Oguri T, Achiwa H, Sato S, et al. The determinants of sensitivity and acquired resistance to gemcitabine differ in non-small cell lung cancer: a role of ABCC5 in gemcitabine sensitivity. Mol Cancer Ther 2006;5:1800-6.
47. Ota I, Sakurai A, Toyoda Y, et al. Association between breast cancer risk and the wild-type allele of human ABC transporter ABCC11. Anticancer Res 2010;30:5189-94.
48. Liu HG, Pan YF, You J, et al. Expression of ABCG2 and its significance in colorectal cancer. Asian Pac J Cancer Prev 2010;11:845-8.
49. Huang EH, Hynes MJ, Zhang T, et al. Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic stem cells (SC) and tracks SC overpopulation during colon tumorigenesis. Cancer Res 2009;69:3382-9.
50. Corti S, Locatelli F, Papadimitriou D, et al. Identification of a primitive brain-derived neural stem cell population based on aldehyde dehydrogenase activity. Stem Cells 2006;24:975-85.
51. Jones RJ, Barber JP, Vala MS, et al. Assessment of aldehyde dehydrogenase in viable cells. Blood 1995;85:2742-6.
52. Chen YC, Chen YW, Hsu HS, et al. Aldehyde dehydrogenase 1 is a putative marker for cancer stem cells in head and neck squamous cancer. Biochem Biophys Res Commun 2009;385:307-13.
53. Ginestier C, Hur MH, Charafe-Jauffret E, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell 2007;1:555–67.
54. Croker AK, Goodale D, Chu J, et al. High aldehyde dehydrogenase and expression of cancer stem cell markers selects for breast cancer cells with enhanced malignant and metastatic ability. J Cell Mol Med 2009;13:2236-52.
55. Al-Hajj M, Wicha MS, Benito-Hernandez A, et al. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A. 2003;100:3983-8.
56. Lawson JC, Blatch GL, Edkins AL. Cancer stem cells in breast cancer and metastasis. Breast Cancer Res Treat 2009;118: 241-54.
57. 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:R25.
58. Charafe-Jauffret E, Ginestier C, Iovino F, et al. Breast cancer cell lines contain functional cancer stem cells with metastatic capacity and a distinct molecular signature. Cancer Res 2009;69:1302-13.
59. Miki J, Rhim JS. Prostate cell cultures as in vitro models for the study of normal stem cells and cancer stem cells. Prostate Cancer Prostatic Dis 2008;11:32-9.
60. Patrawala L, Calhoun T, Schneider-Broussard R, et al. Side population is enriched in tumorigenic, stem-like cancer cells, whereas ABCG2+ and ABCG2- cancer cells are similarly tumorigenic.

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Cancer Res 2005;65:6207-19.
61. Collins AT, Berry PA, Hyde C, et al. Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res 2005;65:10946–51.
62. Pfeiffer MJ, Schalken JA. Stem cell characteristics in prostate cancer cell lines. Eur Urol 2010;57:246-54.
63. Bisson I, Prowse DM. WNT signaling regulates self-renewal and differentiation of prostate cancer cells with stem cell characteristics. Cell Res 2009;19:683-97.
64. 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;6:763-8.
65. Dembinski JL, Krauss S. Characterization and functional analysis of a slow cycling stem cell-like subpopulation in pancreas adenocarcinoma. Clin Exp Metastasis 2009;26:611-23.
66. Yao J, Cai HH, Wei JS, et al. Side population in the pancreatic cancer cell lines SW1990 and CFPAC-1 is enriched with cancer stem-like cells. Oncol Rep 2010;23:1375-82.
67. Shi GM, Xu Y, Fan J, et al. Identification of side population cells in human hepatocellular carcinoma cell lines with stepwise metastatic potentials. J Cancer Res Clin Oncol 2008;134:1155-63.
68. Haraguchi N, Utsunomiya T, Inoue H, et al. Characterization of a side population of cancer cells from human gastrointestinal system. Stem Cells 2006;24:506-13.
69. Chiba T, Kita K, Zheng YW, et al. Side population purified from hepatocellular carcinoma cells harbors cancer stem cell-like properties. Hepatology 2006;44:240-51.
70. Suetsugu A, Nagaki M, Aoki H, et al. Characterization of CD133+ hepatocellular carcinoma cells as cancer stem/progenitor cells. Biochem Biophys Res Commun 2006;351:820-4.
71. Ma S, Chan KW, Hu L, et al. Identification and characterization of tumorigenic liver cancer stem/progenitor cells. Gastroente - rology 2007;132:2542-56.
72. 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 U S A 2004;101:781-6.
73. Shen G, Shen F, Shi Z, et al. Identification of cancer stem-like cells in the C6 glioma cell line and the limitation of current identification methods. In Vitro Cell Dev Biol Anim 2008;44:280-9.
74. Cruz M, Siden A, Tasat DR, Yakisich JS. Are all glioma cells cancer stem cells? J Cancer Sci Ther 2010;2:100-6.
75. Fukaya R, Ohta S, Yamaguchi M, et al. Isolation of cancer stem-like cells from a side population of a human glioblastoma cell line, SK-MG-1. Cancer Lett 2010;291:150-7.
76. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74-108.
77. zur Hausen H. Papillomaviruses causing cancer: evasion from host-cell control in early events in carcinogenesis. J Natl Cancer Inst 2000;92:690-8.
78. Arends MJ, Buckley CH, Wells M. Aetiology, pathogenesis, and pathology of cervical neoplasia. J Clin Pathol 1998;51:96-103.
79. Rajkumar T, Sabitha K, Vijayalakshmi N, et al. Identification and validation of genes involved in cervical tumourigenesis. BMC Cancer 2011;11:80.
80. Ye F, Zhou C., Cheng Q, et al. Stem-cell-abundant proteins Nanog, Nucleostemin and Musashi1 are highly expressed in malignant cervical epithelial cells. BMC Cancer 2008;8:108.
81. Todaro M, Lombardo Y, Stassi G. Evidences of cervical cancer stem cells derived from established cell lines. Cell Cycle 2010;9:1238-9.
82. Feng D, Peng C, Li C, et al. Identification and characterization of cancer stem-like cells from primary carcinoma of the cervix uteri. Oncol Rep 2009;22:1129-34.
83. Bortolomai L, Canevari S, Facetti I, et al. Tumor initiating cells: development and critical characterization of a model derived from the A431 carcinoma cell line forming spheres in suspension. Cell Cycle 2010;9:1194-206.
84. Geng S, Wang Q, Wang J, et al. Isolation and identification of a distinct side population cancer cells in the human epidermal squamous cancer cell line A431. Arch Dermatol Res 2011;303:181-9.
85. Peehl DM, Sellers RG. Cultured stromal cells: an in vitro model of prostatic mesenchymal biology. Prostate 2000;45:115-23.
86. Wei N, Flaschel E, Friehs K, Nattkemper TW. A machine vision system for automated non-invasive assessment of cell viability via dark field microscopy, wavelet feature selection and classification. BMC Bioinformatics 2008;9:449.
87. Bissell MJ. The differentiated state of normal and malignant cells or how to define a "normal" cell in culture. Int Rev Cytol 1981;70:27-100.
88. Bissell MJ, Rizki A, Mian IS. Tissue architecture: the ultimate regulator of breast epithelial function. Curr Opin Cell Biol 2003;15:753-62.
89. Lee J, Kotliarova S, Kotliarov A, et al. Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. Cancer Cell 2006;9:391-403.
90. O'Hare MJ, Ormerod MG, Monaghan P, et al. Characterization in vitro of luminal and myoepithelial cells isolated from the human mammary gland by cell sorting. Differentiation 1991;46:209-21.
91. Zeng X. Human embryonic stem cells: mechanisms to escape replicative senescence? Stem Cell Rev 2007;3:270-9.
92. Petitot F, Lebeau J, Dano L, et al. In vitro aging of rat lung cells: Downregulation of telomerase activity and continuous decrease of telomere length are not incompatibile with malignant transformation. Exp Cell Res 2003;286:30-9.
93. Barrandon Y, Green H. Three clonal types of keratinocyte with different capacities for multiplication. Proc Natl Acad Sci U S A 1987;84:2302-6.
94. Czajkowski R, Placek W, Drewa T, et al. Autologous cultured melanocytes in vitiligo treatment. Dermatol Surg 2007;33:1027-36.
95. Drewa T. The urothelium cell culture on the starch scaffold. Acta Pol Pharm 2006;63:153-5.
96. Papini S, Cecchetti D, Campani D, et al. Isolation and clonal analysis of human epidermal keratinocyte stem cells in long-term culture. Stem Cells 2003;21:481-94.
97. Peehl DM. Primary cell cultures as models of prostate cancer development. Endocr Relat Cancer 2005;12:19-47.
98. Pellegrini G, Ranno R, Stracuzzi G, et al. The control of epidermal stem cells (holoclones) in the treatment of massive full-thickness burns with autologous keratinocytes cultured on fibrin. Transplantation 1999;68:868-79.
99. Chai JK, Sheng ZY, Ma ZF, et al. Growth activity of epidermal cells from different parts of human body. Chin Med J (Engl) 2007;120:1444-7.
100. Bayne CW, Donnelly F, Chapman K, et al. A novel coculture model for benign prostatic hyperplasia expressing both isoforms of 5 alpha-reductase. J Clin Endocrinol Metab 1998;83:206-13.
101. Redvers RP, Kaur P. Serial cultivation of primary adult murine keratinocytes. Methods Mol Biol 2005;289:15-22.
102. Drewa T, Pokrywka Ł, Wolski Z, et al. α-blockade is not effective in decreasing tissue bulk in patients suffering from BPH , an in vitro study. Central Eur J Urol 2009;62:263-5.
103. Drewa T, Woźniak A, Olszewska D, et al. The in vitro study of influence of four novel platinum compounds on rodent melanoma cells. Acta Pol Pharm 2001;58:169-74.
104. Olszewska-Słonina D, Drewa T, Musiałkiewicz D, et al. Comparison of viability of B16 and Cl S91 cells in three cyto-

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toxicity tests: cells counting, MTT and flow cytometry after cytostatic drug treatment. Acta Pol Pharm 2004;61:31-7.
105. Kamohara Y, Haraguchi N, Mimori K, et al. The search for cancer stem cells in hepatocellular carcinoma. Surgery 2008; 144:119-24.
106. Zhou L, Wei X, Cheng L, et al. CD133, one of the markers of cancer stem cells in Hep-2 cell line. Laryngoscope 2007;117: 455-60.
107. Lapidot T, Grunberger T, Vormoor J, et al. Identification of human juvenile chronic myelogenous leukemia stem cells capable of initiating the disease in primary and secondary SCID mice. Blood 1996;88: 2655-64.
108. Tirino V, Desiderio V, d'Aquino R, et al. Detection and characterization of CD133+ cancer stem cells in human solid tumours. PLoS One 2008;3:e3469.
109. Donnenberg VS, Donnenberg AD. Multiple drug resistance in cancer revisited: the cancer stem cell hypothesis. J Clin Pharmacol 2005;45:872-7.
110. Litman T, Brangi M, Hudson E, et al. The multidrug-resistant phenotype associated with overexpression of the new ABC half-transporter, MXR (ABCG2). J Cell Sci 2000;113:2011-21.
111. Modok S, Mellor HR, Callaghan R. Modulation of multidrug resistance efflux pump activity to overcome chemoresistance in cancer. Curr Opin Pharmacol 2006;6:350-4.
112. Wicha MS, Liu S, Dontu G. Cancer stem cells: an old idea--a paradigm shift. Cancer Res 2006;66:1883-90; discussion 1895-6.
113. Kelly PN, Dakic A, Adams JM, et al. Tumor growth need not be driven by rare cancer stem cells. Science 2007;317:337.
114. Yoo MH, Hatfield DL. The cancer stem cell theory: is it correct? Mol Cells 2008;26: 514-6.
115. Chu MW, Siegmund KD, Eckstam CL, Kim JY, et al. Lack of increases in methylation at three CPG-rich genomic loci in non-mitotic adult tissues during aging. BMC Med Genet 2007;31:8:50.
116. Hernandez-Vargas H, Ouzounova M, Calvez-Kelm FL, et al. Methylome analysis reveals Jak-STAT pathway deregulation in putative breast cancer stem cells. Epigenetics 2011;1:6.

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