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$CD34^+$ stem cells in normal placenta tissues and in placenta with intrauterine growth retardation

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Objective: The purpose of this study was to determine and characterize the expression, spreading and location of CD34 -positive cells in normal human placenta and in placenta in cases of intrauterine growth retardation (IUGR).

Patients and methods: Fifteen placentas from healthy parturient women with the normal course of pregnancy and healthy newborn baby, and ten placentas in cases with IUGR, when the baby's weight was lower than it should be for the stage of the pregnancy, were investigated histologically and immunohistochemically by means of horseradish immunoperoxidase-labeled monoclonal mouse anti-human CD34 antibody.

Results: In normal placenta when a healthy baby was born, $CD34^+$ cells were found throughout the villi in the endothelium of the vascular tree. Occasionally, extravascular scattered CD34-positive nucleated cells were found as well. Some CD34-positive cells were present in the blood vessel lumina. Additionally, weaker CD34-positive immunostaining was found in sparse cells in cyto- and syncytiotrophoblasts and in some decidual cells.

In cases with IUGR, a diminished density of trophoblast villi was found. Some villi were pathologically changed. In IUGR, also the intensity of CD34 expression was lower than in normal placenta. Meanwhile, more of decidual cells expressed CD34 immunoreactivity, and usually the intensity of the expression was higher.

Conclusion: The findings obtained in the present study show that there is a great number of $CD34^+$ cells in human placenta, both in normal and in IUGR-complicated pregnancies.

Key words: stem cells, $CD34^+$, human placenta, IUGR

INTRODUCTION

Stem cells display a capacity for self-renewal and have the ability to give rise to a great variety of more specialized progeny (1, 2). Much interest and efforts has focused on the therapeutic potential of stem cell technology to treat presently intractable diseases (3). Some adult stem cells are not lineage-restricted. It is now believed that these cells are able to give rise to other cell types in a new location, not normally present in their organ of origin. Some have claimed that since adult stem cells can be induced to differentiate into multiple cell lineages, embryonic stem cells from blastocysts and aborted fetuses will no longer be needed. The use of human embryonic stem cells has been accompanied by political and ethical obstacles. There are no such problems with adult stem cells, and this raises the possibility of repairing an individual's failing organ by transplanting autologous, *e.g.*, bone marrow stem cells to replace their diseased tissues, although it there a long way to go before this technology can be applied to humans. Tissues that can now be engineered with limited success using stem cells comprise a diverse range, from epithelial surface as skin, cornea and mucosal membrane to skeletal tissues.

The two main characteristics of stem cells are their capability of extensive self-renewal and their potential to differentiate into at least one, and usually more, mature cell type (4). Hematopoietic stem cells (HSC) are capable of differentiation into all of the mature peripheral blood cell types. Until recently, HSC were thought to reside exclusively in the bone marrow, with occasional leaks of these cells into peripheral blood. However, recently cells with HSC potential have been found to reside in nonhematopoietic organs as well, including muscle. Recent studies have shown that there is a higher than

expected plasticity of cells derived from the brain, fat, muscle and skin (5–11).

In the last years, umbilical cord and placental blood have become clinically relevant as an alternative source of hematopoietic stem and progenitor cells for the treatment of malignant and nonmalignant disorders (12). It has been shown (13) that both term and preterm umbilical cord blood contains a significantly higher number of early and committed progenitor cells as compared with adult peripheral blood. It has recently been demonstrated that umbilical cord blood contains sufficient numbers of hematopoietic progenitor cells to engraft larger size children and adult (14).

A major problem with the use of umbilical/placental blood is the limited blood volume that can be collected from a single donor (15). The total collection volume is about 60 ml, range 17–141 ml (16). The reported volumes vary widely, usually ranging from 40 to 200 ml.

Much emphasis is currently being placed on the environment in which a stem cell is placed – its niche.

A niche is a subset of tissue cells and extracellular substrates, which *in vivo* favours the existence of stem cell in the undifferentiated state. Integrins hold the cells in place, and in their absence cells leave the niche through either differentiation or apoptosis (17). Our immunohistochemical observations showed that in human placenta there is a varying number of stem cells. So placenta could serve as an additional source of such cells.

Although great leaps have been taken in the world of stem cell research, many questions remain unanswered. The clinical application of stem cell research has virtually no limits.

The objective of the current study was to analyze the presence of stem cells in human placenta.

PATIENTS AND METHODS

The study was conducted with human placenta. Permission to use human material was obtained from the local Ethical Committee prior to the study. Fresh human placentas were obtained immediately following spontaneous vaginal deliveries or Caesarean sections from the Opole Mother and Child Health Center, Opole, Poland.

Placenta specimens from 15 healthy parturient women with a normal course of pregnancy and healthy newborn baby and ten in the cases of IUGR were fixed in 10% neutral formalin, embedded in paraffin and processed for routine histological and immunohistochemical analysis.

Immunohistochemistry. The primary antibodies used were anti-human CD34 Type III monoclonal mouse IgG_1 (1:100, Cymbus Biotechnology Ltd, Charles Ford, UK).

Paraffin sections 5 μm thick were mounted on DAKO capillary slides (TechMate™, DAKO, Glostrup, Denmark), deparaffinized in xylene and rehydrated in graded ethanol series and 10 mM phosphate-buffered, 0.9 saline, pH 7.4 (PBS). For antigen retrieval, the slides were placed into Antigen Retrieval Buffer™ (DAKO) and microwaved in 10 mM citrate buffer, pH 6.0, in a microwave processing labstation for histology, microMED T/T Mega Histoprocessing Labstation (Milestone Inc., Atlanta, USA), for 10 minutes at 98 °C according to the manufacturer's program, then cooled at room temperature for 30 minutes, washed in PBS and immunostained automatically at 22 °C using the following protocol: 1) the primary antibody, diluted with DAKO ChemMate™ antibody diluent, for 1 hour; 2) secondary antibody containing biotinylated goat anti-mouse IgG antibodies (DAKO) for 30 minutes; 3) peroxidase block 3 times for 5 minutes; 4) peroxidase-conjugated streptavidin for 30 minutes; 5) HRP Substrate Buffer and 6) substrate solution containing 3,3´-diaminobenzidine tetrahydrochloride (ChemMate™ detection kit) for 15 minutes. Between each step, the sections were washed with DAKO ChemMate™ washing buffers three times and dried with absorbent pads.

Replacement of primary antibody with mouse or goat IgG diluted to the same concentration as the primary antibody were used as negative staining controls. After immunostaining, the sections were counterstained with haematoxylin or left without counterstaining, washed, dehydrated in ethanol series, cleared in xylene and mounted in a synthetic mounting medium (Mountex, Histolab Products AB, Gothenburg, Sweden).

Semi-quantitative microscopic assessment of immunohistochemical staining was performed under 400 × magnification (high power field). For general histological evaluation the slides were stained with haematoxylin and eosin.

RESULTS

In normal placenta we found the immunostaining for CD34+ in the endothelial cells, and occasionally in both cyto- and syncytiotrophoblast. In a normal placenta, when a healthy baby was born, CD34-positive cells were found throughout all villi in the endothelium in the vascular tree (Fig. 1). Occasionally scattered extravascular CD34-positive nucleated cells were found as well (Fig. 2). Additionally, weak CD34-positive immunostaining was found in some decidual cells (Fig. 3).

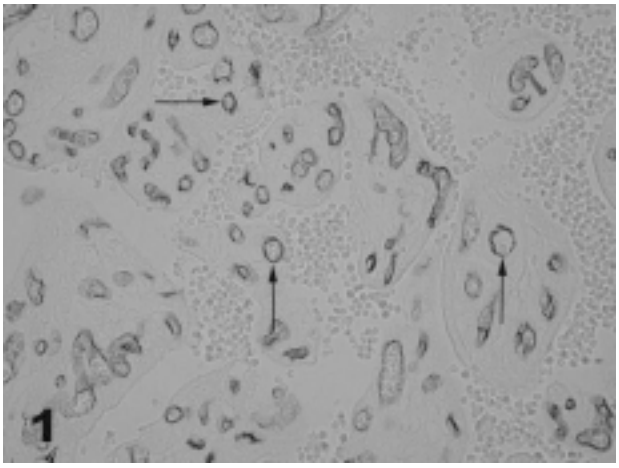

Fig. 1. Expression of CD34 stem cell marker in the endothelium (arrows) of the capillaries in normal human placenta. ×400

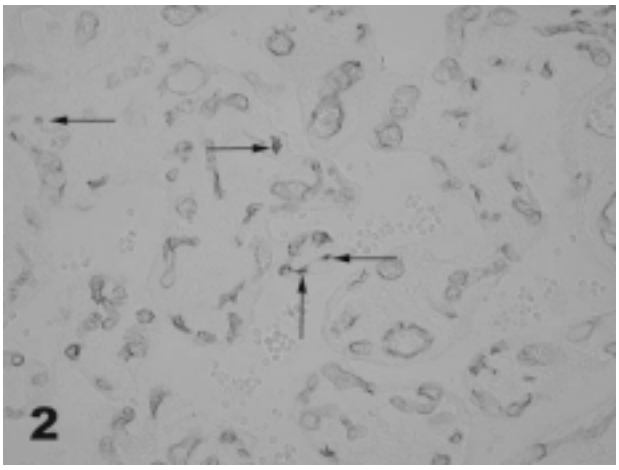

Fig. 2. Solitary CD34-positive cells (arrows) in normal human placenta. ×400

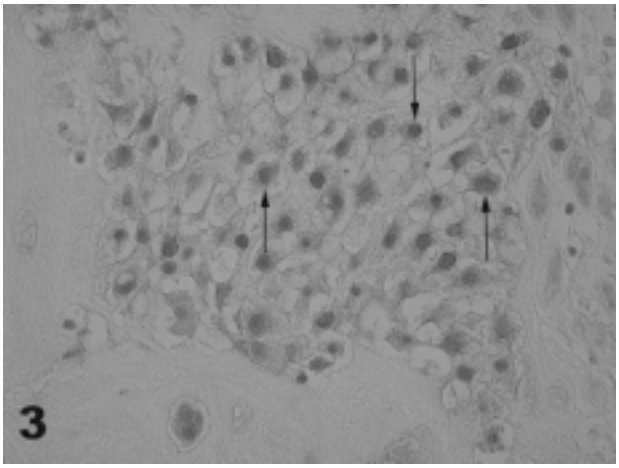

Fig. 3. Decidual $CD34^+$ cells (arrows). Counterstained with haematoxylin. ×400

In IUGR the diameter of the villi was reduced together with a reduction in the amount of villous tissue. The degree of vascularization within terminal villi was reduced. Increased perivillous fibrin deposits were found. The number of cytotrophoblast cells was reduced in IUGR. In parallel, an increased number of syncytiotrophoblast nuclei was noted in the IUGR group. These nuclei showed chromatin clumping. A significant reduction in the vascular surface density of stem and intermediate/terminal villi was found in IUGR. The expression of CD34 immunoreactivity was lower in the capillary endothelial cells of villi as well as in the trophoblast cells in placentas from IUGR pregnancies than in normal pregnancies. Only the decidual cells in the basal plate in IUGR demonstrated slightly higher expression of CD34 (Fig. 4).

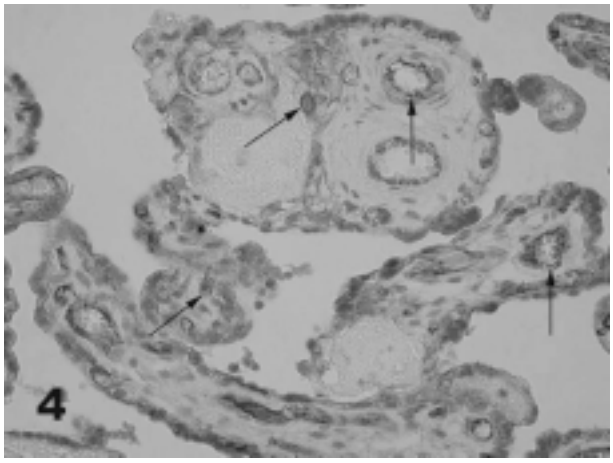

Fig. 4. CD34-positive immunostaining (arrows) in IUGR placenta. Counterstained with haematoxylin. ×400

DISCUSSION

There is currently great excitement and expectation in the stem cell community (18). This originates from the discovery that pluripotent stem cells can be cultivated from human fetal tissue and retain their ability to give rise to a variety of differentiated cell types. The revelation over the past few years of a seemingly multipotent population of precursor cells, distributed through many and perhaps all tissues of the body, have opened new pilot research. The blood vascular system may serve as a conduit, carrying a continuous supply of multipotent cells to sites in the body where they are needed for repair (19). Conversion of blood stem cells to brain, epithelium, liver, muscle or myocardium cells shows the plasticity of adult stem cells.

Within the bone marrow, there is a veritable factory for the production of blood. The existing paradigm has been that the most immature cells in the bone marrow are the HSC which can both self renew and differentiate into all of the different types of hematopoietic cells. Recent data, however, sug-

gest that there are cells in the marrow with the ability to differentiate not only into blood cells, but also into multiple other cell types throughout the body. The phenotype of all these highly plastic cells in the marrow is not known (4). Probably some cells that are already partially committed to hematopoiesis may be able to be reprogrammed to differentiate into other cell types and also the possibility that some of the subpopulations of marrow cells that are enriched for cells with HSC activity may actually contain cells that are less mature than HSC and are not yet committed to the hematopoietic lineage. Rather, they still maintain the ability to differentiate into multiple lineages when exposed to the appropriate stimuli *in vivo* and *in vitro* (20, 21). Direct injection of the marrow cells into the muscle was not necessary for the differentiation of marrow cells into myocytes.

Bone marrow stem cells have been shown to promote repair of myocardial damage following ischemy/reperfusion models of myocardial infarction in mice and rats (22, 23). The same bone marrow subpopulation that is capable of reconstituting the hematopoietic system was able to differentiate into cardiac myocytes, smooth muscle cells and endothelial cells when injected into myocardium after ischemic injury (23). $CD34^+$ human bone marrow cells support repair after myocardial infarction. It is likely that the growth factor administration does induce some marrow-derived cells to differentiate into myocardial endothelial cells at the site of injury.

Endothelial progenitor cells are defined as $CD34^+$ $CD133^+$ Flk-1^+ stem cells, which differentiate into endothelial cells *in vitro* and could be incorporated into sites of pathological neovascularization *in vivo* (24). Vasculogenesis occurs in limited embryonic sites, although angiogenesis, the formation of new blood vessels by sprouting from preexisting ones, occurs in many situations containing embryonic development and pathologic conditions (25). Circulating endothelial cells progenitors may contribute to neoangiogenesis in adult individuals, consistent with vasculogenesis.

In rodents and primates, the uterine epithelium (endometrium) is eroded so that maternal blood comes into direct contact with the trophoblast surface (haemochorial). Primates have a single syncytial layer plus an underlying trophoblast stem cell layer. Trophoblast stem cells are defined as cells that have the potential to give rise to all differentiated trophoblast cell subtypes (26). It depends on presence of signaling specific growth factors. Trophoblast stem cells may not persist (27). The mammalian placenta has evolved to take on many roles dealing with intrauterine life.

Stem cells have varying degrees of potential, ranging from the totipotency (ability to form the embryo and the trophoblast of the placenta) of the fertilized oocyte, to the pluripotency (ability to differentiate into almost all cells) or to the multipotentiality (capability of producing a limited range of differentiated cell lineages appropriate to their location) of most tissue based stem cells, and lastly to the unipotentiality (able to generate only one cell type). Stem cells are known to exist in most organs of the body, usually only 1–2% or less of the total cellularity.

Stem cell transplants could be used to treat a wide variety of pathologies, especially the ones affecting specific cell types such as cardiomyocytes, dopaminergic neurons, and islet β cells that have been destroyed. There is evidence that patients with myocardial infarction recover faster when autologous hematopoietic stem cells are injected directly into the heart.

The stem cell technology is yet in its infancy. The potential benefits of treating diseases by using stem cells are enormous, but several significant challenges still face this technology.

It is known that $CD34^+$ can develop into endothelial progenitor cells and trigger angiogenesis. Controlled differentiation of adult or embryonic stem cells requires the engineering of niches and extracellular signal combinations that would amplify a particular signaling network and allow a uniform and selective differentiation.

The relation between stem cells within embryo and stem cells in extra-embryonic chorion is not profoundly characterized (28). There is no doubt that extra-embryonic stem cells are of great experimental and probably clinical importance. The rapid growth and vascularization of the human placenta are characteristic of early pregnancy and are accomplished in an unusual hypoxic environment.

The findings obtained in the present study show that there is a great number of CD34-positive cells in human placenta, both in m\normal and in IUGR-complicated pregnancies.

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References

1. Watt FM, Hogan BLM. Out of Eden: stem cells and their niches. Science 2000; 287: 1427–30.
2. Anderson DJ, Gage FH, Weissman IL. Can stem cells cross lineage boundaries? Nat Med 2001; 7: 393–5.
3. Orive G, Hernandez RM, Gascon AR et al. Controversies over stem cells. Trends Biotechnol 2003; 21: 109–12.
4. Krause DS. Plasticity of marrow-derived stem cells. Gene Therapy 2002; 9: 754–8.

5. Kucia M, Majka M, Ratajczak MZ. Phenomenon of tissue-specific stem cell plasticity. Adv Cell Biol 2003; 30 (Suppl 21): 3–15.
6. Ferrari G, Cusella-De Angelis G, Coletta M et al. Muscle regeneration by bone marrow-derived myogenic progenitors. Science 1998; 279: 1528–30.
7. Brazelton TR, Rossi FM, Keshet GI, Blau HM. From marrow to brain: expression of neuronal phenotypes in adult mice. Science 2000; 290: 775–9.
8. Theise ND, Nimmakayalu M, Gardner R et al. Liver from bone marrow in humans. Hepatology 2000; 32: 11–6.
9. Krause DS, Theise ND, Collector MI et al. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 2001; 105: 369–77.
10. Jackson KA, Majka SM, Wang H et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest 2001; 107: 1395–402.
11. Toma JG, Akhavan M, Fernandes KJ et al. Isolation of multipotent adult stem cells from the dermis of mammalian skin. Nat Cell Biol 2001; 3: 778–84.
12. Cairo MS, Wagner JE. Placental and/or umbilical cord blood: an alternative source of hematopoietic stem cells for transplantation. Blood 1997; 90: 4665–78.
13. Broxmeyer HE, Gluckman E, Auerbach AD et al. Human umbilical cord blood: A clinically useful source of transplantable hematopoietic stem/progenitor cells. Int J Cell Cloning 1990; 8, Suppl. 1: 76–89.
14. Broxmayer H, Hangoc G, Cooper S et al. Growth characteristics and expansion of human umbilical cord blood and estimation of its potential for transplantation in adults. Proc Natl Acad Sci USA 1992; 89: 4109–13.
15. Nagawa R, Watanaba T, Kawano Y et al. Analysis of maternal and neonatal factors that influence the nucleated and $CD34^+$ cell yield for cord blood banking. Transfusion 2004; 44: 262–7.
16. Belvedere O, Feruglio C, Malangone W et al. Increased blood volume and CD34+CD38- progenitor cell recovery using a novel cord blood collection system. Stem Cells 2000; 18: 245–51.
17. Quesenberry PJ, Becker PS. Stem cell homing: rolling, crawling, and nesting. Proc Natl Acad Sci USA 1998; 95: 15155–7.
18. Preston SL, Alison MR, Forbes SJ et al. The new stem cell biology: something for everyone. J Clin Pathol: Mol Pathol 2003; 56: 86–96.
19. Blau HM, Brazelton TR, Weinmann JM. The evolving concept of a stem cell: entity or function. Cell 2001; 105: 829–41.
20. Pittenger MF, Mackay AM, Beck SC et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284: 143–7.
21. Reyes M, Lund T, Lenvik T et al. Purification and *ex vivo* expansion of postnatal human marrow mesodermal progenitor cells. Blood 2001; 98: 2616–25.
22. Kocher AA, Schuster MD, Szabolcs MJ et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nat Med 2001; 7: 430–6.
23. Orlic D, Kajstura J, Chimenti S et al. Bone marrow cells regenerate infarcted myocardium. Nature 2001; 410: 701–5.
24. Dus D, Paprocka M. Endothelial progenitor cells: origin, characteristics and their future therapeutic use. Adv Cell Biol 2003; 30 (Suppl 2): 25–36.
25. Hirashima M, Kataoka H, Nishikawa S et al. Maturation of embryonic stem cells into endothelial cells In an *in vitro* model of vasculogenesis. Blood 1999; 93: 1253–63.
26. Tanaka S, Kunath T, Hadjantonakis AK et al. Promotion of trophoblast stem cell proliferation by FGF4. Science 1998; 282: 2072–5.
27. Rossant J, Cross JC. Placental development: lesion from mouse mutants. Nat Rev Genet 2000; 2: 528–48.
28. Uy GD, Downs KM, Gardner RL. Inhibition of trophoblast stem cell potential in chorionic ectoderm coincides with occlusion of the ectoplacental cavity in the mouse. Development 2002; 129: 3913–24.

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PLACENTOS $CD34^+$ KAMIENINĖS LĄSTELĖS GIMUS NORMALIAM KŪDIKIUI IR VAISIAUS AUGIMO ATSILIKIMO ATVEJU

S a n t r a u k a

Imunohistochemiškai ištyrėme $CD34^+$ kamieninių ląstelių pasiskirstymą placentoje gimus normaliam kūdikiui bei vaisiaus augimo atsilikimo atvejais. Normalioje placentoje, gimus sveikam kudikiui, CD34 ekspresiją nustatėme visų choriono gaurelių kapiliarų endoteliocituose. Taip pat rastas nedidelis kiekis pavienių ekstravaskulinių CD34 teigiamų ląstelių. Silpna CD34 ekspresija pasižymėjo ir pavienios cito- bei sincitiotrofoblasto, taip pat kai kurios decidualinės ląstelės.

Vaisiaus augimo atsilikimo atvejais gaurelių tankis sumažėjęs, o kai kurie gaureliai patologiškai pakitę. CD34 ekspresija kapiliarų endotelyje silpnesnė, tačiau decidualinės ląstelės daugiausia buvo CD34 teigiamos ir šio antigeno ekspresija kiek ryškesnė.

Tyrimo rezultatai parodė, kad normalioje placentoje ir net esant vaisiaus augimo atsilikimui nemažai placentos ląstelių turi CD34 žymenį.

Raktažodžiai: CD34, kamieninės ląstelės, placenta