ANTICANCER RESEARCH *32*: 1153-1162 (2012)

Reduction of Tumorigenicity by Placental Extracts

ANNETTE M. MARLEAU[1], GREG MCDONALD[2], JAMES KOROPATNICK[3,4], CHIEN-SHING CHEN[5] and DAVID KOOS[6]

[1]*Department of Immunology, Institute for Molecular Medicine, Huntington Beach, CA, U.S.A.;*
[2]*McDonald Animal Hospital, Santa Barbara, CA, U.S.A.;*
[3]*The Lawson Health Research Institute, London Health Sciences Center, London, ON, Canada;*
[4]*Department of Oncology, University of Western Ontario, London, ON, Canada;*
[5]*Loma Linda University School of Medicine, Division of Hematology and Oncology, Loma Linda, CA, U.S.A.;*
[6]*Entest Biomedical, San Diego, CA, U.S.A.*

Abstract. *The influence of adult stem cells on tumor growth is paradoxical. On one hand, angiogenic factors secreted by stem cells are known to be essential for tumor vascularization. On the other hand, stem cell-derived factors can reportedly induce tumor differentiation or direct death of tumor cells. Both the placenta and umbilical cord are rich sources of stem cells with immune modulatory and tissue-healing properties; however, the effects of placental components on cancer cells have not been fully defined. Here we demonstrate that extracts of placental lysates reduce the malignancy of a variety of human tumor cell lines in a species-unrestricted manner. Using a standard model of leukemia cell differentiation, we demonstrated that addition of placental extracts to tumor cells, or co-culture of tumor cells with the CD34$^+$ cells from umbilical cord blood, induced tumor cell differentiation. Inhibition of tumor growth and metastasis in vivo was also observed following administration of placental extracts. These data support the concept of non-toxic biological therapy of cancer using stem cell derivatives, possibly through the induction of tumor cell differentiation.*

The characteristic function of a stem cell is the ability to self-renew and to differentiate. Stem cells differentiate in a tissue-directed manner in response to a wide variety of stimuli; for example, at high altitudes increased numbers of erythrocytes differentiate and during bacterial infections a preferential increase in neutrophil differentitation occurs (1). In addition, stem cells can be artificially induced to replicate through various means including administration of cytokines, growth factors, and small peptides (2). A wide variety of tissue-specific stem cells have been identified, including those in liver (3), skin (4), kidney (5), pancreas (6), stomach (7) and neuronal tissue (8).

There are many similarities between tumor stem cells and normal, non-transformed stem cells, in the sense that both express embryonic features including the ability to migrate (metastasize), suppress immune responses, and proliferate. They differ, however, in that normal stem cells are highly regulated with respect to growth and differentiation while cancer cells are not. At the molecular level, the more aggressive a cancer is, the more de-differentiated and stem cell-like it becomes. Specifically, regions of DNA that are transcriptionally silent or of low activity in mature tissue become active upon neoplastic transformation. For example, the enzyme telomerase is required for cells to escape proliferative senescence (*i.e.* to continue to proliferate at and beyond the Hayflick limit of 40-60 cell divisions). As normal stem cells differentiate into specialized tissue, telomerase expression becomes silenced epigenetically through synergistic involvement of DNA methylation and histone deacetylation (9). Conversely, tumor cells selectively silence genes that suppress malignant characteristics including tumor-initiating capacity. For example, it is known that in prostate cancer, tumor suppressor genes become silenced by methylation (10). In fact, this common phenomenon is observed in a wide variety of histologically distinguishable tumors (11).

Stem cells actively secrete differentiation-inducing factors that are capable of inducing epigenetic reprogramming in cells. Embryonic stem cells reportedly possess the ability to reprogram differentiated cells to take on a de-differentiated phenotype. This concept raises the interesting question of whether embryonic stem cells can induce dedifferentiation of cancer cells, which would be expected to increase their

Correspondence to: Dr. Annette M. Marleau, Department of Immunology, Institute of Molecular Medicine, 16731 Gothard St Unit H, Huntington Beach, CA 92647, U.S.A. Tel: +858 9979435, Fax: +714 5963791, e-mail: amarleau@immed.org

Key Words: Cancer, stem cells, placenta, differentiation, biological therapies, HL-60, LNCap, PC-3, LLC cells.

malignant behavior. There has been controversy surrounding the influence of adult stem cells on tumor growth. Investigators have demonstrated that mesenchymal stem cells can specifically home to tumors (12-14), directly lyse tumors (15), and exert anti proliferative effects *in vitro* (16) and *in vivo* (17). On the other hand, mesenchymal stem cells appear to trigger angiogenesis in tumors (18), stimulate tumor growth and metastasis (19, 20), and can act as fibrosarcoma-initiating cells in animal models of aging (21).

In the current report, we examined the prospect of adult stem cell reprogramming of cancer cell differentiation using cord blood as a source of $CD34^+$ stem cells, as well as placental extracts. Cord blood is a rich source of hematopoietic stem cells capable of reconstituting the hematopoietic lineage in non-obese diabetic/severe combined immunodeficient mice. The potential utilization of cord blood stem cells, such as $CD34^+$ cells, has led to the cryopreservation of such cells for more than a decade (22). Indeed, the clinical use of cord blood-derived stem cells is becoming increasingly acceptable as an alternative to bone marrow transplantation or use of cytokine-mobilized peripheral blood stem cell transplantation as treatment for a wide variety of diseases. This is in part because of a reduced incidence of graft *vs*. host reaction using cord blood stem cells in comparison with other sources (23). Indeed, this was observed in pediatric patients with leukemia lacking suitable major histocompatibility complex (MHC) matched donors (24), and in transplant-treated patients with certain types of anemia or hematopoietic stem cell disorders such as beta-thalassemia major (25). Additionally, the placenta contains stem cell populations that secrete immune-modulatory factors that promote the successful completion of pregnancy. For example, a low molecular weight fraction of placental tissue is known to be immunosuppressive (26) and useful for control of autoimmune reactions. Additionally, the use and safety of placental extracts in cosmetics is well known (27). Currently, placenta-derived stem cells are in phase II clinical trials by the company Pluristem and showed no adverse effects in phase I trials where the cells were administered to patients with end-stage peripheral artery disease in an unmatched, universal donor manner (28). Numerous reports support the use and safety of unmatched cord blood mononuclear cells, which we have previously reviewed (29-33). Early work from the group of Govallo reported inhibition of tumors in patients receiving a "placental vaccine" (34, 35). Although we have previously investigated the possibility of immune modulation with placental extracts (36), to our knowledge, the possibility of a direct anti-cancer effect has not been investigated.

In this study we examined the ability of cord blood and placental extracts to modulate tumor growth *in vitro*. We identified a pro-differentiation activity in cord blood that segregated with $CD34^+$ cells, as well as a similar activity in extracts of placental lysate. Interestingly, the latter were capable of inhibiting metastasis and tumor growth in the Lewis lung carcinoma model of lung cancer *in vivo*.

Materials and Methods

Cell lines and tissue culture. HL-60, LNCaP, PC-3 and Lewis lung carcinoma cells (LLC) (American Type Culture Collection, Manassas, VA, USA) were maintained in RPMI media supplemented with 10% fetal calf serum (FCS) (Life Technologies, Carlsbad, CA, USA), 100 U/ml of penicillin (Life Technologies), and 100 μg/ml of streptomycin (Life Technologies). Cells were cultured at 37˚C in a humidified atmosphere of 5% CO_2. Adherent cells (LNCaP, PC-3 and LLC were passaged by trypsinization, washing to remove trypsin, and replating twice per week or at 80% confluence. For proliferation experiments, cells were plated in 96 well plates (1×10^5 cells per well) in 200 μl of complete RPMI 1640 (Life Technologies) for 3 days in the presence of 10 ng, 50 ng, or 100 ng of placental extract, and pulsed with 1 μCi of [^{3}H]thymidine (Amersham Pharmacia Biotech, Piscataway, NJ, USA) for the last 16 h of culture. Cells were harvested onto glass fiber filters. Incorporated radioactivity was quantified using a Wallac Betaplate liquid scintillation counter (Perkin Elmer Life Sciences, Boston, MA, USA).

Cord blood and $CD34^+$ cell purification. Umbilical cord blood was obtained from consenting mothers undergoing cesarean delivery of healthy, full-term infants, and low-density (less than 1.077 g/ml) cells were isolated by centrifugation on Ficoll-Hypaque medium. $CD34^+$ cell–enriched populations (65-98% $CD34^+$ cells) were obtained by removal of lineage marker-positive cells using a column (n=2); and positive (EasySep®) selection using magnetic beads (n=1; StemCell Technologies Inc., Vancouver, BC Canada). Cells were stimulated overnight for *in vivo* experiments and for 48 hours for *in vitro* experiments at densities less than or equal to 2×10^5 cells/ml in Iscove's medium supplemented with 1% BSA, 10 μg/ml bovine pancreatic insulin, 200 μg/ml human transferrin (BIT; StemCell Technologies Inc.), 0.1 mM 2-mercaptoethanol, 2 mM glutamine, 100 ng/ml FL (Immunex Corp., Thousand Oaks, CA, USA), 100 ng/ml SF, 50 ng/ml Tpo (Genentech Inc.), and 100 ng/ml hIL-6 (provided by S. Rose-John, Christian-Albrechts University, Kiel, Germany). The following day the cells were pelleted, resuspended in fresh growth factor–supplemented medium with 5 μg/ml protamine sulfate and 0.5×10^8-5×10^8 infectious U/ml (MOI=9-140; 140 in experiment 1, 9 and 90 in experiment 2), placed in a 24-well plate coated with 2 μg/cm^2 Retronectin (Takara Bio Inc., Otsu, Shiga, Japan) or with 5 μg/cm^2 fibronectin (Sigma-Aldrich, St. Louis, MO, USA), and were then incubated at 37˚C for 6 hours.

Generation of placental lysate. Term placentas were harvested and the chorion was mechanically separated under sterile conditions. Placentas were collected in ice-cold Dutch modification of RPMI 1640 (Sigma) supplemented with 10% FCS, 2 mM L-glutamine, gentamicin (25 μg/ml), and penicillin/streptomycin (100 U/ml) (complete medium). They were incubated with occasional agitation for 20 min at room temperature in calcium- and magnesium-free HBSS (Life Technologies, Paisley UK) containing 1 mM DTT (Sigma). Purified cell homogenate was prepared by collagenase digestion. Briefly, bacterial collagenase (Advance Biofactures, Lynbrook, NY, USA) digestions were sequentially performed, 4 μl of

50 mM calcium acetate was added, followed by 3 μl (3 units) of bacterial collagenase. Reactions were then incubated for another 60 min at 37˚C. Heparitinase digestions (15 μl) were carried out for 90 min at 37˚C in a pH 7.0 buffer consisting of 100 mM sodium acetate, and 10 mM calcium acetate, using 5 milliunits of enzyme. In those reactions where bacterial collagenase digestion was sequentially performed, 4 μl of 5× collagenase buffer (250 mM Tris-HCl, pH 7.2, 50 mM calcium acetate) was added, followed by 3 μl (3 units) of bacterial collagenase. Reactions were then incubated for another 60 min at 37˚C. Purified cells were subsequently sonicated, centrifuged at 10,000 ×*g* for 3 h, and the supernatant was collected, filter sterilized, and quantified for protein content using the Bradford assay.

Tumor model. Six- to eight-week-old female C57BL/6 mice were purchased from Harlan (The Jackson Laboratory, Bar Harbor, ME, USA). The animals were maintained under standard pathogen-free conditions at the University of Western Ontario (London, Canada) and all experiments were performed in accordance with institutional guidelines for animal care.

For the *in vivo* tumor studies, the Lewis lung carcinoma (LLC) tumor cell line was thawed and expanded by culturing for three passages in Dulbecco's modified Eagle's medium (DMEM) containing 10% FCS, penicillin, and streptomycin. Before injection, the tumor cells were detached by trypsinization, washed once in cold DMEM containing 10% FCS, and once in cold serum-free medium. Cell viability was usually greater than 85%, as determined by trypan blue exclusion. The dorsal skin of C57BL/6 mice was shaved 1 day before injection. The mice were anesthetized by Metofane inhalation, and tumor cells (500,000) trypan blue-excluding cells in 100 μl) were injected intravenously into the tail vein. In the first protocol, one intraperitoneal dose of placental lysate extract (1 μg) was injected one day prior to LLC cell injection. The second protocol involved administration of placental lysate (1 μg) at days -1, 1, 4, and 7 relative to tumor cell inoculation. Control mice were injected with LLC tumor cells without placental extract. Animals were sacrificed 3 weeks after tumor inoculation. To quantify the number of pulmonary metastases in these animals, mice were killed by CO_2 narcosis after which the lungs were removed, rinsed in PBS, and placed in Bouin's fixative for at least 24 h. The fixed lungs were carefully separated into individual lobes with forceps and the numbers of surface metastases (appearing as white foci against a yellow background) were counted in each lobe with the aid of a dissecting microscope at 4× magnification (total metastatic foci), or by naked eye (large metastatic foci).

Heterotopic tumor growth experiments were also conduced in which C57BL/6 mice were inoculated with LLC cells according to the second protocol described above. Briefly, C57BL/6 mice were implanted with 5×10^5 LLC cells subcutaneously in the flank and these mice were either treated with placental lysate extract on days -1, 1, 4 and 7 relative to tumor implantation or were left untreated (controls). At 15, 20, 25 and 50 days following implantation, tumor measurements were taken with a caliper by a blinded observer and the results were expressed as tumor volume (mm^3).

Results

Placental extracts inhibit proliferation of cancer cells. While the concept of bone marrow-induced tumor angiogenesis is well known (37-43), there are reports that bone marrow mesenchymal stem cells inhibit cancer growth through secretion of soluble factors (44-46). One factor that has been shown to selectively inhibit cancer cell proliferation, Reptimed, was isolated from bone marrow through the use of solid phase adsorption C-18 cartridges (47, 48). Here we sought to determine whether a similar protocol might be applicable to identify placenta-derived cancer suppressive activity since previous work has described clinical responses with placental lysates (34, 35). Proliferation of the myelomonocytic cell line HL-60 (Figure 1A), androgen-dependent prostate cancer cell line LNCaP (Figure 1B), androgen-independent prostate cancer cell line PC-3 (Figure 1C) and mouse Lewis Lung Carcinoma (LLC) (Figure 1D) was shown to be inhibited by placental lysate extracts. Inhibition was dose-dependent for all cells except the PC-3 cell line, which demonstrated high sensitivity to the inhibitory effects of the lowest concentration of the placental lysate extract.

Suppression of proliferation associated with induction of differentiation. In the dose escalation studies described in Figure 1 we noted that tumor cell lines treated with placental lysate extract did not display differences in viability compared to non-treated control cells (data not shown). We therefore sought to investigate whether placental extract might induce terminal differentiation of HL-60 cells, a human acute promyelocytic cell line, into mature monocytes. Numerous agents have been shown to induce differentiation of HL-60 cells, including phorbol myristate acetate, granulocyte macrophage colony stimulating factor, and dimethyl sulfoxide (49, 50).

Treatment of HL-60 cells with cord blood nucleated cells for 48 h resulted in a dose-dependent induction of differentiation as determined by the percentage of adherent cells that possessed a monocytic morphology (Figure 2A). To determine whether differentiation was associated with the hematopoietic stem cell compartment, $CD34^+$ cells were isolated from cord blood and co-cultured with HL-60 cells. Much lower concentrations (10-fold less) of $CD34^+$ cells were required to induce a 50% differentiation response compared to unpurified cord blood mononuclear cells (Figure 2B). When cord blood mononuclear cells that were depleted of $CD34^+$ cells were added to cultures, no changes in the frequencies of differentiating HL-60 cells were observed compared to untreated cultures (Figure 2C). These data suggest that the $CD34^+$ stem cell compartment induces cancer cell differentiation.

To determine whether placental lysate extracts possessed a similar ability to induce tumor cell differentiation, increasing doses of material isolated from C-18 cartridges were added to the culture. A dose-dependent stimulation of differentiation of HL-60 cells was observed (Figure 2D). Strikingly, utilization of the same protocol for isolating cord blood mononuclear cell lysate extract revealed a similar inhibitory effect (Figure 2E). Lysate from cord blood mononuclear cells

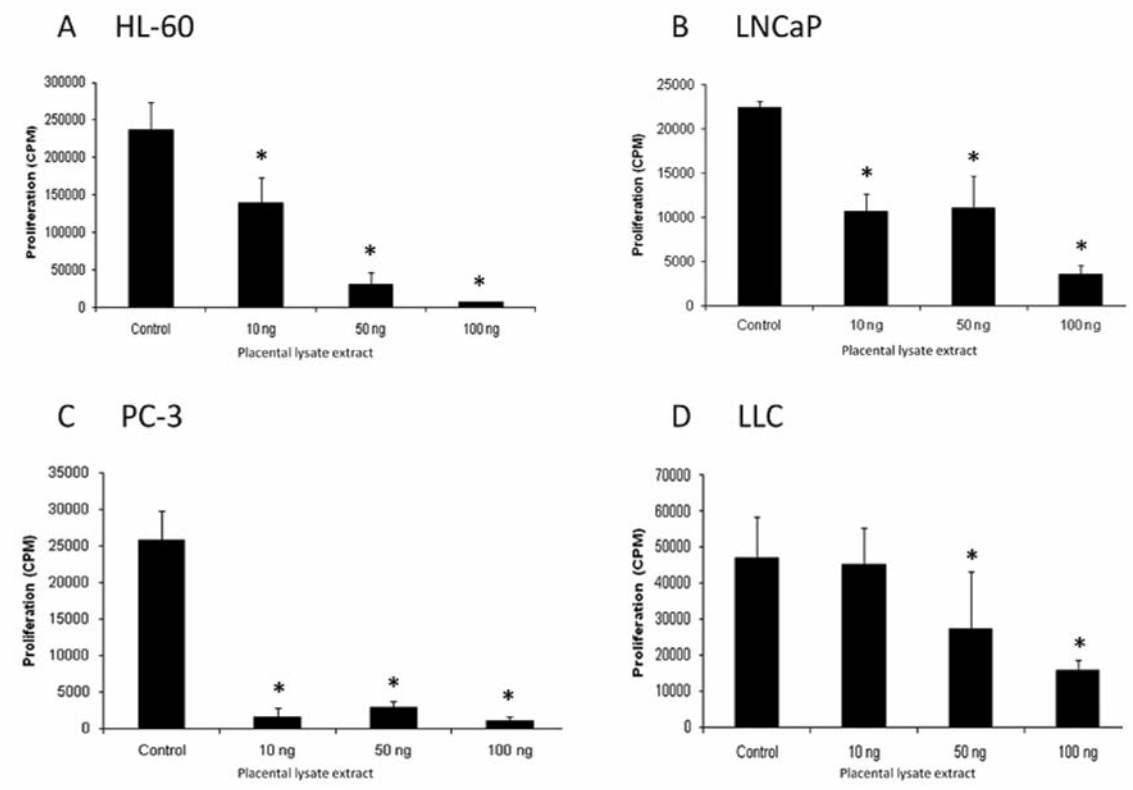

Figure 1. *Placental lysate extract inhibits proliferation of cancer cell lines. HL-60 (A), LNCaP (B), PC-3 (C) and LLC (D) cells were plated (1×10^5 cells per well) in 96 well plates and cultured for 72 h in the presence of the amount of indicated placental lysate extract. Cultures were pulsed with 1 μCi of [^{3}H]thymidine for the last 16 h and proliferation was assessed by scintillation counting. Results are expressed as the mean CPM of triplicate cultures±SEM. (*) denotes a statistically significant difference ($p<0.05$) of placental lysate extract-treated cultures from control (untreated) cultures, as determined using the Student's t-test.*

that were depleted of $CD34^+$ cells did not induce differentiation of HL-60 cells (Figure 2F), once again supporting the idea that inhibition of tumor cell growth is stem cell-dependent. We attempted to generate lysate from purified $CD34^+$ cells using C-18 columns, however the small numbers of isolated cells prevented us from harvesting sufficient concentrations of lysate to perform the experiments.

In vivo effects of placental lysate extracts on LLC tumor model. Previous studies have demonstrated that bone marrow extracts have inhibitory activity on tumors *in vitro* and *in vivo* (47, 48). We therefore used the clinically relevant LLC lung metastasis model with two protocols. In the first protocol, one intraperitoneal dose of placental lysate was administered one day prior to LLC tumor cell injection. Quantification of the numbers of lung metastases 3 weeks later revealed that placental lysate extract had a significant effect on inhibiting tumor growth (Figure 3). In the second protocol, placental lysate extract was administered more frequently, on days -1, 1, 4 and 7 relative to tumor cell inoculation. The results showed that several placental lysate injections had a greater effect on blocking tumor growth as compared to a single injection (Figure 3). We therefore chose to use protocol 2 in an *in vivo* heterotopic tumor model to test the effects of placental lysate extract on tumor growth. C57BL/6 mice were implanted with 5×10^5 LLC cells subcutaneously in the flank. Four mice served as controls and seven mice were treated with placental lysate extract according to protocol 2. Of the four control mice, one died at day 25 and the remaining three succumbed to tumors by day 50. In contrast, all seven treated mice were alive by day 50, of which three had no tumors detectable by palpation (Table I). These results demonstrate that placental lysate has an inhibitory effect on tumor growth *in vivo*.

Discussion

A relationship between tumors and stem cells has always been postulated. However, whether this relationship is mutually beneficial or antagonistic has been a matter of debate. While it is relatively well established that bone

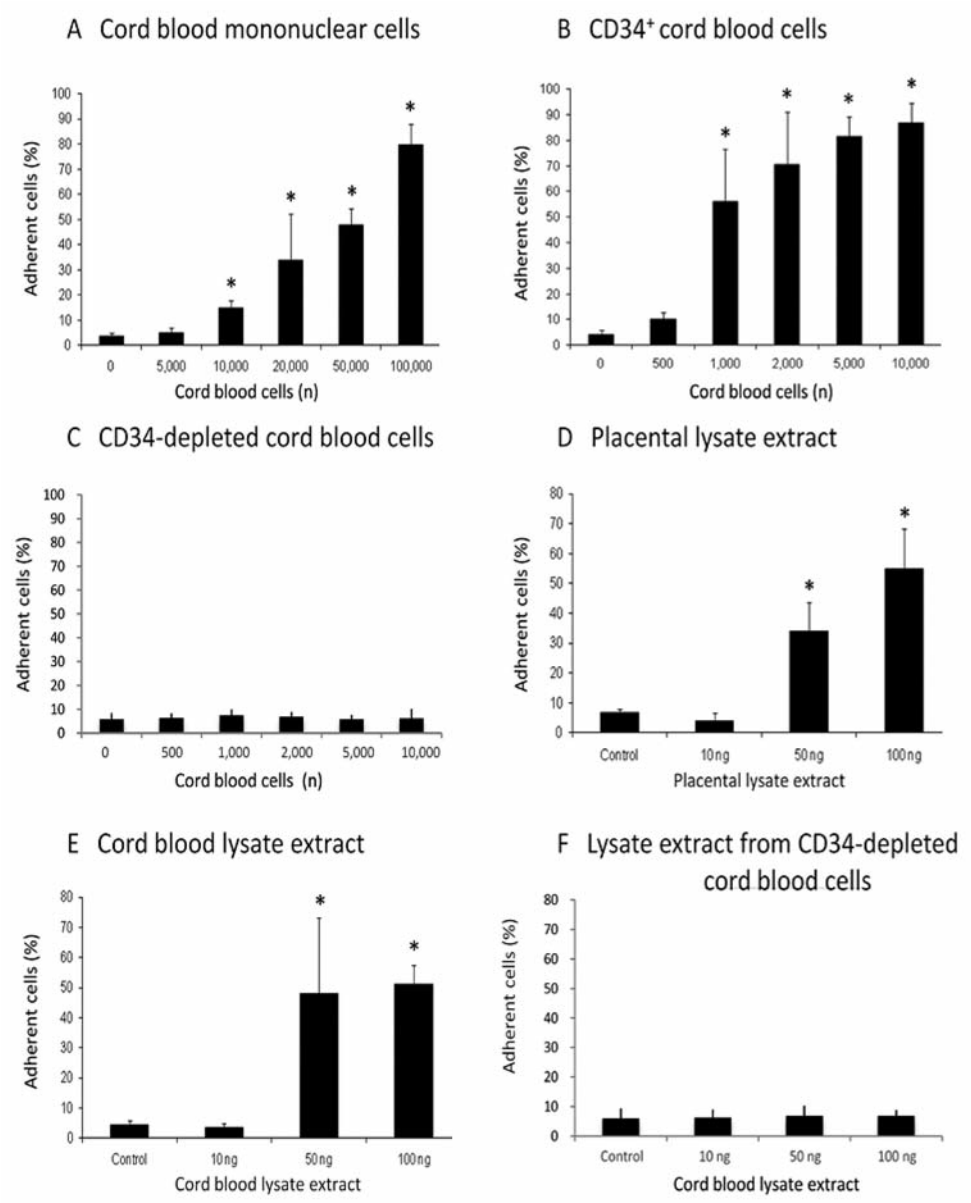

Figure 2. *Cord blood stem cells and placental extracts induce HL-60 differentiation. HL-60 cells were plated (1×10^5 cells per well) in 96-well plates and cultured for 48 h in the presence of the indicated number of cord blood nucleated cells (A); cord blood CD34+ cells (B), CD34-depleted cord blood cells (C), placental lysate extract (D), cord blood lysate extract (E), and lysate extract from CD34-depleted cord blood cells (F). Control wells were not treated with placental or cord blood lysate extracts. Differentiation of HL-60 cells was quantified as the percentage of adherent cells by a blinded observer. Results are expressed as the mean CPM of triplicate cultures±SEM. (*) denotes a statistically significant difference ($p<0.05$) between cultures treated with cells or lysate extracts vs. control (untreated) cultures Student's t-test.*

marrow progenitor cells are involved in tumor vasculogenesis (51, 52), numerous reports also describe inhibition of tumor growth by stem cell administration (53, 54). The emergence of the notion of "cancer stem cells" has brought to light novel mechanisms by which stem cells may affect cancer growth. In one such scenario, cancer stem cells are believed to enter the cell cycle and increase aggressiveness in response to injury. This is the same property that the healthy stem cells exhibit: after injury they undergo an induced reparative response (55). If cancer stem cell aggressiveness is a result of an aberrant healing response, then administration of agents that accelerate healing/inhibit inflammation should reduce cancer aggressiveness. This notion has been supported by studies in which cyclooxygenase-2 inhibitors and acetaminophen (56) have been shown to reduce cancer stem cell proliferation. Other examples include the use of

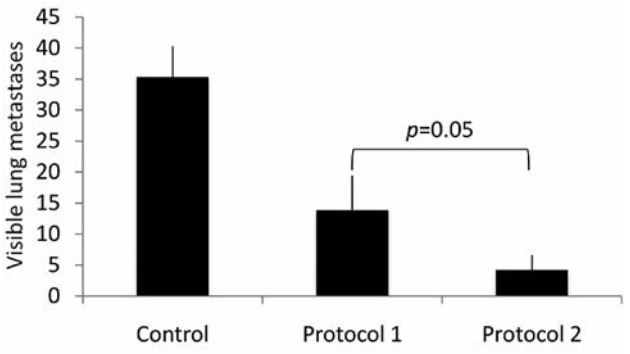

Figure 3. *Inhibition of lung metastasis using placental extract in the Lewis lung carcinoma (LLC) murine model of lung cancer. C57BL/6 mice (8 per group) were injected i.v. with 5×10^5 LLC cells and sacrificed after 3 weeks. Lung histology was performed and metastases were enumerated as described in the Materials and Methods. Animals were subjected to protocol 1 (1 μg placental lysate extract administered 1 day prior to tumor cell inoculation) or protocol 2 (1 μg placental lysate extract administered on days -1, 1, 4, and 7 relative to the day of tumor cell inoculation).*

Table I. *The effects of placental lysate extract on tumor volume. C57BL/6 mice implanted subcutaneously with Lewis lung carcinoma (LLC) cells (5×10^5 cells/recipient) were untreated (control) or treated with placental lysate extract (1 μg) at days –1, 1, 4 and 7 post-tumor implantation. The size of the tumors (mm^3) and the survival were monitored for 50 days post-implantation.*

	Tumor volume (mm^3) at days post-tumor implant			
Animal	Day 15	Day 20	Day 25	Day 50
1 Control	65	133	Dead	–
2 Control	103	212	300	Dead
3 Control	45	205	331	Dead
4 Control	74	122	153	Dead
5 Treated	123	34	36	8
6 Treated	35	48	93	No tumor
7 Treated	65	58	118	32
8 Treated	30	47	1	No tumor
9 Treated	33	54	40	14
10 Treated	62	55	3	No tumor
11 Treated	55	45	38	22

differentiation agents such as bone morphogenetic protein 4 in the treatment of aggressive tumors not curable by chemotherapy (57). Indeed, there may be several other comparisons made between neoplastic and healthy stem cells. For example, the marker CD133 is known to reside on non-malignant tissue resident stem cells. CD133 positive stem cells have been described in liver stem cell cells (oval cells) (58), prostate stem cells (59), muscle stem cells (60), hematopoietic stem cells (61), and stem cells of the small intestine (62). CD133 has also been detected to date on tumor stem cells of the following tissue of origin: colon (63-65), ovarian (66), liver (67), brain (68-70), prostate (71), and head and neck cancer (72). Numerous other markers and properties of healthy stem cells have been associated with cancer stem cells; for example, expression of CD44 (73), c-kit (74), multiple drug resistance protein (75-78), and decay-accelerating factor (79). Thus, one possible anticancer mechanism of placental extracts is by production of factors that mediate tumor cell differentiation.

Early studies demonstrated that bone marrow-derived factors have the ability to inhibit cancer cell proliferation and tumor growth *in vivo* (47, 48). In those studies, factors inhibiting tumor cell proliferation were isolated using solid-phase adsorption C-18 cartridges. In the present study we utilized identical methods in order to determine whether such factors are present in placental cell lysates. We demonstrated inhibition of leukemia, prostate cancer, and lung cancer cell lines by these extracts. We also observed that cord blood mononuclear cells also had similar cancer inhibitory properties that were contained within the $CD34^+$ fraction. Although it is impossible to state whether the activity found in the placental lysate extract is the same as that observed associated with the $CD34^+$ cells, we believe these data provide an important starting point for further investigations.

While tumor cell differentiation may be a mechanism of action of placental lysate extract based on our findings, other mechanisms cannot be ruled out. Numerous agents have been shown to induce differentiation of HL-60 cells and we have not yet demonstrated differentiation in the solid tumor cell lines. Despite this, there is some support for the possibility of differentiation induced by the placental lysate extract. For example, it is reported that stem cells have a propensity to differentiate in the absence of de-differentiation stimuli; accordingly, it may be possible that pro-differentiation-inducing factors are found in the placental isolate. Studies by Kulesa and co-workers showed that injection of melanoma cells into fertilized chicken eggs resulted in differentiation into non-tumor neural crest cells (80). Another possible explanation for the *in vivo* results is that administration of the extracts from human cells induces a xenogeneic response. Indeed, it has been published that human umbilical vein endothelial cell administration in animals and humans elicits an anticancer immune response (81-83).

In conclusion, the current data suggest that placental lysate extracts possess an anticancer activity *in vitro* and *in vivo*. This activity was associated with the $CD34^+$ cell compartment. Further investigation into whether the mesenchymal stem cell compartment in placentas possesses similar inhibitory activity against cancer is required. Given the non-toxic nature of the placental lysate extracts and the possible unique mechanism of action, we believe that future studies are warranted to determine the feasibility of clinical development.

References

1 Attar EC and Scadden DT: Regulation of hematopoietic stem cell growth. Leukemia *18*: 1760-1768, 2004.

2 Ema H and Nakauchi H: Self-renewal and lineage restriction of hematopoietic stem cells. Curr Opin Genet Dev *13*: 508-512, 2003.

3 Craig CE, Quaglia A, Selden C, Lowdell M, Hodgson H and Dhillon AP: The histopathology of regeneration in massive hepatic necrosis. Semin Liver Dis *24*: 49-64, 2004.

4 Kaur P, Li A, Redvers R and Bertoncello I: Keratinocyte stem cell assays: an evolving science. J Investig Dermatol Symp Proc *9*: 238-247, 2004.

5 Rookmaaker MB, Verhaar MC, van Zonneveld AJ and Rabelink TJ: Progenitor cells in the kidney: biology and therapeutic perspectives. Kidney Int *66*: 518-522, 2004.

6 Chaudhari M, Cornelius JG, Schatz D, Peck AB and Ramiya VK: Pancreatic stem cells: a therapeutic agent that may offer the best approach for curing type 1 diabetes. Pediatr Diabetes *2*: 195-202, 2001.

7 Brittan M and Wright NA: Gastrointestinal stem cells. J Pathol *197*: 492-509, 2002.

8 Martino G: How the brain repairs itself: new therapeutic strategies in inflammatory and degenerative CNS disorders. Lancet Neurol *3*: 372-378, 2004.

9 Liu L, Saldanha SN, Pate MS, Andrews LG and Tollefsbol TO: Epigenetic regulation of human telomerase reverse transcriptase promoter activity during cellular differentiation. Genes Chromosomes Cancer *41*: 26-37, 2004.

10 Lodygin D, Diebold J and Hermeking H: Prostate cancer is characterized by epigenetic silencing of 14-3-3sigma expression. Oncogene *23(56)*: 9034-9041, 2004.

11 Mhawech P, Benz A, Cerato C, Greloz V, Assaly M, Desmond JC, Koeffler HP, Lodygin D, Hermeking H, Herrmann F and Schwaller J: Downregulation of 14-3-3sigma in ovary, prostate and endometrial carcinomas is associated with CpG island methylation. Mod Pathol *18(3)*: 340-8, 2005.

12 Stoff-Khalili MA, Rivera AA, Mathis JM, Banerjee NS, Moon AS, Hess A, Rocconi RP, Numnum TM, Everts M, Chow LT, Douglas JT, Siegal GP, Zhu ZB, Bender HG, Dall P, Stoff A, Pereboeva L and Curiel DT: Mesenchymal stem cells as a vehicle for targeted delivery of CRAds to lung metastases of breast carcinoma. Breast Cancer Res Treat *105*: 157-167, 2007.

13 Kucerova L, Altanerova V, Matuskova M, Tyciakova S and Altaner C: Adipose tissue-derived human mesenchymal stem cells mediated prodrug cancer gene therapy. Cancer Res *67*: 6304-6313, 2007.

14 Birnbaum T, Roider J, Schankin CJ, Padovan CS, Schichor C, Goldbrunner R and Straube A: Malignant gliomas actively recruit bone marrow stromal cells by secreting angiogenic cytokines. J Neurooncol *83*: 241-247, 2007.

15 Kang SG, Jeun SS, Lim JY, Kim SM, Yang YS, Oh WI, Huh PW and Park CK: Cytotoxicity of human umbilical cord blood-derived mesenchymal stem cells against human malignant glioma cells. Childs Nerv Syst *24(3)*: 293-302, 2008.

16 Ramasamy R, Lam EW, Soeiro I, Tisato V, Bonnet D and Dazzi F: Mesenchymal stem cells inhibit proliferation and apoptosis of tumor cells: impact on *in vivo* tumor growth. Leukemia *21*: 304-310, 2007.

17 Khakoo AY, Pati S, Anderson SA, Reid W, Elshal MF, Rovira II, Nguyen AT, Malide D, Combs CA, Hall G, Zhang J, Raffeld M, Rogers TB, Stetler-Stevenson W, Frank JA, Reitz M and Finkel T: Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma. J Exp Med *203*: 1235-1247, 2006.

18 Galie M, Konstantinidou G, Peroni D, Scambi I, Marchini C, Lisi V, Krampera M, Magnani P, Merigo F, Montani M, Boschi F, Marzola P, Orrù R, Farace P, Sbarbati A and Amici A: Mesenchymal stem cells share molecular signature with mesenchymal tumor cells and favor early tumor growth in syngeneic mice. Oncogene *27(18)*: 2542-51, 2008.

19 Zhu W, Xu W, Jiang R, Qian H, Chen M, Hu J, Cao W, Han C and Chen Y: Mesenchymal stem cells derived from bone marrow favor tumor cell growth *in vivo*. Exp Mol Pathol *80*: 267-274, 2006.

20 Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R and Weinberg RA: Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature *449*: 557-563, 2007.

21 Li H, Fan X, Kovi RC, Jo Y, Moquin B, Konz R, Stoicov C, Kurt-Jones E, Grossman SR, Lyle S, Rogers AB, Montrose M and Houghton J: Spontaneous Expression of Embryonic Factors and p53 Point Mutations in Aged Mesenchymal Stem Cells: A Model of Age-Related Tumorigenesis In Mice. Cancer Res *67*: 10889-10898, 2007.

22 de Vries EG, Vellenga E, Kluin-Nelemans JC and Mulder NH: The happy destiny of frozen haematopoietic stem cells: from immature stem cells to mature applications. Eur J Cancer *40*: 1987-1992, 2004.

23 Rocha V, Sanz G and Gluckman E: Umbilical cord blood transplantation. Curr Opin Hematol *11*: 375-385, 2004.

24 Michel G, Rocha V, Chevret S, Arcese W, Chan KW, Filipovich A, Takahashi TA,Vowels M, Ortega J, Bordigoni P, Shaw PJ, Yaniv I, Machado A, Pimentel P, Fagioli F, Verdeguer A, Jouet JP, Diez B, Ferreira E, Pasquini R, Rosenthal J, Sievers E, Messina C, Iori AP, Garnier F, Ionescu I, Locatelli F and Gluckman E: Unrelated cord blood transplantation for childhood acute myeloid leukemia: a Eurocord Group analysis. Blood *102*: 4290-4297, 2003.

25 Hall JG, Martin PL, Wood S and Kurtzberg J: Unrelated umbilical cord blood transplantation for an infant with beta-thalassemia major. J Pediatr Hematol Oncol *26*: 382-385, 2004.

26 Chaouat G, Kolb JP, Riviere M and Lankar D: Immunoactive products of placenta. V: Soluble factors from murine placenta can block effector stages of maternal antipaternal cell-mediated immunity. American journal of reproductive immunology and microbiology. Am J Reprod Immunol Microbiol *12(3)*: 70-77, 1986.

27 Nair B and Elmore AR: Final report on the safety assessment of human placental protein, hydrolyzed human placental protein, human placental enzymes, human placental lipids, human umbilical extract, placental protein, hydrolyzed placental protein, placental enzymes, placental lipids, and umbilical extract. Int J Toxicol *21(Suppl 1)*: 81-91, 2002.

28 Prather WR, Toren A, Meiron M, Ofir R, Tschope C and Horwitz EM: The role of placental-derived adherent stromal cell (PLX-PAD) in the treatment of critical limb ischemia. Cytotherapy *11(4)*: 427-434, 2009.

29 Riordan NH, Chan K, Marleau AM and Ichim TE: Cord blood in regenerative medicine: do we need immune suppression? J Transl Med *5*: 8, 2007.
30 Ichim TE, Solano F, Brenes R, Glenn E, Chang J, Chan K and Riordan NH: Placental mesenchymal and cord blood stem cell therapy for dilated cardiomyopathy. Reprod Biomed Online *16*: 898-905, 2008.
31 Yang WZ, Zhang Y, Wu F, Min WP, Minev B, Zhang M, Luo XL, Ramos F, Ichim TE, Riordan NH, Hu X: Safety evaluation of allogeneic umbilical cord blood mononuclear cell therapy for degenerative conditions. J Transl Med *8*: 75, 2010.
32 Ichim TE, Solano F, Lara F, Paris E, Ugalde F, Rodriguez JP, Minev B, Bogin V, Ramos F, Woods EJ, Murphy MP, Patel AN, Harman RJ and Riordan NH: Feasibility of combination allogeneic stem cell therapy for spinal cord injury: a case report. Int Arch Med *3*: 30, 2010.
33 Yang WZ, Zhang Y, Wu F, Zhang M, Cho SC, Li CZ, Li SH, Shu GJ, Sheng YX, Zhao N, Tang Y, Jiang S, Jiang S, Gandjian M, Ichim TE and Hu X: Human umbilical cord blood-derived mononuclear cell transplantation: case series of 30 subjects with hereditary ataxia. J Transl Med *9*: 65, 2011.
34 Harandi A: Immunoplacental therapy, a potential multi-epitope cancer vaccine. Med Hypotheses *66*: 1182-1187, 2006.
35 Corocleanu M: A possible "universal" cancer vaccine that might cause an immune response against emerging cancer cells that originate from any tissue. Med Hypotheses *70*: 381-383, 2008.
36 Zhong Z, Kusznieruk KP, Popov IA, Riordan NH, Izadi H, Yijian L, Sher S, Szczurko OM, Agadjanyan MG, Tullis RH, Harandi A, Reznik BN, Mamikonyan GV and Ichim TE: Induction of antitumor immunity through xenoplacental immunization. J Transl Med *4*: 22, 2006.
37 Melero-Martin JM and Dudley AC: Concise review: vascular stem cells and tumor angiogenesis. Stem Cells *29*: 163-168, 2011.
38 Defresne F, Bouzin C, Grandjean M, Dieu M, Raes M, Hatzopoulos AK, Kupatt C and Feron O: Preconditioned Endothelial Progenitor Cells Reduce Formation of Melanoma Metastases through SPARC-Driven Cell-Cell Interactions and Endocytosis. Cancer Res *71*: 4748-4757, 2011.
39 Takemoto Y, Li TS, Kubo M, Ohshima M, Ueda K, Harada E, Enoki T, Okamoto M, Mizukami Y, Murata T and Hamano K: Operative injury accelerates tumor growth by inducing mobilization and recruitment of bone marrow-derived stem cells. Surgery *149*: 792-800, 2011.
40 Real C, Remedio L, Caiado F, Igreja C, Borges C, Trindade A, Pinto-do-Ó P, Yagita H, Duarte A and Dias S: Bone marrow-derived endothelial progenitors expressing Delta-like 4 (Dll4) regulate tumor angiogenesis. PloS One *6*: e18323, 2011.
41 Suzuki K, Sun R, Origuchi M, Kanehira M, Takahata T, Itoh J, Umezawa A, Kijima H, Fukuda S and Saijo Y: Mesenchymal stromal cells promote tumor growth through the enhancement of neovascularization. Mol Med *17*: 579-587, 2011.
42 Li A, Cheng XJ, Moro A, Singh RK, Hines OJ and Eibl G: CXCR2-dependent endothelial progenitor cell mobilization in pancreatic cancer growth. Transl Oncol *4*: 20-28, 2011.
43 Fang S and Salven P: Stem cells in tumor angiogenesis. J Mol Cell Cardiol *50*: 290-295, 2011.
44 Jiao H, Yang B, Guan F, Li J, Shan H, Song L, Hu X, Liang S, Du Y and Jiang C: The mixed human umbilical cord blood-derived mesenchymal stem cells show higher antitumor effect against C6 cells than the single *in vitro*. Neurol Res *33*: 405-414, 2011.
45 Dasari VR, Velpula KK, Kaur K, Fassett D, Klopfenstein JD, Dinh DH, Gujrati M and Rao JS: Cord blood stem cell-mediated induction of apoptosis in glioma downregulates X-linked inhibitor of apoptosis protein (XIAP). PloS One *5*: e11813, 2010.
46 Kang SG, Jeun SS, Lim JY, Kim SM, Yang YS, Oh WI, Huh PW and Park CK: Cytotoxicity of human umbilical cord blood-derived mesenchymal stem cells against human malignant glioma cells. Childs Nerv Syst *24*: 293-302, 2008.
47 DeKoter RP, Parsons MF, Fong WG, Lin CH, Khalil W, Howson-Jan K and Singhal SK: Suppression of myelopoiesis and myeloid leukemia cell line proliferation by a novel bone marrow-derived factor, reptimed. Cell Immunol *175*: 120-127, 1997.
48 Khandaker MH, Kadhim SA, Ichim TE, Howson-Jan K, Chin J and Singhal SK: Prevention of bladder tumor formation in mice by a novel bone marrow-derived factor, reptimed. Anticancer Res *20*: 183-189, 2000.
49 Ashkenazi A and Marks RS: Luminol-dependent chemiluminescence of human phagocyte cell lines: comparison between DMSO differentiated PLB 985 and HL 60 cells. Luminescence *24*: 171-177, 2009.
50 Mazzei GJ, Bernasconi LM, Lewis C, Mermod JJ, Kindler V and Shaw AR: Human granulocyte-macrophage colony-stimulating factor plus phorbol myristate acetate stimulate a promyelocytic cell line to produce an IL-1 inhibitor. J Immunol *145*: 585-591, 1990.
51 Le Bourhis X, Romon R and Hondermarck H: Role of endothelial progenitor cells in breast cancer angiogenesis: from fundamental research to clinical ramifications. Breast Cancer Res Treat *120*: 17-24, 2010.
52 Dome B, Dobos J, Tovari J, Paku S, Kovacs G, Ostoros G and Timar J: Circulating bone marrow-derived endothelial progenitor cells: characterization, mobilization, and therapeutic considerations in malignant disease. Cytometry A *73*: 186-193, 2008.
53 Dong W, Du J, Shen H, Gao D, Li Z, Wang G, Mu X and Liu Q: Administration of embryonic stem cells generates effective antitumor immunity in mice with minor and heavy tumor load. Cancer Immunol Immunother *59*: 1697-1705, 2010.
54 Han X, Meng X, Yin Z, Rogers A, Zhong J, Rillema P, Jackson JA, Ichim TE, Minev B, Carrier E, Patel AN, Murphy MP, Min WP and Riordan NH: Inhibition of intracranial glioma growth by endometrial regenerative cells. Cell Cycle 8: 606-610, 2009.
55 Cottage CT, Bailey B, Fischer KM, Avitable D, Collins B, Tuck S, Quijada P, Gude N, Alvarez R, Muraski J, Sussman MA: Cardiac progenitor cell cycling stimulated by pim-1 kinase. Circ Res *106*: 891-901, 2010.
56 Takehara M, Hoshino T, Namba T, Yamakawa N and Mizushima T: Acetaminophen-induced differentiation of human breast cancer stem cells and inhibition of tumor xenograft growth in mice. Biochem Pharmacol *81*: 1124-1135, 2011.
57 Piccirillo SG, Reynolds BA, Zanetti N, Lamorte G, Binda E, Broggi G, Brem H, Olivi A, Dimeco F and Vescovi AL: Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. Nature *444*: 761-765, 2006.
58 Rountree CB, Barsky L, Ge S, Zhu J, Senadheera S and Crooks GM: A CD133-expressing murine liver oval cell population with bilineage potential. Stem Cells *25*: 2419-2429, 2007.
59 Richardson GD, Robson CN, Lang SH, Neal DE, Maitland NJ and Collins AT: CD133, a novel marker for human prostatic epithelial stem cells. J Cell Sci *117*: 3539-3545, 2004.

60 Torrente Y, Belicchi M, Sampaolesi M, Pisati F, Meregalli M, D'Antona G, Tonlorenzi R, Porretti L, Gavina M, Mamchaoui K, Pellegrino MA, Furling D, Mouly V, Butler-Browne GS, Bottinelli R, Cossu G and Bresolin N: Human circulating AC133(+) stem cells restore dystrophin expression and ameliorate function in dystrophic skeletal muscle. J Clin Invest *114*: 182-195, 2004.
61 Wognum AW, Eaves AC and Thomas TE: Identification and isolation of hematopoietic stem cells. Arch Med Res *34*: 461-475, 2003.
62 Katoh Y and Katoh M: Comparative genomics on PROM1 gene encoding stem cell marker CD133. Int J Mol Med *19*: 967-970, 2007.
63 O'Brien CA, Pollett A, Gallinger S and Dick JE: A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature *445*: 106-110, 2007.
64 Ieta K, Tanaka F, Haraguchi N, Kita Y, Sakashita H, Mimori K, Matsumoto T, Inoue H, Kuwano H and Mori M: Biological and Genetic Characteristics of Tumor-Initiating Cells in Colon Cancer. Ann Surg Oncol *15*: 638-48, 2008.
65 Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C and De Maria R: Identification and expansion of human colon-cancer-initiating cells. Nature *445*: 111-115, 2007.
66 Ferrandina G, Bonanno G, Pierelli L, Perillo A, Procoli A, Mariotti A, Corallo M, Martinelli E, Rutella S, Paglia A, Zannoni G, Mancuso S and Scambia G: Expression of CD133-1 and CD133-2 in ovarian cancer. Int J Gynecol Cancer *18(3)*: 506-14, 2007.
67 Yin S, Li J, Hu C, Chen X, Yao M, Yan M, Jiang G, Ge C, Xie H, Wan D, Yang S, Zheng S and Gu J: CD133 positive hepatocellular carcinoma cells possess high capacity for tumorigenicity. Int J Cancer *120*: 1444-1450, 2007.
68 Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J and Dirks PB: Identification of a cancer stem cell in human brain tumors. Cancer Res *63*: 5821-5828, 2003.
69 Hemmati HD, Nakano I, Lazareff JA, Masterman-Smith M, Geschwind DH, Bronner-Fraser M and Kornblum HI: Cancerous stem cells can arise from pediatric brain tumors. Proc Natl Acad Sci USA *100*: 15178-15183, 2003.
70 Beier D, Hau P, Proescholdt M, Lohmeier A, Wischhusen J, Oefner PJ, Aigner L, Brawanski A, Bogdahn U and Beier CP: CD133(+) and CD133(–) glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. Cancer Res *67*: 4010-4015, 2007.
71 Wei C, Guomin W, Yujun L and Ruizhe Q: Cancer Stem-like Cells in Human Prostate Carcinoma Cells DU145: The Seeds of the Cell Line? Cancer Biol Ther *6*: 763-768, 2007.
72 Harper LJ, Piper K, Common J, Fortune F and Mackenzie IC: Stem cell patterns in cell lines derived from head and neck squamous cell carcinoma. J Oral Pathol Med *36*: 594-603, 2007.
73 Sales KM, Winslet MC and Seifalian AM: Stem Cells and Cancer: An Overview. Stem Cell Rev *3(4)*: 249-55, 2007.
74 Lennartsson J and Ronnstrand L: The stem cell factor receptor/c-Kit as a drug target in cancer. Curr Cancer Drug Targets *6*: 65-75, 2006.
75 Ho MM, Ng AV, Lam S and Hung JY: Side population in human lung cancer cell lines and tumors is enriched with stem-like cancer cells. Cancer Res *67*: 4827-4833, 2007.
76 Salmaggi A, Boiardi A, Gelati M, Russo A, Calatozzolo C, Ciusani E, Sciacca FL, Ottolina A, Parati EA, La Porta C, Alessandri G, Marras C, Croci D and De Rossi M: Glioblastoma-derived tumorospheres identify a population of tumor stem-like cells with angiogenic potential and enhanced multidrug resistance phenotype. Glia *54*: 850-860, 2006.
77 Szotek PP, Pieretti-Vanmarcke R, Masiakos PT, Dinulescu DM, Connolly D, Foster R, Dombkowski D, Preffer F, Maclaughlin DT and Donahoe PK: Ovarian cancer side population defines cells with stem cell-like characteristics and Mullerian Inhibiting Substance responsiveness. Proc Natl Acad Sci USA *103*: 11154-11159, 2006.
78 Donnenberg VS and Donnenberg AD: Multiple drug resistance in cancer revisited: the cancer stem cell hypothesis. J Clin Pharmacol *45*: 872-877, 2005.
79 Xu JX, Morii E, Liu Y, Nakamichi N, Ikeda J, Kimura H and Aozasa K: High tolerance to apoptotic stimuli induced by serum depletion and ceramide in side-population cells: high expression of CD55 as a novel character for side-population. Exp Cell Res *313*: 1877-1885, 2007.
80 Kulesa PM, Kasemeier-Kulesa JC, Teddy JM, Margaryan NV, Seftor EA, Seftor RE and Hendrix MJ: Reprogramming metastatic melanoma cells to assume a neural crest cell-like phenotype in an embryonic microenvironment. Proc Natl Acad Sci USA *103*: 3752-3757, 2006.
81 Okaji Y, Tsuno NH, Tanaka M, Yoneyama S, Matsuhashi M, Kitayama J, Saito S, Nagura Y, Tsuchiya T, Yamada J, Tanaka J, Yoshikawa N, Nishikawa T, Shuno Y, Todo T, Saito N, Takahashi K and Nagawa H: Pilot study of anti-angiogenic vaccine using fixed whole endothelium in patients with progressive malignancy after failure of conventional therapy. Eur J Cancer *44*: 383-390, 2008.
82 Okaji Y, Tsuno NH, Kitayama J, Saito S, Takahashi T, Kawai K, Yazawa K, Asakage M, Hori N, Watanabe T, Shibata Y, Takahashi K and Nagawa H: Vaccination with autologous endothelium inhibits angiogenesis and metastasis of colon cancer through autoimmunity. Cancer Sci *95*: 85-90, 2004.
83 Chen XY, Zhang W, Wu S, Bi F, Su YJ, Tan XY, Liu JN and Zhang J: Vaccination with viable human umbilical vein endothelial cells prevents metastatic tumors by attack on tumor vasculature with both cellular and humoral immunity. Clin Cancer Res *12*: 5834-5840, 2006.

Received December 17, 2011
Revised February 25, 2012
Accepted February 29, 2012