Contribution of hematopoetic stem cells to the homeostasis and regeneration of the uterine epithelium and the epidermis

Doctoral thesis

Dr. Krisztián Németh

Semmelweis University

Molecular Medical Chemistry School of Doctoral Studies

Mentor: Dr. Sarolta Kárpáti, MD, PhD, DSc
Co-mentor: Dr. Éva Mezey, MD, PhD

Official Reviewers:
Dr. Sára Tóth, PhD
Dr. László Kóbori, MD, PhD

Comprehensive exam committee:
President: Dr. György Nagy, MD, PhD, DSc
Members: Dr. Emília Madarász, PhD, DSc
Dr. Gergely Szakács, MD, PhD

Budapest

2011

Table of Contents

List of Abbreviations

ABC	ATP Binding Cassette
ALL	Acute Lymphoid Leukemia
AML	Acute Myeloid Leukemia
BAC	Bacterial Artificial Chromosome
Beta-Gal	Beta-galactosidase
BFU-E	Burst Forming Unit Erythroblasts
BM	Bone Marrow
BMDC	Bone Marrow Derived Cell
BMP4	Bone Morphogenetic Protein 4
BMT	Bone Marrow Transplantation
BrdU	Bromodeoxyuridine
CFU-E	Colony Forming Unit Eryhtroblasts
CFU-GEMM	Granulocyte-Erythroid-Macrophage-Megakaryocyte
CK	Cytokeratine
CLP	Common Lymphoid Progenitor
CML	Chronic Myeloid Leukemia
CMP	Common Myeloid Progenitor
CNS	Central Nervous System
DAPI	4,6-diamidino- 2-phenylindole
DMD	Duchenne's Muscular Dystrophy
DNA	Deoxyribonucleic Acid
EB	Embryoid Body
EGFP	Enhanced Green Fluorescent Protein
EPU	Epidermal Proliferative Unit
ES	Embryonic Stem
ESC	Epidermal Stem Cell
FACS	Fluorescent activated cell sorting
FGF2	Fibroblast Growth Factor 2
FISH	Fluorescent in situ Hybridisation
GCSF	Granuylocyte Colony-Stimulating Factor
GFP	Green Frluorescent Protein
GLUT2	Glucose Transporter 2
GVHD	Graft-versus Host Disease
HFSC	Hair Follicule Stem Cell
HSC	Hematopoetic Stem Cell
IFSC	Interfollicular Stem Cell
iPSC	Induced Pluripotent Stem Cells
IRES	Internal Ribosomal Entry Site
Lin	Lineage
MCSP	Melanoma Chondroitin Sulfate Proteoglycan
MSC	Mesenchymal Stem Cell
MUD	Matched Unrelated Donor
OPN	Osteopontin
PBS	Phosphate Buffered Saline
PBSC	Peripheral Blood Stem Cell
PCR	Polymerase Chain Reaction

RFP	Red Fluorescent Protein
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SHH	Sonic Hedgehog
SP	Side Population
TA	Transient Amplifying
TBI	Total Body Irradiation
UV	Ultraviolet
X-Gal	3-indolyl-beta-D-galactopyranoside
YFP	Yellow Fluorescent Protein

1. Introduction

1.1 Definition of stem cells

It's hard to find a universally acceptable definition for "stem cells". Nevertheless there are certain characteristics in both developing and adult multicellular organisms that help distinguish stem cells from other tissue resident cells. For example, stem cells possess the unique ability to undergo assymetric cell divisions. In other words they can renew themselves while giving rise to an identical stem cell as well as a progeny, which is restricted in both their mitotic potential and the range of terminally differentiated cells they can become (1). To illustrate this essential feature of stem cells let's take a closer look at the production of mature red blood cells **(Fig. 1)**. Hematopoietic stem cells (HSCs), the stem cells responsible for the production of all blood cell lineages, are located in the bone marrow. After the first division, hematopoietic stem cells will give rise to an HSC which is identical to the mother stem cells and a progeny which is committed to serve either as a progenitor for lymphoid cells (common lymphoid progenitor or CLP) or as a precursor for all myeloid cells, erythrocytes and platelets (common myeloid progenitor or CMP). CMPs will again divide and become further specialized progenitors (Colony Forming Unit Granulocyte-Erythroid-Macrophage-Megakaryocyte or CFU-GEMM) capable to create only a subset of myeloid cells (neutrophil granulocytes and monocytes) as well as erythrocytes and platelets. Burst Forming Unit Erythroblasts (BFU-E) and Colony Forming Unit Eryhtroblasts (CFU-E) are direct mono-potential progenitors of red blood cells. Following well characterized differentiation stages (proertytroblasts, orthochromatophil erythroblasts, reticulocytes) CFU-E-s will subsequently become mature erythrocytes capable of carrying oxygen (2-4).

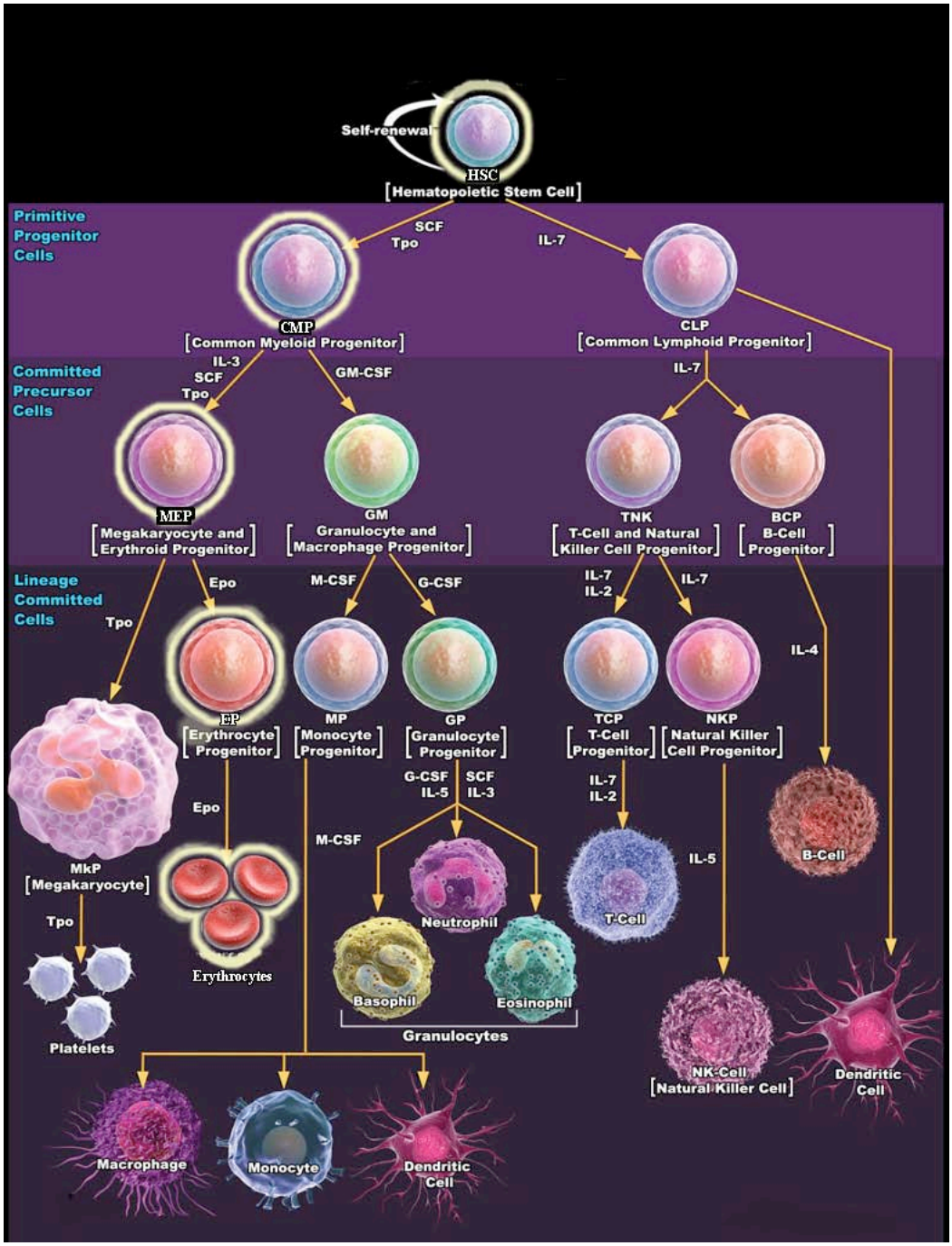

Figure 1. Differentiation of HSCs into mature blood cells. Cells of the differentiation path of the erythrocyte lineage are highlighted in yellow. Adopted and modified from the following website: www.biolegend.com.

1.2 Categories of stem cells based on their potency

Stem cells can be classified into three broad categories, based on their ability to differentiate (5).

- *Totipotent stem cells* are found only in early embryos, where each cell is able to build a complete organism (e.g., identical twins) and to produce extra-embryonic tissues such as the placenta.
- *Pluripotent stem cells* exist in the undifferentiated inner cell mass of the blastocyst and can form any (over 200 different) cell types found in the human body.
- *Multipotent stem cells* are derived from fetal tissue, cord blood, and adult organisms and are able to give rise to a subset of cell lineages such as hematopoietic stem cells which can produce all the different blood cells.
- *Unipotent stem cells* are able to become only one mature cell type (i.e. epidermal stem cells that make keratinocyte).

1.3 Categories of stem cells based on their source of origin

1.3.1 Embryonic stem (ES) cells:

Definition of ES cells

Approximately 30 hours after the oocyte is fertilized the zygote undergoes repeated mitotic divisions resulting in a rapid increase in the number of cells (6). These dividing cells are called blastomers. When there are 12 to 32 of them the developing human is called a morula (7). Shortly after the morula enters the uterus (about 4 days after fertilization) fluid passes from the uterinal cavity through the zona pellucida and forms a blastocystic cavity. As fluid increases in the blastocystic cavity it separates the blastomers into two parts: 1. A thin outer cell layer (the throphoblast) which gives rise to the embryonic part of the placenta and a group of centrally located blastomers (the inner cell mass) which gives rise to the embryo. At this stage the human conceptus is a blastocyst (8).

Human ES cells are cultured cell lines derived from the inner cell mass of the blastocyst **(Fig. 2)** developed from fertilized eggs following *in vitro* fertilization (9). If the blastocyst would be implanted back into the mother's uterus, it could develop into a

fetus and then a newborn baby. Because not all eggs will fertilize and develop properly into blastocysts during an *in vitro* fertilization procedure, more eggs are fertilized than are required in order to maximize the chances of having a successful pregnancy. Those blastocysts that are not implanted for pregnancy are usually frozen for future use by the couple that produced them, or are ultimately discarded. It is these to be discarded blastocysts that have been used to derive human embryonic stem cell lines in the laboratory, after approval of the donors and oversight by the appropriate institutional review boards or ethics committees (10).

Basic features of ES cells

- Can be grown indefinitely in an undifferentiated state (a feature corresponding with high telomerase activity),
- In culture spontaneously form ball-like structures termed "embryoid bodies", which consist of a core of mitotically active and differentiating ES cells and a periphery of fully differentiated cells from all three germ layers,
- Capable to differentiate into any type of cell, tissue, organ in the human body (more then 220 cell types), a characteristic described as pluripotency,
- Clonogenic, i.e. a single ES cell can give rise to a colony of genetically identical cells, or clones, which have the same properties as the original cell,
- Exhibit and maintain a stable diploid karyotype,
- High level of Oct3/4 and Nanog transcriptional factors (both of which are critical in maintaining pluripotency),
- Expression of distinct cell surface antigens: SSEA-1, SSEA-3, SSEA-4 and Alkaline phosphatase (11).

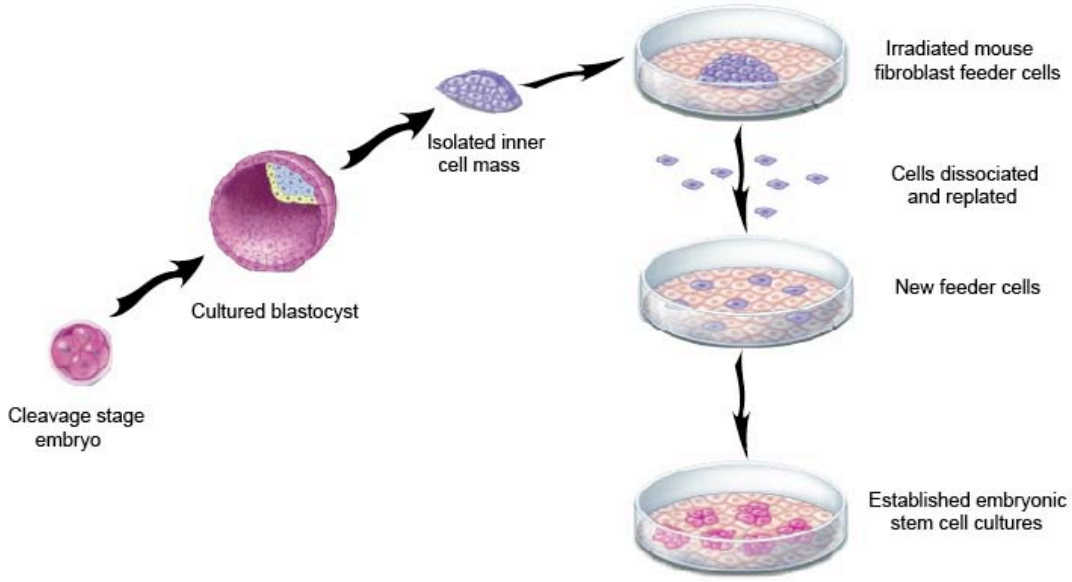

Figure 2. Derivation and culture technique of ES cells. The figure shows the schematic process of the establishment and culture of ES cells. First the inner cell mass is isolated from a developing blastocyst around day 5-7 of the embryonic development. The isolated cells are placed into culture together with fibroblast feeder cells that support the growth of the initial ES cell colonies. Once ES cells start growing the cells are passaged and subcultured on a new layer of feeder cells. Adopted and modified from http://faculty.ksu.edu.sa.

Assays to test the pluripotent nature of ES cells

- One test called “tetraploid complementation assay” is conducted by injecting ES cells derived from the inner cell mass of one blastocyst into the cavity of another, tetraploid blastocyst (every chromosome exists in four copies in each cell). The "combination" embryos are then transferred to the uterus of a pseudopregnant female mouse, and the resulting progeny are called chimeras. The fetus is exclusively derived from the ES cell, while the extra-embryonic tissues are exclusively derived from the tetraploid cells (12).
- Another method for determining the pluripotency of ES cells is to inject the cells into genetically matched or immune-deficient adult mice (under the skin or the kidney capsule), so that the tissue will not be rejected. In the host animal, the injected ES cells develop into benign tumors called teratomas (13). When

examined under a microscope these tumors contain cell types derived from all three primary germ layers of the embryo—endoderm, mesoderm, and ectoderm. Teratomas typically contain gut-like structures such as layers of epithelial cells and smooth muscle; skeletal or cardiac muscle (which may contract spontaneously); neural tissue; cartilage or bone; and sometimes hair (14). Thus, ES cells that have been maintained for a long period *in vitro* can behave as pluripotent cells *in vivo*. They can participate in normal embryogenesis by differentiating into any cell type in the body, and they can also differentiate into a wide range of cell types in an adult animal.

- A third technique to demonstrate pluripotency is to allow ES cells *in vitro* to differentiate spontaneously or to direct their differentiation along specific pathways. The former is usually accomplished by removing feeder layers and adding different growth factors (basic fibroblast growth factor, transforming growth factor β, activin-A, bone morphogenic protein 4, hepatocyte growth factor, epidermal growth factor, nerve growth factor, and retinoic acid) to the culture medium (15). Within a few days after changing the culture conditions, ES cells aggregate and may form embryoid bodies (EBs). In many ways, EBs in the culture dish resemble teratomas that are observed in the animal. EBs consist of a disorganized array of differentiated or partially differentiated cell types that are derived from the three primary germ layers of the embryo—the endoderm, mesoderm, and ectoderm.

Potential use of ES cells

There are at least three basic opportunities presented by embryonic stem cell research:

- Research could lead to the development of innovative *replacement or transplantation therapies* for diseases such as diabetes - where the insulin producing pancreatic beta-cells should be replaced (16)-, Parkinson's disease - where the missing dopamine producing brain cells should replaced (17), heart infarction - where necrotized myocardium should be replaced (18) or congenital malformations where major body wall defects could be repaired.
- ES cells could be used as a surrogate in the *screening and testing of drugs (19),*

(20). When developing a new drug to treat heart disease for instance, ES cell derived cardiomyocytes can be utilized to test pharmacological efficacy of the new agent (21). Similarly, to rule out cardiac toxicity of a drug, ES derived heart cells could be used instead of animal cells that are currently applied for this purpose (22).

- Studying ES cells can provide a deeper *understanding of cell differentiation and development (23)*, with possible consequences for the treatment of diseases such as cancer (24).

Possible side effects and concerns

Rejection of the implanted ES derived products

ES cells - and any differentiated cells, tissues or organs created from them - are derived from a genetically non-identical source hence are considered allogeneic. Once implanted into the recipient's body the host immune system will mount an immune response against the immunologically incompatible cells initiating a process that might lead to the destruction of the introduced graft (25). Therefore most likely, along with the transplantation of ES derived tissues, a life-long immunosuppressive therapy will be necessary (26).

Formation of tumors

Although there are several techniques available to drive differentiation of isolated ES cells in vitro, we are still far from understanding and being able to control the entire process. One of the most important dangers of ES cell transplantation is that once the differentiation cascade is initiated and a target cell type or tissue is created there is no guarantee that an uncontrolled proliferation will not take place (27) that could lead to tumor formation.

Ethical issues

The harvesting of embryonic stem cells requires the destruction of the embryos from which they are harvested. Issues about the value of life emerge in the question of whether life for those already existing should be improved at the seeming expense of a prospective human life that has just come into being. This issue is a subject of fierce debate listing pros and cons since the establishment of the first embryonic stem cell

line (28).

1.3.2 Induced pluripotent stem cells (iPSCs)

Definition

IPSCs are pluripotent stem cells derived from genetically reprogrammed non-pluripotent (typically adult somatic) cells that are forced to express genes and factors important for maintaining the defining properties of embryonic stem cells. Mouse iPSCs were first reported in 2006 (29), followed by human iPSCs in 2007 (30).

The first reprogramming experiment involved retroviral-mediated overexpression of twenty four well-defined pluripotency regulators in mouse embryonic fibroblasts, and led to the emergence of cells that morphologically resemble ES cells. Subsequent experiments, in which factors were eliminated from the original mix, identified a smaller number of genes necessary for the generation of pluripotent stem cells. Oct-3/4 and certain members of the Sox gene family (Sox1, Sox2, Sox3, and Sox15) were the crucial transcriptional regulators involved in the induction process. Absence of any of these factors results in failed induction. Additional genes, however, like certain members of the Klf family (Klf1, Klf2, Klf4, and Klf5), the Myc family (C-myc, L-myc, and N-myc), Nanog, and LIN28, have been found to increase the induction efficiency (31-33).

Crucial genes involved in the production of iPSCs

- *Oct-3/4:* Oct-3/4 is a family member of the octamer transcription factors, and plays a critical role in maintaining pluripotency. The absence of Oct-3/4 in blastomeres and embryonic stem cells leads to spontaneous trophoblast differentiation preventing further development into any embryonal structures (34).
- *Sox* family: SOX genes bind to the minor groove in DNA, and belong to a super-family of genes characterized by a homologous sequence called the HMG (high mobility group) box (35). The Sox family of genes is associated with maintaining pluripotency similar to Oct-3/4, although it is associated with multipotent and unipotent stem cells in contrast with Oct-3/4, which is exclusively expressed in pluripotent stem cells. While Sox2 was the initial gene used for induction by Takahashi et al. (29), other genes in the Sox family have

been shown to possess similar properties in the induction process. Sox1 generates iPS cells with a similar efficiency as Sox2, and the genes Sox3, Sox15, and Sox18 also yield iPSCs, although not as efficiently (36).

- *Klf* (Kruppel like factors) family: Klf4 of the Klf family of genes was initially identified by Yamanaka et al. (29) and confirmed by Welstead et al. (37) as a factor for the generation of mouse-, as well as human iPSCs (30). However, Thomson et al. reported that Klf4 was unnecessary for generation of human iPSCs (38).
- *Myc* family: The Myc family consists of proto-oncogenes that primarily play a role in cancer formation (39) and among them c-myc has been implicated in the generation of mouse (29) and human (30) iPSCs. It's role, however, became questioned recently when several groups reported that c-myc was in fact unnecessary for generation of human iPSCs (31; 40; 41). Furthermore, the use of the "myc" family of genes to generate iPSCs for therapeutical use is also troubling since many of the mice transplanted with c-myc-induced iPSCs developed lethal teratomas (42).
- *Nanog:* Nanog is a newly identified homeodomain-bearing transcriptional factor (43) with a so far undecided role in iPSC induction. In embryonic stem cells, Nanog, along with Oct-3/4 and Sox2, is necessary to promote pluripotency (44), but it was reported not to be needed to induce iPSCs (29). On the other hand it was also shown that Nanog can be used as one of the factors to generate iPSCs (44).

Characteristics of iPSCs

- Unlimited mitotic capacity with high telomerase activity,
- embryoid body formation in vitro,
- potential to differentiate into a wide array of tissues,
- expression of specific stem cell markers and transcripition factors,
- formation of tumors containing cells from all three germ layers when transplanted into mice (teratomas),
- positive tetraploid complementation test (see above at the discription of ES cells) (45-47).

1.3.3 Miscellaneous stem cells from the pre-or perinatal life

Fetal Stem Cells

The developing organism is referred to as a fetus from approximately 10 weeks of gestation. As their name suggests, fetal stem cells are taken from the fetus. Most tissues in a fetus contain stem cells that drive the rapid growth and development of the organs. Like adult stem cells, fetal stem cells are generally tissue-specific, and generate the mature cell types within the particular tissue or organ in which they are found (48).

Amniotic stem cells

Amniotic stem cells are multipotent stem cells of mesenchymal origin extracted from amniotic fluid or prepared from the placenta. Amniotic stem cells are able to differentiate into various tissue types such as skin, cartilage, cardiac tissue, nerves, muscle, and bone (49) .

Cord Blood Stem Cells

At birth the blood in the umbilical cord is rich in blood-forming stem cells. The applications of cord blood are similar to those of adult bone marrow and these cells are currently used to treat diseases and conditions of the blood or to restore the blood cells after treatment for specific cancers. Like the stem cells in adult bone marrow, cord blood stem cells are also tissue-specific being able to differentiate into hematopoietic and skeletal lineages (50).

1.3.4 Adult Stem Cells

Cell biologists have long realized that most cells do not live as long as organisms do; thus, cells in almost every tissue need to be renewed/replaced during the natural lifespan of the organism. Depending on the turnover rate of cells in any given organ, this process can be very frequent or very rare. Epithelial cells in the mouth and the GI tract are constantly exposed to a variety of insults (such as heat, cold, extreme changes in pH, strong spices etc.) and have a very fast turnover rate; nerve cells get wired during embryonal development and either do not turn over or have a very low turnover rate. The rest of the tissues are somewhere in between. This kind of tissue regeneration relies on undifferentiated tissue-specific stem cells (also known as somatic

stem cells; postnatal or adult stem cells) that are found in all adult animals and humans and multiply by cell division. They replenish cells that die from old age or injury and regenerate those that have been damaged. Typically, there are a very small number of stem cells in each tissue, and they are thought to reside in a specific area called a "stem cell niche".

Stem cell niche

Definition of the stem cell niche

In 1978, R. Schofield published his observations demonstrating that hematopoietic cells derived from the spleen display decreased proliferative potential when compared to hematopoietic stem cells from the bone marrow (51). According to his hypothesis the main reason for this is that stem cells were no longer in association with a complement of cells, a "niche," which supports long-term stem cell activity. This idea that specialized environments within tissues can preserve proliferative potential and block maturation of adult stem cells was the first description of the stem cell niche hypothesis. This model predicts that removal of stem cells from the niche results in loss of stem cell identity, self-renewal capacity, and the onset of differentiation. As such, the niche would provide a mechanism to precisely balance the production of stem cells and progenitor cells to maintain tissue homeostasis. Therefore, a stem cell niche is not defined solely by the presence of stem cells but also by the ability of the environment to regulate stem cell behavior.

Factors necessary for the maintenance of the stem cell niche

A. Cellular components

A stem cell niche is a 3 dimensional structure created by special supporting cells and extracellular material (52). These cells surround stem cells and provide important signals that help maintain a balance between self-renewal and differentiation towards more mature cell populations. Supporting cells are not universal throughout the body, they show specific phenotypic and functional differences depending on the stem cell niche they are form. Below are a few examples:

- In the bone marrow osteoblasts and bone marrow stromal fibroblasts together

create an environment **(Fig. 3)** that is capable of supporting hematopoietic stem cell functions (53).

- In the skin dermal connective tissue cells around the bulge region form a niche to guide asymmetric division of epidermal stem cells (54).
- In the testis Sertioli cells surround spermatogonia and assist production of sperm cells (55).

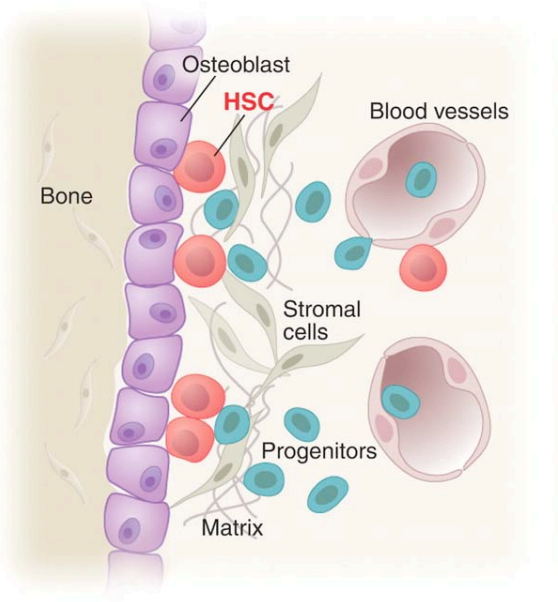

Figure 3. Illustration of the stem cell niche theory. Hematopoetic Stem Cells develop in the bone marrow stem cell niche created by the osteoblasts and the stromal fibroblasts. Adopted from Moore KA et al (2006) Stem Cells and Their Niches. Science, Vol. 311 no. 5769 pp. 1880-1885.

B. Non-cellular components

Extracellular matrix

Matrix components provide localizing niche elements that can contribute stimulatory- or impose inhibitory influences on the stem-cell pool.

Beta-integrins

Integrins are obligate heterodimers containing two distinct chains, called the α (alpha) and β (beta) subunits (56). Integrins function as receptors that mediate attachment between a cell and the tissues surrounding it, which may be other cells or the extracellular matrix components (57). In the skin, for instance, β-1 integrins are known

to be differentially expressed on primitive cells and they participate in constrained localization of a stem-cell population through presumed interaction with matrix glycoprotein ligands (58).

Tenascin C

Tenascin-C is a large hexameric extracellular matrix glycoprotein originally isolated from embryonic tissues (59). It is typically seen at sites of epithelial-mesenchymal interaction in mature tissues (60). In the nervous system, the absence of tenascin C alters neural stem-cell number and function in the subventricular zone (61). Tenascin C seems to modulate stem-cell response to fibroblast growth factor 2 (FGF2) and bone morphogenetic protein 4 (BMP4), resulting in an increased potential to generate glial cells (62), (63). Since tenascin C deletion has been shown to affect primitive cell populations in the haematopoietic system as well, it seems likely that tenascin C affects several stem-cell niches (63),(64).

Osteopontin

Osteopontin (OPN) is a highly negatively charged, Arg–Gly–Asp (RGD)-containing sialoprotein (65). It has now been demonstrated to contribute to the regulation of HSCs (66). OPN interacts with several receptors known to be on HSCs, including CD44 (67).

Sonic-hedgehog

Hedgehog family members have a role in topographic organization and formation of niches in development (68). Sonic hedgehog (SHH) is essential for hair-follicle formation, and the localization of specific subsets of epidermal stem cells to either the follicular niche or the interfollicular epidermis (69). In addition, SHH participates in specific spatially restricted events within established niches. Hair represents a uniquely dynamic physical association of elements within a niche, due to the change in distance during the hair cycle of the dermal papilla from the bulge region where at least some stem cells reside. In the developing intestine, SHH and Indian hedgehog contribute to architectural patterning, playing a key part in assembly, dimensions and regularity of the crypts where stem cells reside (70). Therefore, HH contributions to stem cell control are morphogenic, creating a niche, and regulating stem-cell fate within that niche.

Ephrins

The term Ephrins is a collective term for ligands of a large family of tyrosine kinase

receptors known as Eph receptors (71). Graduated expression of ephrin B1 and, reciprocally, its transmembrane tyrosine kinase receptors, EphB2 and EphB3, have been elegantly shown to spatially organize mouse intestinal epithelial cells. Disrupted expression of the receptors leads to aberrant organization of crypt and villus cells, including cells at the interposition between them, which are defined as stem cells (72).

Basic characteristics of adult stem cells

Slow-cycling feature in vivo

The slow-cycling attribute is particularly important biologically because it conserves the cell's proliferative potential and minimizes DNA replication-related errors (73). The slow-cycling stem cells can be identified experimentally as the “label-retaining cells” (74). This is done by long-term labeling of all of the cells with a DNA building block such as [3H]thymidine or bromodeoxyuridine (BrdU), followed by a chase period that results in the dilution of the label from all of the rapidly cycling cells, but not from the slow-cycling stem cells (75).

Repopulation potential in vivo

In this assay the cells are removed from the living animal, characterized and labeled in vitro (alternatively cells from a genetically tagged animal could be used) followed by their subsequent transplantation into another animal. Stem cells should be able to repopulate their tissue of origin (76).

Cell surface markers

Most stem cells possess a special combination of cell surface antigens that make them unique among a mixed population of tissue resident cells. Using these markers stem cells can be identified by immunostaining in histological sections in situ, or isolated and enriched by Fluorescent Activated Cell Sorting (FACS) following digestion of the target tissue (77-79).

Types of adult stem cells

The hematopoietic stem cell is currently the best characterized multipotent adult stem cell, and was first isolated from the mouse bone marrow more then 20 years ago. Clonogenic in vivo and in vitro assays suggested that high levels of purity are achievable for these cells. (80). Mesenchymal stem cells (MSCs), which give rise to multiple mesenchymal lineages (bone, fat and cartilage), can be isolated from the bone marrow as well. MSCs historically have been isolated through cell culture techniques, and only recently have phenotypic markers for the prospective isolation of these cells been described (81; 82). Specific markers of other tissue-resident stem cells, including the peripheral nervous system (83), central nervous system (84), and myocardium, have also been described (85). Interestingly, high activity of ATP binding cassette (ABC) transporter proteins appears to be a common, though not exclusive, marker of adult stem cell populations (86-88). Although the biological significance of this property is unclear, it has been a useful tool in the enrichment of stem cells from multiple tissues by virtue of their enhanced efflux of the fluorescent dye Hoechst 33342 (the so called "side population," SP, phenotype) (89). Other candidate tissue-specific stem cell populations, for which phenotypic markers are lacking or ambiguous, have been provisionally defined by positional or anatomical criteria; these include muscle satellite cells (reside beneath the basal lamina of mature muscle fibers) (90); epidermal stem cells (located predominantly in or near the bulge region of the hair follicle in haired epidermis) (91); and intestinal stem cells (believed to reside near the bottom of the intestinal crypts) (92). For other tissues, including the liver and pancreas, the identity of resident stem cell population(s), and even whether regeneration of these tissues in adults is stem cell mediated, is still debated (93-96).

Epidermal stem cells

The epidermis is the outer layer of the skin. Histologically it is a stratified squamous epithelium primarily composed of keratinocytes: cells that express the characteristic intermediate filament known as keratin. It has a basal layer of proliferative keratinocytes that periodically detach from the basal membrane, withdraw from the cell cycle, and initiate a program of terminal differentiation, moving upward to the skin

surface. Upon commitment to terminal differentiation, keratinocytes progress through different stages and form three distinct layers: the spinous, the granular, and the stratum corneum. Eventually, keratinocytes loose the nuclei and are continuously shed from the skin surface (97).

Epidermal stem cells (ESCs) are responsible for maintaining epidermal homeostasis and for repairing the tissue after injuries (98). There are two distinct pools of ESCs: the interfollicular stem cells (IFSC) of the basal layer, and the hair follicle stem cells (HFSC) in the bulge region.

Interfollicular stem cells

In mice the IFSCs are found in patches at the basis of the so-called Epidermal Proliferative Unit or EPUs. As they divide they give rise to transient amplifying (TA) cells, which are still active mitotically but have only a limited capacity to proliferate. Finally, these TA cells become post-mitotic mature keratinocytes. In the human scalp and foreskin area as well as in the skin surrounding the breasts ESCs are found adjacent to the tip of the dermal papilla whereas in the palms and soles ESCs are evident in the rete ridges. IFSCs in both mouse and human skin show high expression of alpha -6 and beta-1 integrins and low expression of CD71 (transferrin receptor). Human IFSCs can be also distinguished by the presence of melanoma chondroitin sulfate proteoglycan (MCSP) and the EGF receptor antagonist Lrig1 (99).

Hair follicle stem cells

The HFSCs are found in the upper third of the external root sheath under the site of attachment of the hair erector muscle. This area is most commonly referred to as the bulge region. Mouse bulge cells express CD34 and K15 as the most reliable markers, while human bulge cells preferentially express CD200, K15, and K19.

There are numerous studies that suggest that the bulge region is the home to the most quiescent epidermal cell population and hence is the ultimate source of epidermal stem cells. In other words, stem cells found in the bulge region become not only hair follicles but also serve as a source for IFSCs and thereby the whole epidermis (100).

Hematopoietic stem cells

HSCs are multipotent stem cells that give rise to all the blood cell types including myeloid (monocytes, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets), and lymphoid lineages (T-cells, B-cells, NK-cells) (101).

Localization

In developing embryos, blood formation occurs in well-defined areas of the yolk sac, called blood islands. As development progresses, the place of blood formation slowly shifts to the spleen, liver and lymph nodes. When bone marrow develops, it eventually assumes the task of forming most of the blood cells for the entire organism. In children, haematopoiesis occurs in the marrow of the long bones such as the femur and tibia. In adults, it occurs mainly in the pelvis, cranium, vertebrae, and sternum. In some cases, the liver, thymus, and spleen may resume their haematopoietic function, if necessary, a phenomenon called extramedullary haematopoiesis (102).

Markers

Although the literature is diverse in defining exact markers for hematopoietic stem cells, there are some markers that are universally accepted and commonly used to isolate (or at least highly enrich) HSCs from the bone marrow. In mice almost all HSC purification strategies revolve around the cell surface phenotype of positive selection for the markers c-Kit and Sca-1 and negative selection for the so called lineage markers, markers of mature hematopoietic cell lineages (typically B220, CD4, CD8, Gr-1, Mac-1 and Ter-119). In humans lineage negative CD34+ and CD38- cells are considered HSCs (103).

Mesenchymal stem cells

There are many terms currently used in the literature to refer to non-hematopoietic cells derived from the bone marrow. They are most frequently called mesenchymal stem cells (MSCs) (104) or bone marrow stromal cells (BMSCs) (105); other variations on the theme are mesenchymal stromal cells (106), mesenchymal stromal stem cells,

mesenchymal progenitor cells (107), etc. However, independently of how they are called, they are best characterized by how they are produced and screened.

Characterization

Isolation is based on BMSCs' adherence to plastic, and the fact that BMSCs, unlike HSCs, do not require the addition of cytokines and/or growth factors to the media. When they are cultured in vitro, BMSCs acquire a fibroblast-like phenotype. They lack hematopoietic and endothelial lineage markers (CD45, CD34, CD14, and CD31, respectively) but are positive for a wide variety of other cell surface molecules (CD29, CD73, CD90, CD105, CD106, etc.). Unfortunately, none of these positive markers are unique for BMSCs, thus one must use them in combination with a negative selection (to separate out hematopoietic cells) to identify BMSCs in a mixed population of bone marrow cells. After they are isolated, BMSCs can be differentiated towards osteogeneic, adipogeneic or chondrogeneic lineages in vitro **(Fig. 4)**, whereas when they are transplanted into immunocompromised host animals, they can support hematopoiesis in vivo (108).

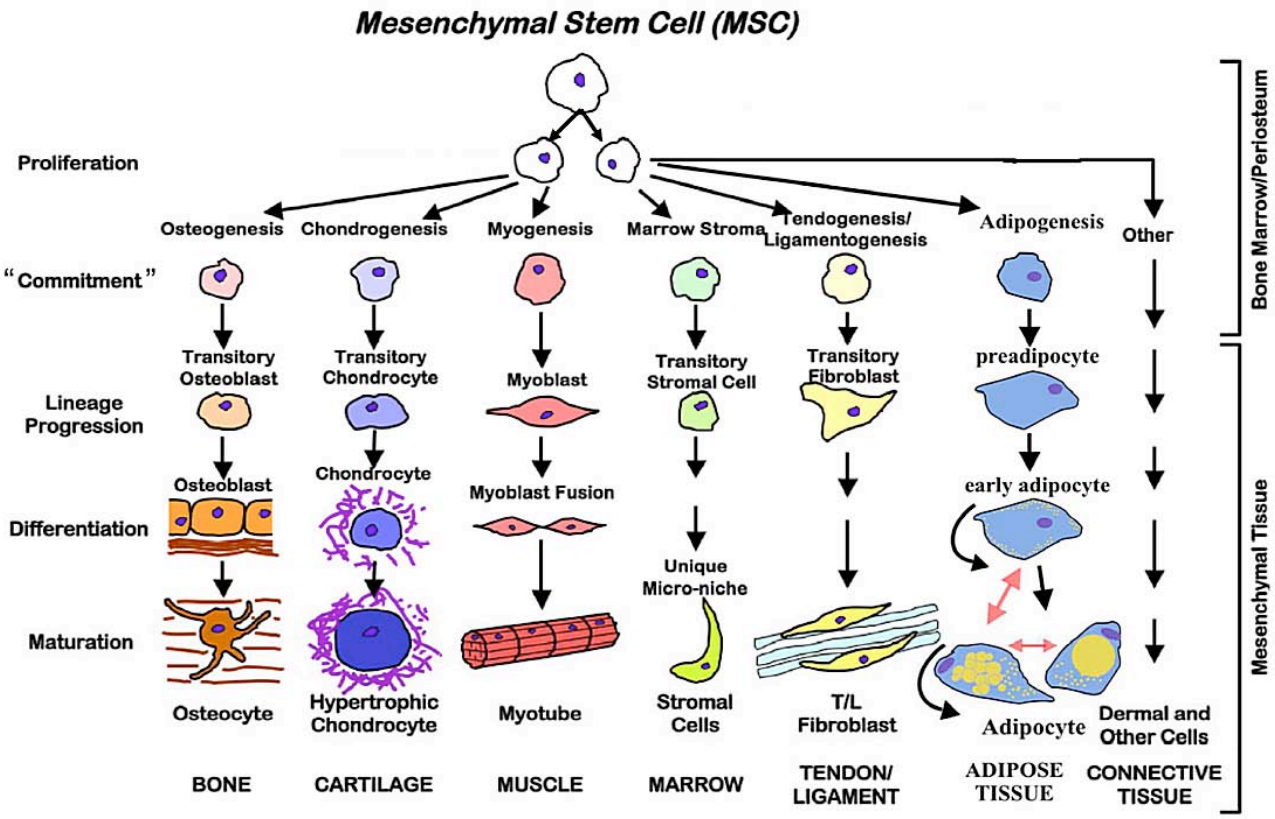

Figure 4. Differentiation of Mesenchymal Stem Cells. In this figure the differentiation potential of MSCs is illustrated. Upon specific, well-defined stimuli MSCs initiate lineage specific differentiation pathways that enable them to become a wide variety of mature skeletal cell types. Adopted and modified from www.discoverymedicine.com.

Functions

In addition to regulating normal skeletal homeostasis, MSCs also play an important role in fracture repair. Bone fracture or injury initiates a series of cellular and molecular pathways that commence with hematoma formation and an inflammatory cascade that regulates MSCs activity leading to fracture healing and the reestablishment of skeletal integrity (109).

The other well recognized function of MSCs is support of hematopoiesis by continuously recreating and safeguarding the microenvironment (stem cell niche) necessary for proper blood cell production. They do so by producing a wide array of different cytokines (IL-6, TGF- β, etc.), chemokines (CCL2), and growth factors (G-CSF, HGF, etc.) (110).

1.4 Germ layer derivatives and the central dogma

A germ layer is a collection of primitive cells which appear early during the development of the human embryo following gastrulation. In accordance with their positions in the spherical type of gastrula three kinds of germ layers are recognizable. The ectoderm also known as the epiblast, the mesoderm also called the mesoblast and the endoderm often referred to as the hypoblast. During organogenesis these layers will create the various organ systems (111). **Table 1** summarizes the origins of different organs.

Until recently the general understanding was that after embryonic development cells cannot change their germ-line fate and become cells bearing characteristics of another tissue. In the last couple years, however, it became clear that this dogma cannot survive the scientific surge of the last decades. There have been several well-documented and controlled studies using the tools of modern biology and genetics showing that already differentiated cells or tissue specific stem cells can indeed adopt phenotypic and functional characteristics of different tissues - even outside of their own germ layers. These types of fate conversions are referred to as transdifferentiation.

Tissue	**Adult derivatives**
Ectodermal germ layer	
Surface ectoderm	Epidermis and its appendages (hair and nails), subcutaneous glands, mammary glands, enamel of teeth
Neural Ectoderm	Central Nervous System (brain, spinal cord)
Neural Crest	Cells of the spinal cranial (V, VII, IX, X) and autonomic ganglia, ensheathing cells of the peripheral nervous system, pigment cells of the dermis, bone, connective tissues, and muscles of pharyngeal arch origin, suprarenal medulla and meninges of the brain and spinal cord
Mesodermal germ layer	
Prechordal Plate and Paraxial Mesoderm	Several skull bones, head and eye muscles
Cardiac mesoderm	Heart
Notochord	Intervertebral discs
Somites	Vertebrae, ribs, skeletal muscles, dorsal dermis
Somatic mesoderm	Sternum, bones of the limbs, ventral dermis
Intermediate mesoderm	Somatic gonad, adrenal cortex, kidneys, ureters, genital ducts
Splanchnic mesoderm	Connective tissue of the cardiovascular system and the gut
Endodermal germ layer	Epithelial lining of the airways (larynx, trachea, bronchi, lungs), gut, bladder, most of the urethra, tympanic cavity, and the auditory tube; liver, pancreas, thyroid and parathyroid glands, thymus, tonsils

Table 1. The most important adult derivatives of embryonal structures are listed

1.4.1 Criteria for transdifferentation

- Transition of a cell of a specific tissue lineage into a different lineage must be demonstrated possibly by multiple convincing methods. Such demonstration requires identification and quantification of the original as well as the ”transdifferentiated” cell types, showing appropriate expression of donor-specific and tissue-specific markers, with proper integration into the target tissue and preferably concomitant demonstration of tissue-specific function.
- The lineage transition should be accompanied by “reprogramming” of the transdifferentiated nucleus, such that genes characteristic of the original tissue of origin are silenced and transcription of genes specific to the new cell fate are activated.
- In order to assess the intrinsic developmental potential of a particular cell type, the cells should be minimally manipulated, and preferably analyzed without any intervention (this includes labeling of the cells, since almost all labels used can affect cell function) that may change their gene expression profile and/or chromatin configuration.
- Ideally, transplantation should be done with a single adult stem cell; otherwise, contamination by precursors of other lineages could be responsible for the finding of unexpected cell types.
- Given the possibility that certain cell types can transfer genetic material via cell fusion, claims of transdifferentiation must exclude the possibility that donor markers were acquired from unrelated cell types by such a mechanism (4; 112; 113).

1.4.2 Methods to study transdifferentiation

Detection of donor Y chromosome

Human cells contain 23 pairs of chromosomes for a total of 46. There are 22 pairs of autosomes and one pair of sex chromosomes represented by an X chromosome and a Y chromosome. In females two X chromosomes are found while in males an X and a Y chromosome together determine the gender. If cells are prepared from a male and transplanted into a female, they will be unique owing to the presence of the Y

chromosome. In other words, in the recipient's body, male cells can be distinguished from female cells using detection methods that visualize the presence of the Y chromosome. The most widely utilized method for this is in situ hybridization where radioactive or non-radioactive (digoxigenin labeled) probes bind to specific regions on the Y chromosome to identify male cells (114-116).

Detection of beta-galactosidase activity

The lacZ gene of E. coli, encoding for beta-galactosidase (beta-Gal), is used extensively as a reporter of gene expression in a variety of systems. The ROSA26 mice carry a LacZ-neoR insertion on Chromosome 6, which drives beta-Gal expression to all cells of these animals. This means that if we transplant cells from a ROSA26 animal into a wild-type recipient the transplanted cells and their progenies can be found by detecting enzymatic activity of beta-Gal in histological sections. This enzyme exhibits maximal activity at neutral to alkaline pH (optimal pH 7.0 – 7.5) that enables discriminating it from the endogenous mammalian beta-galactosidase (optimal pH 3.0 – 6.0). Another advantage of the beta-Gal system is the availability of the precipitating beta-Gal substrate 3-indolyl-beta-D-galactopyranoside (X-Gal). In combination with a specific iron buffer, X-Gal offers an easy-to-use histochemical procedure, enabling detection of individual cells expressing a bacterial lacZ gene by light microscopy. It yields a blue stain within positive cells without producing any background. One of the drawbacks of the technique though is that it is very challenging to look for another marker in the same cells due to technical difficulties (117; 118).

Detection of fluorescent reporter gene products

Similarly to the beta-Gal system, other reporter mice showing universal expression of fluoresecent proteins can also be utilized as donor cells. Green Fluorescent Protein (GFP) isolated from the jellyfish Aequorea victoria was the first fluorescent protein discovered. It revolutionized the technology of molecular tracking and soon became one of the most widely used tool in molecular and cell biology. Other examples of frequently used fluorescent proteins are RFP (red fluorescent protein) or YFP (yellow fluorescent protein); each is linked to the promoter of a house keeping gene, beta-actin. When fluorescent stem cells are transplanted into naïve mice, they (and any cells

differentiating or trans-differentiating from them) can be identified on histological sections based on their fluorescence upon Ultraviolat (UV) light exposure. Fluorescent labeling has further advantages, such as enabling co-labeling with other markers and other fluorochromes as well as the ability of using confocal microscopic analysis of the tissues (119-122).

1.4.3 Transdifferentation of bone marrow cells into non-hematopoietic cell types

Tissues with slow turnover rate

Central nervous system

Until about 20 years ago, it was the general notion that after the developmental period ends, neurons cannot be regenerated. This principle was overturned when neuronal stem cells were first discovered and a whole new field of neurobiology was born (123). Many studies have been published on adult neurogenesis and several outstanding reviews have appeared on this subject (124; 125). These stem cells are present in the embryonal brain and develop together with the brain itself keeping the potential of producing additional neurons post-natally during physiological processes, such as learning and exercise (126). Even more surprising were data suggesting that circulating cells from the blood might be able to enter the brain and develop into new neural cells there, including microglia, macroglia and even neurons (114; 127; 128) in rodents' brains. These findings met a lot of skepticism due to the possible technical problems that could have been responsible for the results. Many groups continued to study the possible regenerative and/or neuroprotective effect of BM transplantation or injection of different populations of BM cells into the Central nervous system (CNS) following a variety of injuries including, but not restricted to, stroke, trauma, degenerative diseases and most workers concluded that BM-derived cells indeed help the healing process. The most widely accepted mechanism of this healing is that the BM cells enter the CNS, migrate to the injury site, and release neuroprotective and survival factors as well as anti-inflammatory agents as well as help recruit neuronal stem cells to the site (129-133). Studies of post-mortem human samples confirmed the rodent results and showed that female patients who received gender mismatched BM transplants had Y chromosome containing neural cells (including neurons) present in their brains (115; 134-136). These cells--with the exception of Purkinje cells (134)--were diploid,

suggesting that they were not the products of cell fusion (135). These human data suggests that transdifferentiation is a real, naturally occurring phenomenon - even if it is very rare. In any event, because bone marrow cells are able to enter the brain and travel to areas of need, it has been suggested that they might be used as vectors for missing factors (e.g., enzymes) and/or agents that could selectively kill cancer cells (130; 137-139). These exciting possibilities deserve to be further explored in detail.

Liver

Petersen et al. identified BM as a source of hepatic cells in female rats following transplantation with male cells using the Y chromosome as a marker of BM derivatives and dipeptidyl peptidase IV enzyme, and L21-6 antigen to identify liver cells (140). Using archived specimens of gender mismatched BM or liver transplanted humans, Theise et al showed that 4-40% of the liver hepatocytes arose from exogenous cells (141) confirming the animal data. A year later, another group reported that intravenous injection of adult bone marrow cells, rescued the Fah (-/-) mouse, an animal model of tyrosinemia, and showed that only purified hematopoietic stem cells gave rise to hepatic regeneration (142). Using the same system and the Fah (-/-) mice, Vassilopoulos and his group has demonstrated that the new hepatocytes are generated by fusion of hematopoietic and liver cells (143) and following two more publications with similar conclusions, (139; 144) the possibility of transdifferentiation was ignored and fusion favored in all instances. It is worthwhile to take a closer look at the problem in the light of data known about liver regeneration.

Guidotti et al. (145) have shown that cultured hepatocytes commonly replicate their genomes in the absence of cell division. The resulting cells are tetraploid, and if the above process repeats itself, their children or grandchildren can even be octaploid. The *Fah*-/- mice have a neonatal lethal phenotype resulting from hepatic dysfunc-tion and require treatment with 2-(2-nitro-4-tri-fluoro-methylbenzoyl)-1,3 cyclo -hexamedione to survive. Drug treatment was suspended after the mice were transplanted, and surely this was stress-ful to the *Fah*-/- hepatocytes (143; 146). To understand the importance of this stress, we would have to know why, among all cells of the body, the liver cells are polyploid. Hepatocytes are the body's first line of defense against harmful agents in the diet, and polyploidy may permit them to manufacture detoxifying enzymes at

higher levels. Furthermore, it may also protect them from loss of recessive oncogenes and malignant transformation; the more copies of a gene you have, the less likely it is that all of these will be lost. Given these facts, stress could induce liver cells to fuse as part of an organ-specific defense mechanism. In any case, fusion may be much less common in cells that are prone to remain diploid throughout their lives, and the suggestion that the fusion of stem cells to differentiated cells could account for all of the evidence presented in favor of transdifferentiation is very likely to be incorrect. The issue has been discussed in several reviews (137; 138; 147; 148) and it seems likely that both mechanisms (i.e. transdifferentiation and fusion) may play a role in tissue regeneration by exogenous cells.

Pancreas

The regeneration of pancreatic beta cells has been a hot topic for decades due to the increasing prevalence of diabetes and the need for better treatments. Thus Kodama et al. triggered both interest and controversy when they published a study showing successful treatment of NOD mice with end-stage diabetes by injection of donor splenocytes and complete Freund's adjuvant. This treatment appeared to eliminate the autoimmune response and permanently restore normoglycemia. This was accompanied by the reappearance of pancreatic beta cells. Kodama demonstrated that donor male or labeled splenocytes rapidly differentiated into islet and ductal epithelial cells within the pancreas in diabetic NOD females (149). While others have successfully reversed diabetes using Kodama's protocol (150-152), the mechanism of the effect is still debated. Ianus et al. reached similar conclusion to Kodama using bone marrow cells from male mice that express enhanced green fluorescent protein (EGFP) if the insulin gene is actively transcribed. When these cells were transplanted into lethally irradiated recipient female mice, Y chromosome and EGFP double-positive cells were seen in the pancreatic islets of recipients. EGFP could not be detected in either bone marrow cells or circulating peripheral nucleated cells. The EGFP positive islet cells were shown to express insulin, glucose transporter 2 (GLUT2), and transcription factors typically found in pancreatic beta cells. These results suggest that there are cells in the BM (and in the spleen) with the capacity to differentiate into functionally competent pancreatic endocrine beta cells and they might represent a potential for cell-based treatment of

diabetes mellitus (153).

Lung

In her important paper in 2001, Krause reported that one single CD34+ cell is capable of repopulating the BM and giving rise to a variety of tissue cells including lung epithelium in mice (154). Three groups later studied the relevance of the above finding in human biopsied or archived material from female patients who received gender mismatched BM or lung transplants using the Y chromosome as a marker. All groups concluded that, indeed, there is chimerism in both epithelium and endothelium. One group found 2.5-8% epithelial and 37.5-42.3% endothelial donor contributions (155). Similarly, Mattsson et al. found 2-6% Y positive and surfactant positive lung epithelial cells following non-myeloablative HSC transplantations (156). In the lungs of male patients who had received female lung transplants, Y chromosome positive, surfactant positive type II pneumocytes were found. Using concomitant X and Y chromosome hybridization the authors excluded fusion as a possible explanation for the finding (157). In her new studies in mice Krause examined the effect of radiation damage to the lung on the level of BM-derived cell engraftment and concluded that lung injury stimulates both hematopoietic and stromal BM cells to contribute to the injured lung epithelium (158).

Kidney

The kidney is a very complex organ composed of more than 20 different cell types. These cells also differ in their proliferation rate and more than one type of tissue resident stem cell was found to help maintain the balance of proliferation in health and in injury. It was almost 40 years ago - way before we talked about the plasticity of adult stem cells - when Sinclair published his observations on the endothelial cells of 40 human kidneys transplanted to recipients of the opposite sex and showed that the donor endothelium had persisted. However, in three poorly functioning damaged grafts a high proportion of the endothelial cells in capillaries and veins seemed to derive from the host. He suggested that the repopulated endothelium possibly derived from circulating cells (159). With the technical development and the increased knowledge about stem cell biology many groups continued to study the contribution of circulating

cells to the kidney in patients who received gender mismatched organ transplants. Several groups have reported the presence of Y chromosome–positive renal tubular cells ranging from 0.6–6.8% in one study (160) and about 1% in the other (161) in kidneys of male patients who received kidneys from female recipient. These studies also suggested that cells outside of the kidney are able to be recruited and replenish tubular epithelium and they conclude that kidney injury seems to increase the number of cells that are recruited from the circulation. Rodent studies using GFP+ BM transplanted into lethally irradiated mice and rats also concluded that circulating BM-derived cells could differentiate into glomeruli mesangial cells (162; 163). In an interesting study, bone marrow-derived cells were also examined for their ability to repair a genetic collagen defect in mice. The authors used a model of Alport syndrome, when genes encoding the type IV collagen have mutations that result in structural basement membrane defect leading to proteinuria and eventually renal failure. They demonstrated that in a mouse with a similar defect, the transplanted lacZ positive and gender mismatched BM gave rise to cells that can replace podocytes and mesangial cells and can partially restore glomerular function (164). They found 6.2% beta-Gal positive cells per glomerulus at 12 weeks of age and the number seemed to be increasing, reaching over 10% by week 20. The authors did not rule out fusion as a mechanism and suggest that kidney injury needs to be present for the BM cells to be recruited. The possible sources and ways of how the different compartments of the kidneys can regenerate are nicely summarized in recent reviews (165; 166). Among these possibilities it seems very likely that BMSCs contribute to kidney regeneration through the release of growth factors, anti-inflammatory cytokines and anti-apoptotic factors (167; 168).

Skeletal Muscle

In 1998, Ferrari et al. reported that BM cells contribute to myogenesis in genetically diseased muscle of mice with muscular dystrophy (169). They did not define the nature of the cells that differentiated into myocytes, but their work initiated studies aimed to do just that. Gussoni and his group first reported in an animal model of Duchenne's muscular dystrophy (DMD), that the intravenous injection of either normal haematopoietic stem cells or a novel population of muscle-derived stem cells into

irradiated animals results in the incorporation of donor-derived nuclei into muscle, and the partial restoration of dystrophin expression in the affected muscle (170). He later published an interesting case report showing that a person, who received gender-mismatched BM as a child and later developed DMD, presented donor nuclei in muscle biopsies within a small number of muscle myofibers (0.5-0.9%). These data documented the occurrence of BM cells fusing with skeletal muscle cells and maintaining the fused DNA for over a decade (171). Corbel et al. transplanted isolated GFP+ HSCs into GFP- recipients and studied their possible contribution to healthy as well as diseased muscle (172). Their results showed that in a portion of the healthy animals a few GFP+ myocytes could be found, but when a toxin was used to damage the anterior tibial muscle, all animals seemed to incorporate the donor HSCs into their regenerating muscle. The authors did not rule out fusion as a possible explanation of the findings. In a similar experiment, Abedi et al. transplanted animals with different populations of GFP+ bone marrow-derived cells and looked for GFP+ myocytes in toxin-injured *tibialis anterior* muscles. The authors concluded that HSCs rather than mesenchymal cells or more differentiated hematopoietic cells preferentially participated in skeletal muscle regeneration (173).

Cardiac muscle

Heart attacks are the leading cause of death and kill close to half a million people in the US every year. New treatments that might help victims survive and achieve a good quality of life receive attention from the public and research communities alike. Thus in 2001, when Orlic reported that he injected Lin(-) c-kit(+) BMCs from syngeneic mice into the hearts of animals that had acute infarcts, and that these cells differentiated into myocytes and vascular structures (174), many groups started to work on cellular repair in heart disease. In a subsequent article the same group reported that cytokine-mobilized BM cells caused a significant drop in mortality rate and improvement of function following myocardial infarctions. They observed large numbers of new myocytes and capillaries in the damaged heart (175). Nygren has demonstrated that BM-derived cells fuse with cardiomyocytes in infarcted mouse hearts (176). Bone marrow-derived stromal cells on the other hand were shown very early to be able to differentiate into cardiomyocytes in vitro and in vivo (177; 178). Based on testing

different fractions of hematopoietic cells, Doyonnas concluded that the c-kit-positive immature myelo-monocytic precursors are responsible for the newly generated myocytes following local intramuscular injection of the cells (179). A few years ago in a very elegant study Rota et al. injected BMSCs at the border of the infarct and used a variety of techniques to conclude that BMSCs engraft into the injured heart and become functional cardiomyocytes (as shown by electrical activity and mechanical function of the heart) in the mouse (180). After initial enthusiasm based on the above rodent data there were disappointing results in human trials: CD34+ cells did not appear to proliferate or transdifferentiate into cardiomyocytes (181; 182). Thiele et a. examined gender mismatched transplant patients and found a lower incidence (1.6%) of chimeric cardiomyocytes after allografting with PBSC (peripheral blood stem cell) than after full BM transplants (5.3% of donor-derived cells) (183). Assmus et al. showed that in patients the intracoronary infusion of BMSCs results in a significant reduction of postinfarction heart failure (184). At this time there are clinical data on over 1000 patients with heart disease who received stem cell therapy and the data have been carefully analyzed (185). This includes a variety of cell types (whole bone marrow; HSC, BMSC) and routes of administration. The consensus is that the procedure seems to be relatively safe. The cardiac function shows a modest improvement on the average. Although there is still a debate about the mechanism of action (transdifferentiation, fusion, paracrine effects or a combination of any of the above) but based on the safety it is surely worthwhile to try to learn much more about the biology of these stem cells to be able to optimize their efficiency in healing the injured heart. No matter what mechanism will turn out to be responsible, it is the final interest of all patients to pursue and understand the process that can potentially help them.

Tissues with high turnover rate

Oral mucosa

PCR analyses of short tandem repeats from blood and mouthwash samples were performed to determine the genotypes of recipient vs. donor cells in 17 BM transplant patients (186). To the surprise of the researchers, donor DNA was detected even after all blood cells were removed from mouthwash samples and they concluded that this must be due to technical problems. In a large number of patients using the same

technique another group found 5-63% chimerism (cells of donor origin) in buccal swab samples. Tran et al. used buccal scrapings on microscope slides and Y chromosomal Fluorescent in situ Hybridisation (FISH) combined with cytokeratin as an epithelial marker to look for donor derived cells in gender mismatched bone marrow transplant female patients (187). Since all of the samples had chimeric cell populations (0.8-12.7% donor-derived) they further analyzed the cells for fusion and found only two potentially fused cells (0.01%), suggesting that the newly formed buccal cells are not a result of cell fusion. Finally, they used microsatellite markers to see if the buccal cell Y chromosome is identical to the donor or to the son of one patient. The Y chromosome showed a 100% identity to the donor, and none with the son, confirming that the cells must be of donor BM or HSC origin.

Gastrointestinal tract

Due to the relatively rapid turnover of gastrointestinal epithelial cells, several groups searched for a contribution to this cell population by exogenous cells. Following irradiation and BM transplantation donor derived myofibroblasts and epithelium in the small intestine and colon was found in both humans and mice (188; 189) using Y chromosome FISH. The number of donor-derived cells seemed to increase if ulcer or inflammation (due to graft-versus-host disease also known as GVHD) was present (189). Contrary to these observations, Meignin could not demonstrate any epithelial cells in the duodenum of BM transplanted patients who developed GVHD (190). In a very thorough study using a rigorous three-dimensional analysis on single sections of colon biopsies triple-stained with donor-specific, epithelial-specific, and hematopoietic-specific markers Spyridonidis demonstrated that chimerism of colon epithelium occurs after human hematopoietic cell transplantation. The authors excluded cell fusion as the possible underlying mechanism of the findings. They observed that tissue damage enhances engraftment by donor-derived epithelial cells (191).

Uterine endometrium

The human endometrium is a dynamic remodelling tissue undergoing more than 400 cycles of shedding, regeneration, and differentiation during a woman's reproductive years (192). Each month 4 – 10 mm of mucosal tissue grows within 4 – 10 days in the follicular (proliferative) stage of the menstrual cycle under the influence of increasing circulating estrogen levels. In non-menstruating species (e.g. mice), the endometrium undergoes cycles of growth and apoptosis during the estrus cycle, rather than physical shedding (193).

Taylor wondered whether circulating cells contributed to this process in humans. He used tissue samples from 4 HLA mismatched transplant recipients to look for donor HLA markers by means of immunostaining and RT-PCR. All 4 women had donor HLA in their epithelial (0.2-48%) and stromal (0.3-52%) cell populations (194) and Taylor concluded that non-uterine cells contribute to endometrial regeneration. In a other study in 2007 Du et al. detected very low level of chimerism (<0.01%) in mouse uterine epithelium following bone marrow transplantation. Hence the rate at which bone marrow derived cells can contribute to the development of uterine epithelium seems extremely variable. This can be due to several factors, including the age of the studied specimen, and the experimental techniques used.

Skin epidermal cells/hair follicles

Our skin is continually challenged by a variety of environmental hazards. The epidermis, the outermost skin layer, provides the first line of defense and protection. The epidermis includes a variety of specialized cell types; however, the major cell type, also responsible for constructing the protective barrier, is the epidermal keratinocyte (97).

Although the capability of bone marrow derived stem cells to become skin keratinocytes has been studied by several research groups, there is still an ongoing debate regarding the existence of this phenomenon. Many laboratories concluded that bone marrow stem cells play an important role in the repopulation of skin keratinocytes, while others detected only a few or no transdifferentiated cells in the epidermis (195-206). The reason for these seemingly contradictory data is multifold and most likely

stems from the following practical issues.

- There are fundamental differences between animal and human models.
- Collection time of human skin specimens after bone marrow transplantation might be too short (average 3-4 years).
- There are several techniques used to study transdifferentiation in the skin, some of which might not allow proper assessment of donor chimerism among host keratinocytes.
- Keratinocyte culture methods:
    - Enzymatic digestion and subsequent in vitro processing can preselect for more abundant host derived keratinocytes,
    - DNA collected from a multi-colony derived cell culture (for PCR analysis of chimerism) mostly represents cells derived from keratinocyte stem cells, or transient amplifying cells, not from post-mitotic keratinoctyes, which account for the majority of keratinocytes in the epidermis.
- Studies using plucked hair:
    - Depending on the actual patient, the scalp area studied, and the hair collection method, hair plucking can result in the evaluation of very different cell populations (epithelial cells, as well as fibroblasts, or immune cells coming from the dermal papilla) even though the examined hair part is always called hair bulb. As a result of this cellular heterogeneity, PCR studies done on hair bulb DNA might not be the proper way to evaluate transdifferentiation.

2. Specific aims

In order to address the seemingly contradictory literature data on the transdifferentiation potential of hematopoetic stem cells, we decided to study the contribution of these cells to highly proliferating epithelial tissues (where one would expect a greater need for hematopoetic stem cell derived support in order to maintain tissue homeostasis).

More specifically, we aimed:

1/a. To examine bone marrow contribution to the uterus epithelium, using a standard bone marrow transplantation assay,

1/b. To develop a mouse model which enables us to detect transdifferentiation of bone marrow derived hematopoietic cells without the need of bone marrow transplantation, and preconditioning with chemotherapeutic agents or whole body irradiation,

1/c. To evaulate bone marrow hematopoetic stem cell contribution to the uterus epithelium utilizing this new model,

2. To reevaluate the potential of human hematopoietic stem cells to become skin keratinocytes using punch biopsy specimens collected from the hairy occipital area and the non-hairy back skin at least 10 years after bone marrow transplantation. In order to avoid technical pitfalls described earlier, in situ histologic analysis combining Y chromosome hybridisation and immunostaining is planned to be performed.

3. Methods

3.1 Mouse experiments

3.1.1 Bone Marrow Transplantation Studies

Allogenic bone marrow transplantation was used to detect progeny of GFP+ BM-derived cells. Briefly, recipient *C57BL/6J* mice (*n*=6) received whole-body irradiation (900 rad) in two separate doses (450 rad each time) 8 hours apart. Donor cells were prepared from the femurs and tibiae of a GFP+ mouse (C57BL/6-Tg [ACTB-EGFP] 1Osb/J, 003291; Jackson Laboratory, Bar Harbor, ME, http://www. jax.org). 2 hours after the second irradiation recipient animals were injected intravenously with one million donor bone marrow cells in 0.3 ml PBS. At later time points, transplanted animals were sacrificed and tissues harvested as described above.

3.1.2 Transgenic Mouse Studies

To determine whether CD45 cell progeny contributes to the repopulation of the uterine epithelium under physiological conditions, we generated a novel transgenic mouse. First, we introduced Cre recombinase cDNA into a bacterial artificial chromosome (BAC) containing the complete mouse *CD45* gene (**Fig. 5A**). The recombinant cDNA was inserted downstream of an internal ribosomal entry site (IRES), which in turn was placed downstream of the last coding exon of CD45. The resulting BAC was used to make transgenic mice (*CD45/Cre*). In such mice, Cre recombinase is produced in any cell that expresses CD45 or expressed it at any time during embryonal development. These *CD45/ Cre* animals were then bred with the *Z/EG* double reporter mice that express lacZ ubiquitously. In the presence of Cre recombinase, the *lacZ* gene and the transcriptional stop following it are excised **(Fig. 5B)**, activating the expression of a second reporter, the enhanced green fluorescent protein. In the offsprings of these mice all cells that expressed CD45 at any timepoint during development or adulthood will become and stay green (i.e. once the Cre-recombinase excises the stop sequence, the GFP will be “turned-on” as long as the cell lives). Only first-generation Cre transgenics were used because the transgene is prone to rearrangement. Thus, in subsequent generations, ectopic expression, even ubiquitous expression, can be seen.

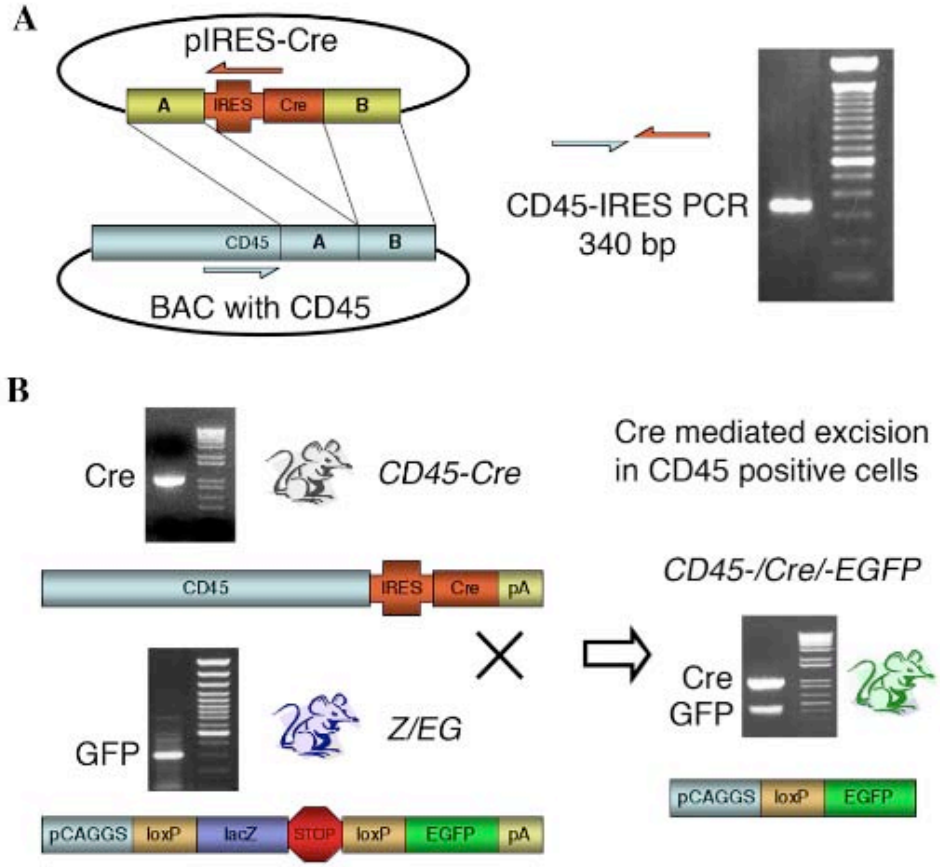

Figure 5. GFP is expressed in CD45+ cells of the double-transgenic (*CD45/Cre-Z/EG*) mice. **(A):** Upon homologous recombination, the IRES- Cre recombination cassette flanked by two homologous fragments (A and B) was inserted into exon 33 of the *CD45* gene in a BAC containing the entire coding region. The recombinant construct was confirmed to be correct by restriction mapping, PCR with primers flanking the recombination fragments, and sequencing. **(B):** To track the fate of CD45+ cells, we created a double-transgenic strain by breeding *CD45/Cre* mice with the double-reporter *Z/EG* mice. In the crossbred mice, every CD45+ cell expresses the Cre recombinase that will excise the floxed lacZ cassette, thus enabling the activation of GFP. Regardless of the future fate of CD45+ cells, GFP will be expressed continuously throughout their life spans.

3.1.3 Construction of Recombination Cassette

The recombination cassette was constructed by cloning the IRES- Cre-frt-pgk/em7/Neo/bpA-frt expression cassette from p459 (a gift from Neal G. Copeland) with two flanking polymerase chain reaction (PCR)-amplified recombination fragments

(A and B) into the pBC KS+ shuttle vector (Invitrogen, Carlsbad, CA, http://www. invitrogen.com). Fragments A (263 base pairs [bp]: 3,305–3,567; GenBank accession number nm_011210) and B (319 bp: 3,568– 3,886; from nm_011210) were designed to flank the insertion site for the expression cassette upstream of the stop codon at the 3' end of exon 33 of the *CD45* gene **(Fig. 5A)**. The plasmid was introduced into *Escherichia coli* using heat shock, single ampicillin-resistant colonies were selected and propagated, and the plasmid was isolated by mini-prep and linearized for homologous recombination.

3.1.4 Homologous Recombination

BAC 131H7 (from the RPCI-23 mouse BAC library; Invitrogen) was used for these studies. This BAC is approximately 190,000 bp long. There are approximately 30,000 bp of DNA 5' to the first exon of CD45 and 50,000 bp 3' to the last exon. The recombination cassette was inserted to the BAC by homologous recombination [14] (Fig. 1A). Briefly, the BAC was electroporated into *E. coli* EL250, which hosts a temperature-inducible prophage that facilitates recombination. BAC-containing bacteria were incubated at 42°C for 15 minutes and transformed with the linearized recombination cassette by means of electroporation. Double-resistant (kanamycin-chloramphenicol) colonies were selected and propagated, and the recombinant BAC was isolated using a NucleoBond BAC Maxi Kit (BD Biosciences, San Diego, (bdbiosciences.com). Homologous recombination was confirmed by enzymatic digestion, PCR, and DNA sequencing **(Fig. 5A)**.

3.1.5 Production of Double-Transgenic Mice

Recombined BAC DNA was microinjected into *C57BL/6J* zygotes that were implanted into estrogen-primed foster mothers, and transgenic founders were selected by PCR using the following primers amplifying Cre: 5'-ccggtcgatgcaacgagtgatgagg-3' and 5'-gcgttaatg- gctaatcgccatcttcc-3'. The resulting *CD45/Cre* mice were bred with *C57BL/6J* mice. Subsequently, *CD45/Cre* offspring were bred with heterozygous *Z/EG* double reporter mice (Jackson Laboratory) that express GFP upon Cre-mediated recombination, and their offspring were screened by PCR for Cre using the primers described above and for GFP (5-gggcgatgccacctacggcaagctgaccct-3'and 5-ccgtcctccttgaagtcgatgcccttcagc-3) **(Fig. 5B)**. Double-transgenic (*CD45/ Cre-Z/EG*)

animals were selected and used for the studies reported. The majority of nucleated cells in the blood of the double-transgenic mice produced from all four founder lines were GFP positive, and their progeny seemed GFP positive as well (**Fig. 6A–6D**). Thus, although we did not study this extensively, we observed no line-to-line variation, and we have no evidence that the site of integration of the recombinant BAC affected Cre expression.

3.1.6 Animal Procedures

Animal care and procedures were approved by the Animal Care and Use Committee of National Institute of Mental Health, NIH. Double-transgenic (*CD45/Cre-Z/EG*) animals were anesthetized, sacrificed by perfusion, and examined at different ages: 18-day-old embryos (*n*=2), 3-day-old pups (*n*=2), 6-week-old young adult (*n*=1), 12-week-old adults (*n*=2), 12-week-old pregnant animals (*n*=2), and 20-week-old adult (*n*=1). Tissues were fixed by transcardiac perfusion or immersion (used for fixing embryos) with Zamboni solution, harvested, and processed for immunostaining.

3.1.7 Histology

Perfused tissues were cryoprotected by immersion in 20% sucrose solution and frozen. Twelve-µm-thick sections were cut at -24°C in a Leica cryostat (Heerbrugg, Switzerland, http://www.leica.com), thaw-mounted, and stained using the following reagents: 1:1,000 anti-GFP antibody (A11122;MolecularProbes, http://probes.invitrogen.com) followed by 1:1,000 Alexa-488 conjugated anti-rabbit antibody (A31565; Molecular Probes), 1:100 anti-CD45 antibody (ab3088-100; Abcam, Cambridge, MA, http://www.abcam.com) followed by 1:1,000 Alexa-594-conjugated anti-rat antibody (A11007; Molecular Probes), 1:20,000 4,6-diamidino- 2-phenylindole (DAPI) (D1306; Molecular Probes), and 1:200 epithelium specific biotinylated *Lotus tetragonolobus* lectin (B-1325; Vector Laboratories, Burlingame, CA, http://www.vectorlabs.com) followed by 1:1,000 Alexa-594-conjugated streptavidin (S11227; Molecular Probes) or 1:1,000 horseradish-peroxidase-conjugated streptavidin and 1:10,000 [EM6] Alexa-350-conjugated tyramide (T20937; Molecular Probes). As a second marker, we also used a wide-spectrum cytokeratin antibody (MU-131-UC; BioGenex, San Ramon, CA, http:// www.biogenex.com) at a 1:200 dilution followed by an anti-mouse IgG conjugated to Alexa-594 (1:1,000) to confirm the

characterization of epithelial cells. The specificity of the GFP immunostaining was also confirmed by a second anti-GFP antibody (1:2,000; AB16901; Chemicon, Temecula, CA, http://www.chemicon.com) that detects a different epitope of GFP and showed results identical to those described before (data not shown). Controls were performed using secondary antibodies after omitting the primary antibodies. Detailed controls and variations of GFP stainings are described in Toth et al. [15]. Random areas of sections were photographed at low magnification, and epithelial cells (approximately 500–1,000 per animal) were counted by two independent investigators. The number of GFP-positive cells was expressed as a percentage of all epithelial nuclei based on DAPI and *Lotus* staining.

3.1.8 In Situ Hybridization Histochemistry

To confirm the results of immunostaining we also demonstrated the presence of GFP mRNA in the uterine epithelial cells using a specific radiolabelled probe in 12-µm fixed sections. The primers that included a T7 and a T3 polymerase sites were constructed to generate a template complementary to the GFP mRNA between nucleotides 1,524 and 1,823 (accession no. AB234879). This template was then transcribed using the Ambion (Austin, TX, http://www.ambion.com) Maxiscript transcription kit (catalog number 1324) following the manufacturer's instructions. After hybridization, the slides were dipped into autoradiographic emulsion (Nuclear Track Emulsion B; Kodak, Rochester, NY, http://www.kodak.com) and developed 2 weeks later. A Giemsa background staining was applied, and the slides were dried and coverslipped. The sections were viewed with a Leica DMI6000 inverted microscope, and images were captured using Volocity software (PerkinElmer Life and Analytical Sciences, Boston, http://www.perkinelmer.com).

3.1.9 Fluorescence In Situ Hybridization in Combination with GFP Immunostaining

To examine if the detected GFP positive cells might be a result of cell fusion we wanted to determine the number of X chromosomes in these cells. The STARFISH kit (catalog no. 1597-KD-50; Cambio, Cambridge, U.K., http://www.cambio.co.uk) was used to detect the X chromosomes in 6-µm thin sections of fixed uterine epithelium. Following microwave-induced antigen retrieval, GFP immunostaining was performed

as described above. Then the sections were dehydrated and stained according to the Cambio protocol. A Cy3-labeled chromosomal paint probe was used for final visualization. Random areas of eight sections of uterine epithelium from one of the pregnant mice were chosen, and 865 cell nuclei were examined, focusing throughout the whole thickness of the section to count the number of X chromosomes per nuclei. Of these nuclei, 240 belonged to GFP+ epithelium, and we found no evidence of fusion; that is, we did not see any nondividing nucleus with four X chromosomes. In fact, all the nuclei that were uncut in the sections exhibited two X chromosomes.

3.1.10 Flow Cytometry

After preincubation with anti-mouse CD16/32 (Caltag Laboratories, Burlingame, CA, http://www.caltag.com) to block the Fc receptor, peripheral blood mononuclear cells were stained with the anti-mouse CD45 R-phycoerythrin-conjugated antibody (catalog no. MCD4504; Caltag). Rat R-phycoerythrin-conjugated IgG2b (Caltag) was used as an isotype control. CD45 staining and EGFP direct fluorescence were analyzed using flow cytomety (Becton, Dickinson and Company, San Jose, CA, http://www.bd.com).

3.2 Human studies

3.2.1 Specimen collection

Incisional biopsies (1 cm x 0.5 cm x 0.5 cm) were taken under local anesthesia (Lidocain-Adrenalin USP) and frozen in embedding media. We enrolled 6 patients with gender- mismatched transplants, one female patient who received female BM and one non- transplanted male control (**Table 2**). From all patients, three sections were taken from the back skin biopsies and three samples from the scalp biopsy. All patients participated voluntarily in the study and were enrolled in a protocol approved by the local ethical committee (TUKEB, 214/2004).

	Patient #1		Patient #2		Patient #3		Patient #4		Patient #5		Patient #6	
	Scalp	Back	Scalp	Back	Scalp	Back	Scalp	Back	Scalp	Back	Scalp	Back
% CK- Y+ cells	11.7±3.0	6.8±4.4	13.6±2.4	13.6±2.0	11.1±5.1	3.7±1.5	12.9±6.0	8.5±0.4	7.5±2.7	10.7±2.8	17.5±2.2	13.4±5.4
% CK+ Y+ clls	0.83±0.3	0.54±0.4	1.32±0.2	0.92±0.5	0.68±0.2	0.32±0.2	1±0.3	0.62±0.5	0.37±0.3	0.38±0.0.1	1.78±0.03	1.08±0.2
Disease	ALL		AML		AML		AML		ALL		CML	
Age at BMT	44		27		46		45		48		27	
Years to biopsy	15		12		13		15		16		15	
Donor	sib		sib		sib		sib		sib		MUD	
BM ablation	TBI+Cy		bu		TBI+Cy		TBI+Cy		TBI+Cy		TBI+Cy	

Table 2. Summary of individual patient characteristics. ALL, acute lymphoid leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; TBI + Cy, total body irradiation and cyclosporine; bu, busulphan; sib, sibling; MUD, matching unrelated donor; BMT, bone marrow transplants.

The biopsies were cut in a cryostat at 10 μm thickness, the sections were placed on poly-l-lysine coated slides and stored frozen at -80°C until ready for processing. For processing, the tissue was removed from the freezer and without thawing, placed into 4% paraformaldehyde/1xPBS for 10 min. The slides were then rinsed 3X3 min in 1XPBS followed by 10min in 1X Universal Power Block (Cat. #HK085-5K BioGenex San Ramon, CA).

3.2.2 Combined double immunofluorescence and Y Chromosome FISH

Three antibodies were used to identify keratinocytes (Pancytokeratin against cytokeratin 1,5,10 and 14), hematopoietic cells (CD45) and proliferating cells (Ki67) in combination with the Y chromosome that was labeled using in situ hybridization and a human Y chromosome specific probe.

Cytokeratin immunofluorescence

After rinsing the slides quickly in deionized water followed by a 1XPBS rinse, the slides were processed as indicated above except using a mouse anti-human cytokeratin (Pan-CK against cytokeratin 1,5,10 and 14) antibody (Cat. #VP-C417, Vector Labs, Inc., Burlingame, CA) at a dilution of 1:150. This was followed by incubation in mouse SuperPicture (Cat. #87-9163 Invitrogen Corp., Camarillo, CA). Positive staining was visualized in green with a tyramide plus FITC system (Cat. # NEL741001KT Perkin-

Elmer, Inc., Waltham, MA). Prior to the second immunostaining, the slides were briefly microwaved in 10 mM Citrate pH 6.1 for 5 min. at 50% power. This procedure blocks any unreacted peroxidase molecules (potentially leftover from the first immunostaining), which could possibly give rise to non-specific staining.

Ki67 and CD45 immunofluorescence

The slides were then incubated with a cell proliferation antibody, rabbit anti-Ki67 (Cat. #15580 GeneTex , Inc. Irvine CA) or a mouse anti-human CD45 antibody (BD Biosciences Cat #555485) at a 1:200 dilution, overnight at 4°C. The next day the slides were rinsed 3X3min. in 1XPBS. Endogenous peroxidase was blocked for 10 min. using a commercially prepared solution (Cat.#S2001, Dakocytomation Carpinteria, CA). Rabbit SuperPicture (Cat.#87-9263 Invitrogen Corp. Camarillo, CA) was applied to the sections for 30 min. at room temperature. After rinsing 3X3 min. in PBS, positively stained cells were visualized in far red (artificial yellow color) using a tyramide plus CY5 system (Cat. # NEL745001KT Perkin-Elmer, Inc. Waltham, MA) at a dilution of 1:1000 for 10 min. The slides were then rinsed in several changes of 1XPBS.

Y Chromosome FISH

Following the double immunostaining, the slides were processed for non-radioactive *in situ* hybridization to detect the Y chromosome. The template for this probe was generated from human genomic DNA by using primers that amplified a 1.3-kb long DNA piece of the human Y chromosome. The probe was labeled with digoxigenin-uridine 5' – triphosphate (Cat. #1 175 025 Roche Applied Science, Indianapolis, IN). The digoxigenin labeled probe was then applied to the slides and hybridized at 81°C for 12 min. followed by 30 min. at 55°C. Slides were then washed in a decreasing concentration of SSC, including a (65°C 0.01% SSC) high stringency wash. A peroxidase conjugated anti-digoxigenin antibody (Cat. #11 207 733 910 Roche Applied Science, Indianapolis, IN) was then applied at 1:400. Positive Y chromosome signal was visualized in red by applying the high affinity substrate of HRP, Alexa-tyramide-594 (Perkin-Elmers). Finally, DAPI (Cat.# D1306, Invitrogen, Corp., Camarillo, CA) was used as a general nuclear stain.

3.2.3 Quantitation

We used three non-consecutive sections (at least 300 μm apart) from each biopsy sample and took pictures of either the whole available section or every second field depending on how large the sections were. In general we ended up with at least 20 fields from each sample and counted all the cell nuclei (stained with DAPI) using the NIH ImageJ program (Mac Vs 1.43). We then manually counted all Y positive cells as well as the double Y and CK positive cells. Because of the limited number of available samples (and the relatively big inter-individual variability observed) we show each data individually instead of performing statistical analysis.

4. RESULTS

4.1 Contribution of bone marrow to the uterus epithelium

4.1.1 Analysis of Blood Cells in the CD45/Cre/Z/EG Mice

As expected, in adult *CD45/Cre-Z/EG* mice, CD45+cells, such as lymphocytes, (**Fig. 6A–6D**) and the progeny of CD45+cells, such as Kupffer cells and microglia (data not shown), were all GFP-positive. Most, but not all, of the white cells in blood that could be stained with an anti-CD45 antibody were also GFP positive. In some cells, the fluorescent signal may have been too weak to detect, or excision of the floxed lacZ cassette may not have occurred because expression of the Cre recombinase was poor. FACS analysis of peripheral blood showed that the majority (65%–85% in several animals tested) of the CD45-positive white blood cells of double-transgenic mice expressed GFP (**Fig. 6A**).

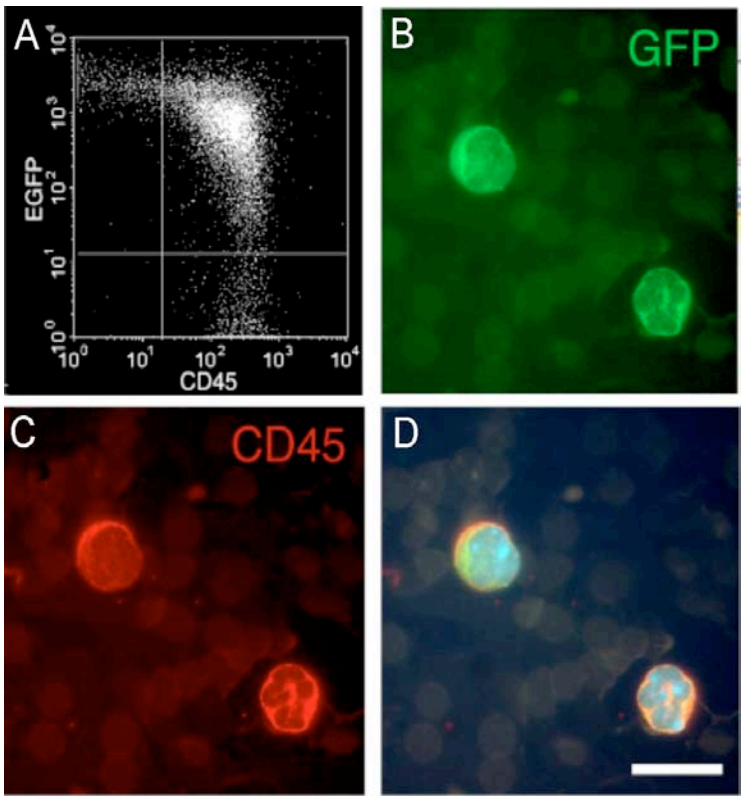

Figure 6. (A): FACS analysis of peripheral blood of a double-transgenic animal showing a high percentage (85%) of double-positive blood cells using green fluorescence for GFP and red for CD45. **(B–D):** White blood cells immunostained with GFP (green) **(B)**, CD45 (red) **(C)**, and an overlay of **(B)** and **(C)** with added 4,6-diamidino-2-phenylindole (nuclear-blue) staining **(D)**. Scale bar = 15 um.

4.1.2 Analysis of Uterine Histology in Long-Term Irradiated and GFP Bone Marrow-Transplanted Mice

Before taking advantage of our newly developed *CD45/Cre-Z/EG* mouse model we decided to assess the transdifferentiation potential of bone marrow derived cells into uterus epithelium using the widely accepted bone marrow transplantation system. Irradiated mice are known to keep on cycling, although the length of the cycles is more variable (207). Eight to 12 months after we transplanted GFP-tagged bone marrow cells (including hematopoietic, mesenchymal, and any other BM stem cell populations) into six irradiated female mice, green cells were detected in the uterine endometrial epithelium and stroma of five of the six animals (**Fig. 7A, 7C**). These bone marrow-derived GFP+ epithelial cells bound *L. tetragonolobus* lectin, a marker with affinity for glycoprotein on the luminal surface of epithelial cells (208). The GFP+ epithelial (i.e., immunopositive for *L. tetragonolobus* [**Fig. 7D, 7E**]) cells did not express the common leukocyte antigen CD45, but numerous CD45+/GFP+ cells were detected in the uterine stroma (Fig. 7A), which is known to harbor CD45+ lymphoid cells (209). Immunostaining using a pan-cytokeratin (cytokeratins [CKs] 8, 18, and 19) antibody also showed colocalization with GFP (**Fig. 7B**), further confirming the epithelial character of the GFP- positive cells.

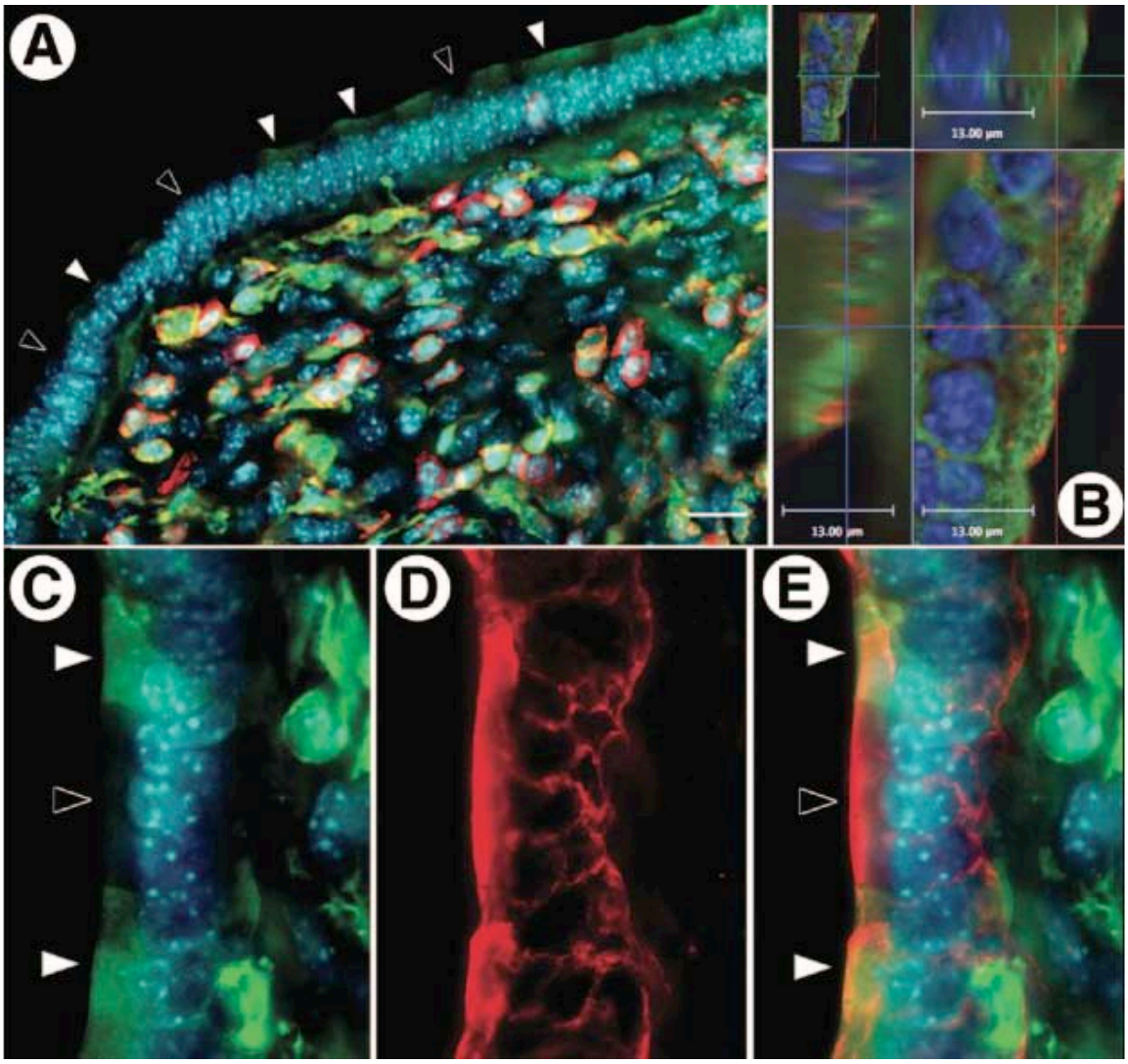

Figure 7. Endometrium of a mouse previously (10 months before) transplanted with enhanced green fluorescence protein bone marrow (BM) following irradiation. Green fluorescent protein (GFP) is shown in green, CD45 **(A)** and *L. tetragonolobus* **(C, D)** in red, and 4,6-diamidino-2-phenylindole (DAPI), the nuclear marker, in blue. **(A):** GFP+ and CD45+ uterine epithelium in the recipient endometrium shows that BM cells can contribute to the regeneration of uterine epithelial cells. CD45+/GFP+ hematopoietic cells are present in the stromal layer. **(B):** Colocalization of a pan-cytokeratin immunostaining (in red) with GFP (in green) in epithelial cells. The image shows one level of a Z-stack in three-panel view. The stack was captured at 0.5-um intervals, and iterative restoration was performed using Volocity 4.0 software (PerkinElmer) and a Leica DMI6000 inverted microscope. **(C–E):** GFP-positive nucleated (DAPI staining) cells appeared in the epithelial layer **(C)**, colocalized with *L. tetragonolobus*, a uterine epithelial marker **(D)**. **(E):** Overlay of **(C)** and **(D)**. Solid arrowheads

indicate GFP+; open arrowheads indicate GFP+ uterine epithelial cells. Scale bar in **(A)** = 20 um **(A)** and 14 um **(C–E)**.

4.1.3 Analysis of Uterine Histology in the Virgin and Pregnant CD45/Cre/Z/EG Mice

Resident macrophages and lymphocytes in the endometrial stroma are known to express CD45 (209), and indeed, we saw many CD45/GFP+ cells in the stroma of the transplanted mice and of the *CD45/Cre-Z/EG* mice (**Figs. 7A, 8B**). We also found GFP+ cells in the epithelial layer of the endometrium (**Figs. 7, 8A**). Although these GFP+cells could be stained with *L. tetragonolobus* lectin (**Fig. 7D, 7E, 8C, 8D**) and with anti- keratin-8, -18, and 19 antibody (**Fig. 7B**), another marker for epithelial cells, they could not be stained with anti-CD45 antibody (**Figs. 7A, 8B**). Expression of GFP mRNA in these cells was further confirmed by in situ hybridization histochemistry (**Fig. 8E, 8F**). Thus, CD45+ cells appear to give rise to epithelial cells in the uterus of nonirradiated transgenic animals.

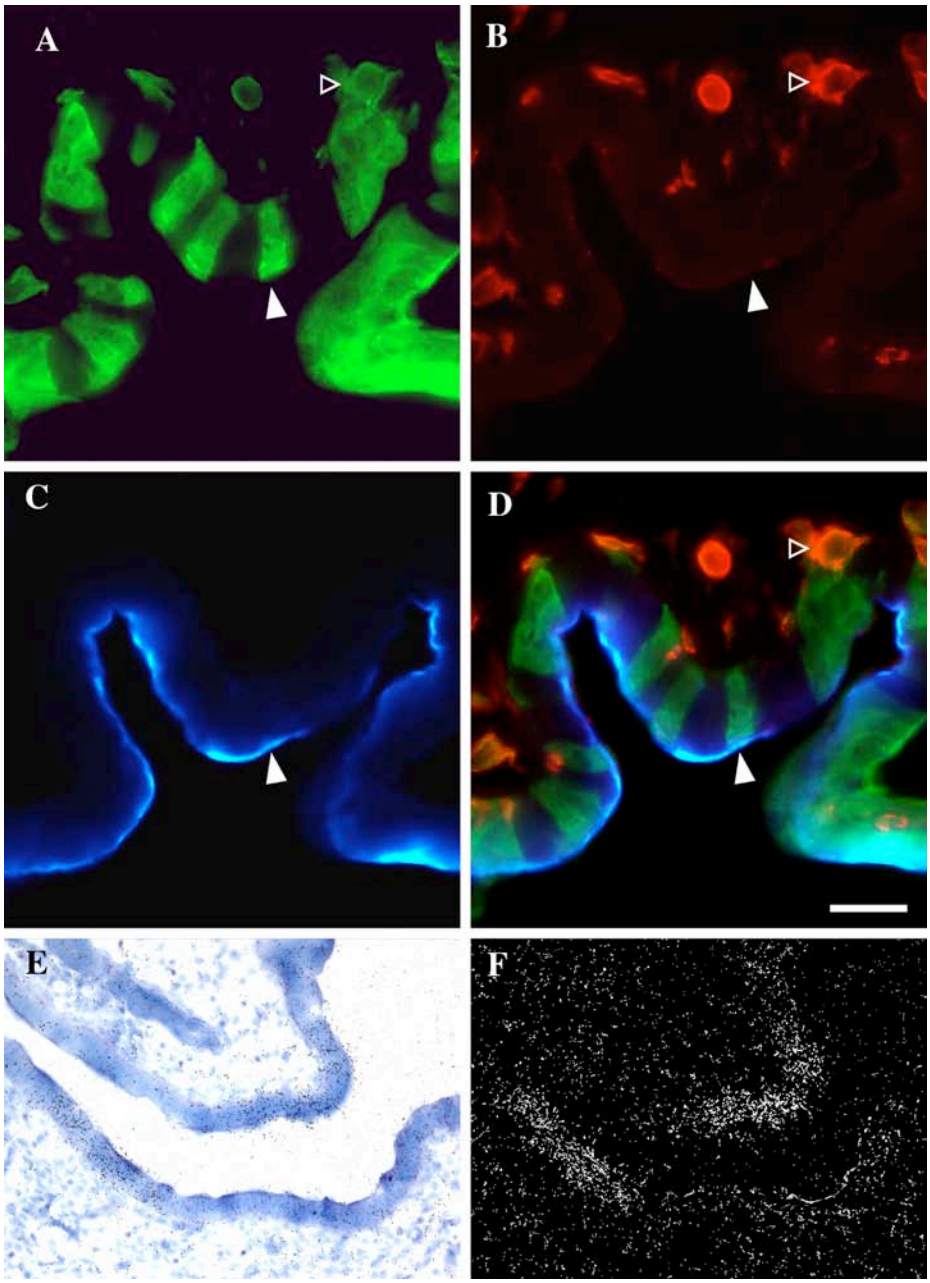

Figure 8. Green fluorescent protein (GFP)-expressing uterine epithelium of a double-transgenic (*CD45/Cre-Z/EG*) mouse. We observed GFP-positive (green) epithelial cells in the uterus **(A)** that did not express CD45 (red) **(B)** but bound the uterine epithelial marker *L. tetragonolobus* (blue) **(C). (D):** Overlay of the three stainings **(A–C)**. **(A–D):** Solid arrowheads point to GFP+/CD45+ uterine epithelial cell; open arrowheads indicate GFP+/CD45+ stromal cell. **(E, F):** Uterine epithelium expressing GFP mRNA in bright-field **(E)** and dark-field **(F)** illumination. Scale bar = 20 um **(A–D)** and 50 um **(E–F)**. Abbreviation: GFP, green fluorescent protein.

To determine the rate at which CD45+ cells contribute to the uterine epithelium and the extent of this contribution, we studied *CD45/Cre-Z/EG* animals at different ages. In mice, the estrous cycle is 5–6 days long (207; 210). In contrast to humans, the epithelium does not shed, but it exhibits continuous degeneration and regeneration [20]. In the course of the cycle, epithelial cells undergo vacuolar degeneration and apoptosis and are replaced by newly generated cells (211). The first cycle in mice occurs at 4–6 weeks of age. Consequently, a 1-week-old pup has not cycled yet, and 6-, 12-, and 20-week old animals have gone through approximately 2, 10, and 26 cycles, respectively. In the double-transgenic mice, we detected many CD45/GFP+ cells in the uterine stroma (**Fig. 9**) at all ages examined (1, 6, 12, and 20 weeks). Although no GFP+ epithelial cells could be detected in the uterine epithelium in 1- and 6-week-old animals (**Fig. 9A, 9B**), 0.5% of the epithelial cells in 12-week-old mice and 6% in a 20-week-old animal were GFP+ (**Fig. 9C– 9F**). The GFP+ epithelial cells were found in patches, suggesting that clonal expansion of individual progenitor cells gave rise to islands of epithelial cells. Thus, although we did not have enough animals at each time point to do a statistical analysis, we think that the number of uterine epithelial cells produced from CD45 precursors increases with age and is related to the number of estrous cycles through which the animals have passed.

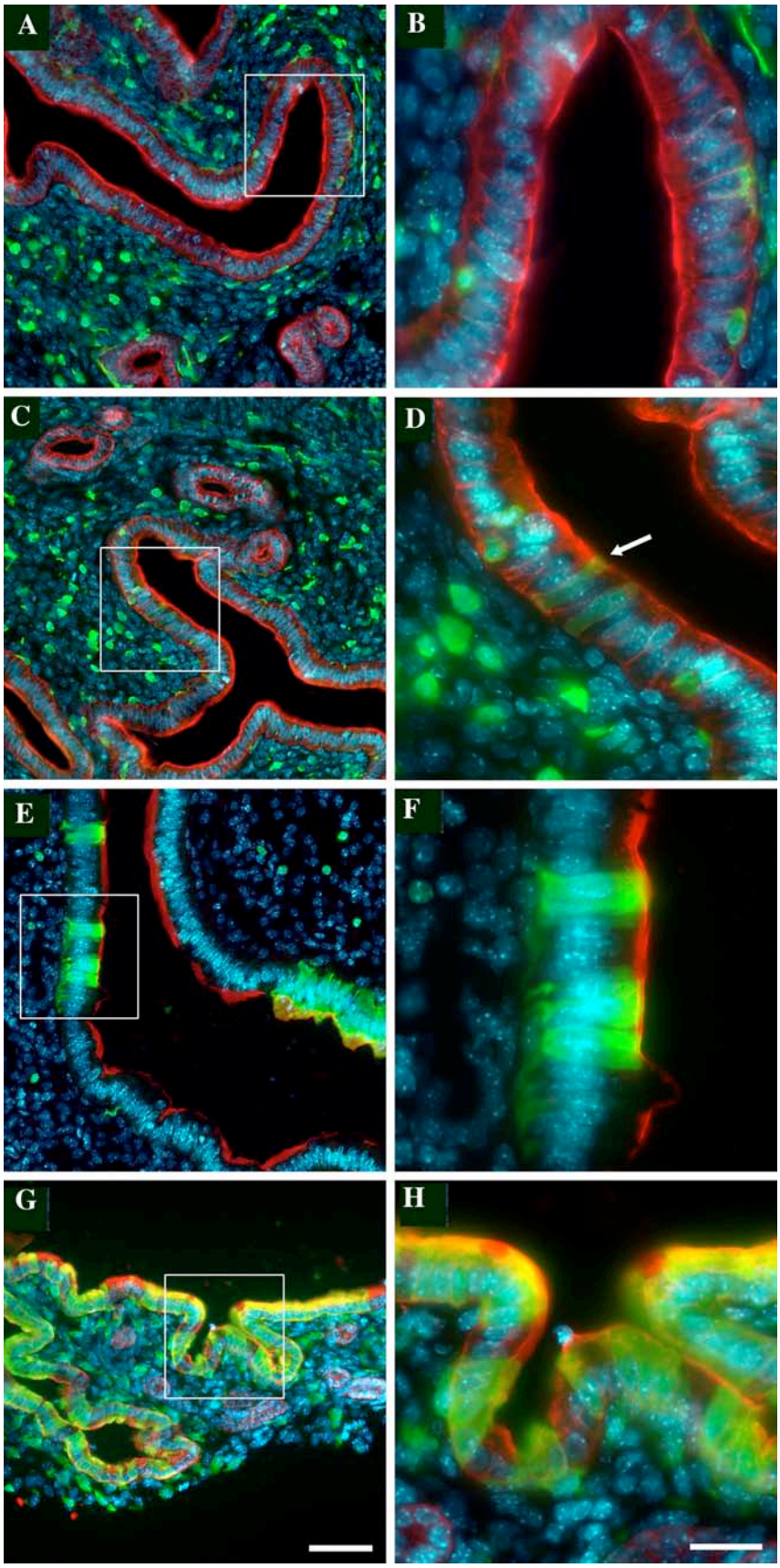

Figure 9. Green fluorescent protein (GFP)-expressing (green) uterine epithelial cells from double-transgenic animals of different ages and pregnant mice. All nuclei were stained in blue with 4,6-diamidino-2-phenylindole. **(A, B):** No GFP-positive (green) uterine epithelial (red staining represents *L. tetragonolobus*, the epithelial marker) cells were detected in 6-week-old animals. **(C, D):** Sporadic GFP-positive uterine epithelial cells were present at 12 weeks of age. Arrow indicates a GFP+ uterine epithelial cell. **(E, F):** At 20 weeks of age, 6% of uterine epithelial cells expressed GFP. **(G, H):** In 12-week-old pregnant mice, there was a robust increase in the number of GFP-expressing cells: 82% of the uterine epithelial cells were GFP+. Scale bars = 60 um (left column) and 20 um (right column).

During pregnancy, the uterus undergoes marked proliferation. The endometrial surface of the pregnant mouse is approximately 25 times larger than that of a nonpregnant animal, and we wondered whether CD45+ cells might contribute to this increase. We were surprised to find that in one pregnant *CD45/ Cre-Z/EG* mouse, the vast majority (82%) of uterine epithelial cells were also GFP+ (**Fig. 9G, 9H**). Our observations indicate that CD45+ progenitors could be the source of most of the new epithelial cells in the pregnant uterus. Based on double staining of GFP and the X chromosome, these cells seem unlikely to be the products of cell-fusion events, since they never contain more than two X chromosomes (**Fig. 10**).

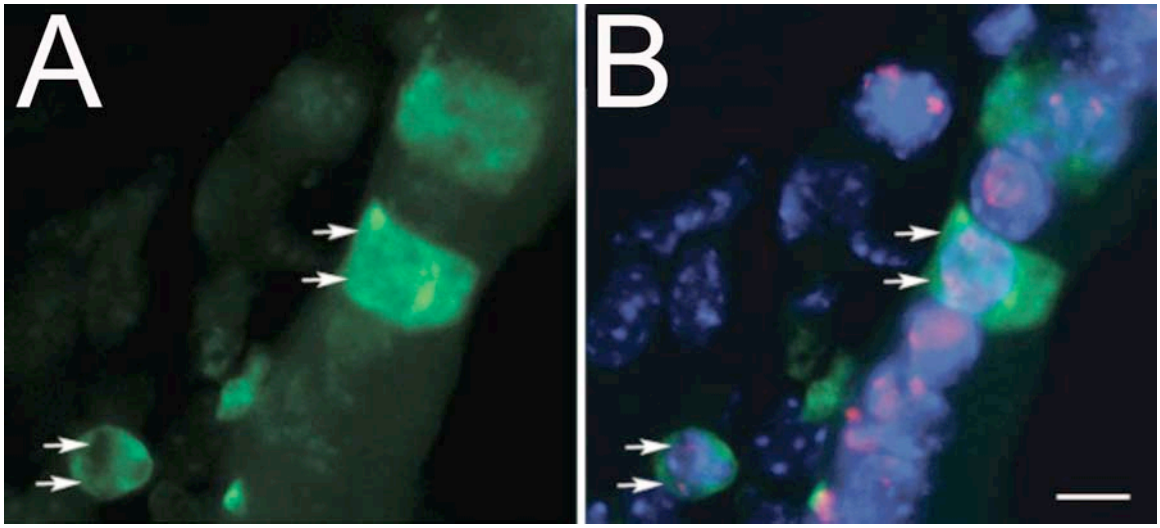

Figure 10. Arrows point to nuclei (DAPI, blue) of green fluorescent protein (GFP)-expressing (green) uterine epithelial cells **(A)** from double-transgenic animals that have two X chromosomes (red dots) **(B)**. GFP was visualized with a tyramide-fluorescein isothio-cyanate conjugate, whereas the X chromosome was labeled by a Cy3- conjugated chromosomal paint probe. Scale bar = 10 um.

4.2 Chimerism in the skin following bone marrow transplantation

We first established – similarly to our previous studies – that the sensitivity of the Y chromosome hybridization is over 90% (i.e. the % of cells in a male section that are labeled with the Y chromosome) (**Fig.11A**) and the specificity of the probe is 100% (i.e. no positive nuclei were found in the female patient samples).

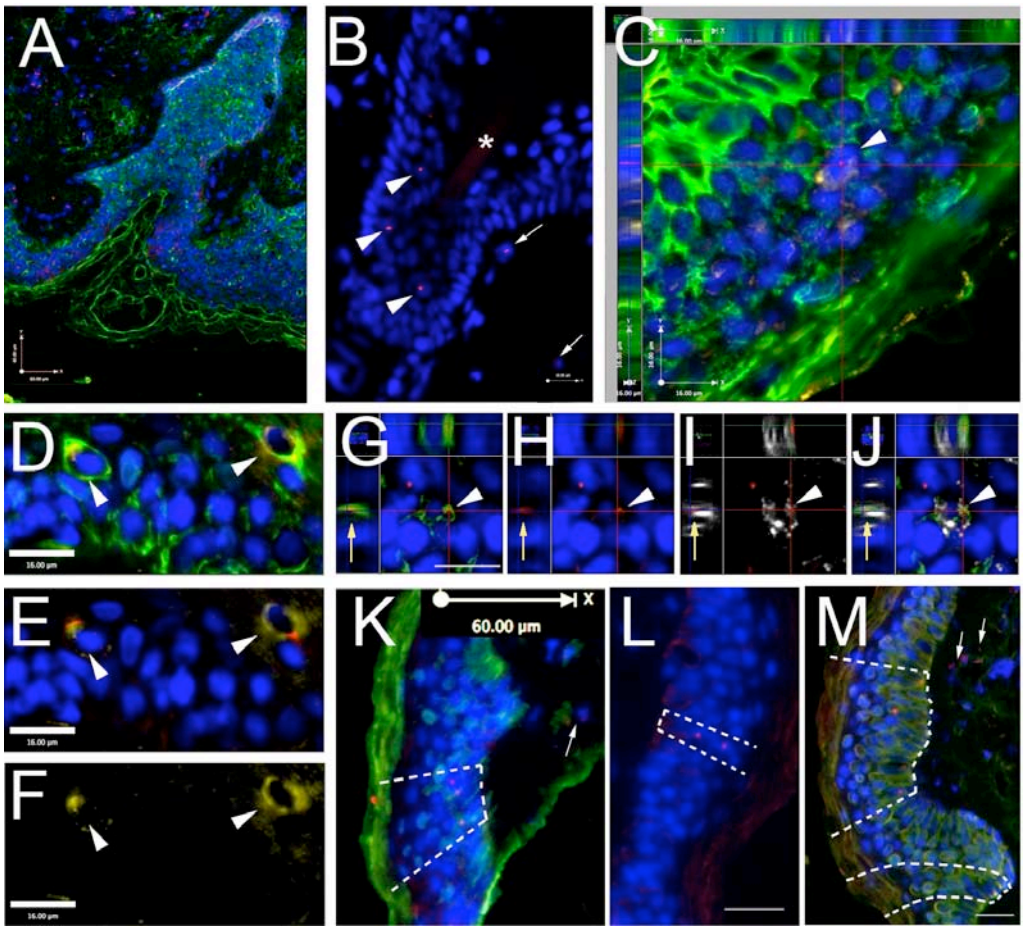

Figure 11. Presence of donor-derived cells in the skin of patients 12–16 years following gender-mismatched BM transplant. In all panels blue color is DAPI (chromosomal marker for nuclei), green labels cytokeratin (fluorescein isothiocyanate), red dots label Y chromosome (Alexafluor-594), and yellow (**C**, **D–F**) and white (**G–J**) label the proliferation marker Ki-67 (CY5). (**A**) Section of a male control scalp showing that the majority of nuclei exhibit the Y chromo- some (sensitivity) and that no red dots are visible outside nuclei (specificity). (**B**) Section of the back skin of patient no. 2 showing several Y+ cells in the hair follicle (arrowheads) and 2 cells outside the follicle (arrows). The asterisk is over a hair. (**C**) One focal level of a Z series from the scalp biopsy of patient no. 5. The arrowhead points at a cell that is

keratinocyte positive as shown in the left-side panel, bears the Y chromosome, and is also positive for the proliferation marker, Ki67. In the side panels, the red Y chromosome appears as a line on the blue DAPI nucleus, which is then lined by yellow (Ki67) and a green (CK) staining. (**D–F**) Patient no. 4, scalp biopsy demonstrates 2 cells (arrowheads) in close proximity, both are positive for all 3 markers (Y chromosome, CK, and Ki67). **D** is an overlay of all colors; **E** shows the Ki67 staining, with Y and **F** showing only the Ki67 staining. All 3 panels are in the same focal plane of a Z series at 0.5 mm optical thickness. (**G–J**) Back skin of patient no. 6 showing 1 cell (arrowhead) in a z-series that labels for CK (**G**), Y chromosome (**H**), Ki67 (**I**), and an overlay of all markers (**J**). The yellow arrow shows the cell in the side view where the colocalization of all markers is clearly visible. (**K–M**) Sections of 3 different samples showing patchy distribution (areas are outlined with white dotted lines) of Y chromosome-positive cells strongly suggesting a clonal origin. Scale bar: **A** = 60 mm, **B**=34mm, **C–J**=16mm, **K**=60mm, **L**, **M**=40mm.

In all samples studied, we observed Y chromosome positive nuclei in epithelial layers and hair follicles (**Fig.11B**) as well as in the epidermal layers, and used the CD45 immunostaining to exclude cells of hematopoietic origin. Most of the Y+ cells in the epithelial layer also stained for CK confirming the epithelial nature of those cells (Fig.1C). When Ki67 was added as a nuclear proliferation marker, we observed rare occasional cells that were triple positive (Y chromosome, cytokeratin and Ki67) (**Fig.11. D-J**). In two patients we observed a specific distribution of Y positive epithelial cells: the Y positive cells seem to group in one area across the heights of the epidermal layer leaving long stretches of epithelium on both sides containing exclusively host derived cells. We used regular microscopic images for the quantification, but performed Z series at the interval of 0.5 μm in our images to demonstrate the exact localization of the Y label, the CK and the Ki67 to a certain cell.
Our results demonstrated the presence of double positive (Y and CK) cells in all samples examined (**Fig.11**), with a relatively small patient-to-patient variation. The percentage of Y+ epidermal cells varied between 0.37-1.78% (scalp) and 0.32-1.08% (back), indicating a slightly, but consistently higher number in the scalp than in the back samples. In addition to the double positive cells we also counted the number of epidermal donor- derived cells that were not epithelial in nature (i.e. not CK positive). The number of these cells, most of them CD45 positive, varied between 7.5 and 17.5%

(scalp) and 3.7 and 13.6 (back) (**Table.2 and Fig. 12**). The histology of the hybridized sections showed a columnal distribution of groups of Y positive keratinocytes in between long stretches of cells of exclusively host origin (**Fig11.K-M**).

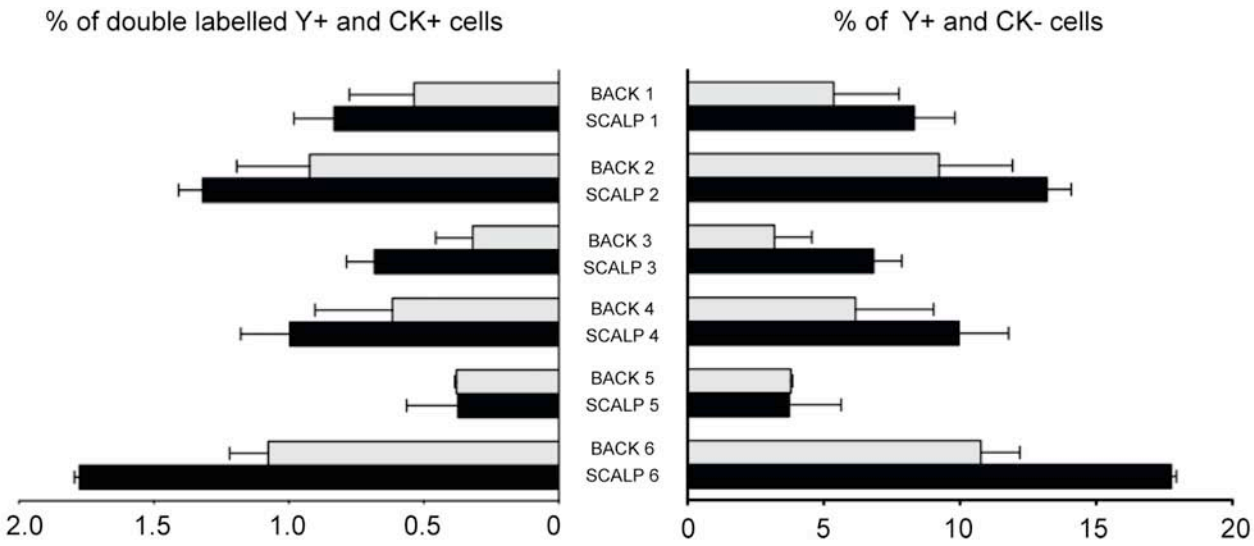

Figure 12. Columnar graph showing the percentage of Y chromosome-positive cells among the cytokeratin-positive (left panel) and the cytokeratin-negative (right panel) cells. Data regarding the scalp are represented by black columns, and data re- presenting the data regarding the back skin biopsies are represented by gray columns. Standard error of the mean of 3 samples is shown.

5. Discussion

5.1. Contribution of bone marrow to the uterus epithelium

Eight to 12 months following the bone marrow transplantation using GFP-positive BM, the GFP-positive cells were detected in the endometrial epithelium and stroma of recipient animals. Bone marrow-derived GFP+ cells bound *L. tetragonolobus* lectin, which is an indicator of their epithelial nature. These GFP+ epithelial cells, however, did not express the common leukocyte antigen CD45, whereas numerous CD45+/GFP+ cells were detected in the uterine stroma, which is known to harbor CD45+ lymphoid cells (209). These findings clearly demonstrate that upon transplantation, BM cells enter the uterus and differentiate into epithelial cells there. The BM cells are unlikely to have fused with uterine cells after colonizing the organ because the GFP+ cells all appear to be diploid. BM cells are heterogeneous, however, and transplantation of tagged cells did not not allow us to determine which sort of BM cell might give rise to the labeled epithelial cells in the recipient mice or whether irradiating the animals affected what we observed.

CD45 is expressed in hematopoietic cells and their derivatives (212) including muscle satellite cells (213). As expected, in the *CD45/Cre-Z/EG* mice, CD45+ cells (such as lymphocytes and the derivatives of CD45+ cells (such as tissue resident macrophages, Kupffer cells, and microglia [data not shown]) were GFP-positive. Most, but not all, white cells in the blood that could be stained with an anti-CD45 antibody were also GFP-positive.

To determine the rate at which CD45+ cells contribute to the uterine epithelium and the extent of this contribution, we studied female *CD45/Cre-Z/EG* animals at different ages. The number of uterine epithelial cells produced from CD45+ precursors seemed to increase with time and correlate with the number of estrous cycles through which the animals have passed.

During pregnancy, endometrial surface of the rodent uterus is approximately 25 times larger than that of a nonpregnant animal, and this change happens in a very short time. We wondered whether CD45+ cells might contribute to this increase and were

surprised to find that in a pregnant *CD45/Cre- Z/EG* mouse, the vast majority (82%) of uterine epithelial cells were also GFP+. Our observations indicate that CD45+ progenitors maybe the source of most of the new epithelial cells in the pregnant uterus.

Stem cells residing in the uterus were thought to be responsible for the expansion of epithelial cells that accompanies pregnancy (214). The nature of these stem cells, whether they are replenished, and how replenishment might occur have been a matter of speculation, but a population of "label-retaining" putative stem cells (LRCs) has recently been observed in the uterus (215; 216). Chan reported that these LRCs are frequently localized near vessels in the mouse endometrium (216). Since our results show that CD45+ cells give rise to epithelial cells, we suggest that circulating CD45 cells provide a renewable pool of epithelial precursors in the uterus. The fact that Chan et al. (214) did not detect CD45 in LRCs suggests that endometrial stem cells turn the marker off after they select their fates.

Circulating hematopoietic stem or progenitor cells might enter the stroma and, when they are needed, migrate toward the lumen, where they could serve to regenerate the epithelium. Alternatively, hematopoietic cells could colonize the stromal layer during fetal life and reside there from birth onwards, entering the epithelium on demand. CD45 was not thought to be produced by cells other than hematopoietic stem cells and their progeny (217), but recent work suggests that it may be made transiently by oligodendrocyte precursors during development (218), and several groups have demonstrated that lung epithelial cells might derive from hematopoietic stem cells in injury models (219-221). Uterine epithelial progenitors might also transiently express CD45 and then turn it off before they differentiate. Were this the case, their progeny would be GFP+ in the double-transgenic mice that we generated, but given the lack of GFP expression in the uterine epithelium during early postnatal development and during the first several estrous cycles, it seems unlikely that CD45 is activated in resident epithelial progenitors. Alternatively, if the BAC-based expression of Cre recombinase "leaks" briefly when the epithelial population expands, the cells could turn green. Since all of CD45's introns and long 5' and 3' flanking sequences are present in the BAC that we used (described in Materials and Methods), and since several founder animals gave similar results to those reported above, we feel that leakiness is unlikely to have been a problem. Instead, we prefer the hypothesis that

circulating CD45+ BM cells are responsible for the phenomena that we observed. The fact that GFP-tagged bone marrow cells transplanted into adult *C57BL/6J* mice generate patches of GFP+ uterine epithelium supports this hypothesis.

We also observed GFP+ and GFP mRNA-positive uterine epithelial cells from animals studied 8–12 months after they underwent irradiation and transplantation with green bone marrow, suggesting that the colonization of the uterus by CD45 - expressing cells and their conversion into uterine epithelium can take place after development is completed. Although whole-body irradiation prior to BM transplantation affects fertility and changes the length/number of estrus cycles in mice, uterine function seems to remain quite intact, and turnover of the epithelium does not stop (207). This appears to be true in humans too; in fact, women who have received myeloablative doses of radiation can become pregnant and carry their fetus to term (222).

If BM progenitor cells do in fact contribute to the uterine epithelium, it is possible that alterations in these cells might result in clinical problems. For example, extramedullary hematopoiesis in the endometrium, although rare, is known to occur (223) and could be caused by CD45+ cells there. Furthermore, we agree with Gargett (224) that endometriosis might be caused by misplaced endometrial stem/progenitor cells, specifically CD45+ derivatives. Du and Taylor suggested in their recent work that bone marrow-derived cells contribute to the endometrium in a mouse model of endometriosis (225). This chronic, painful condition results when endometrium-like tissue grows on the uterus, ovaries, fallopian tubes, uterine ligaments, abdominal lining, or lower part of the large intestine. In addition, such tissue has been seen in the axillary glands, lung, retina, sciatic nerve, skin, and even brain.

Like the endometrium, these ectopic growths respond to hormones; they grow, swell with blood, and degenerate in a cyclic manner. Since their breakdown products have no way to exit the body, however, they cause severe menstrual, abdominal, and lower back pain, and—in up to 40% of women suffering from it—scarring and infertility. Endometriosis is thought to have a genetic component; it is associated with autoimmune diseases, and it can be induced in rhesus monkeys by treatment with dioxin (226). We feel that regardless of the abnormality (genetic, immunological, or hormonal) that drives the process, changes in CD45+ BM-derived cells could cause them to colonize sites other than the uterine lining and to differentiate and proliferate

once they are there. If this is the case, one wonders whether the cells are conditioned in the endometrium and subsequently escape and take up residence in other, nearby tissues (227). Alternatively, stem cells may occasionally make inappropriate fate choices in tissues (e.g., lung or brain) where they migrate via the blood. The fact that endometriosis has been reported in males might seem to lend support to the second of these suggestions; however, it appears that in some cases the growths may arise in the prostatic utricle, a uterine remnant found in men (228).

Clearly, it would be interesting to know whether CD45+cells are important for regenerating tissues other than the endometrium and what cues are used to attract them into any given organ and to drive their differentiation. The *CD45/Cre-Z/EG* mouse strain may serve as a useful model for such studies.

5.2. Chimerism in the skin following bone marrow transplantation

In addition to the constant epidermal renewal, the so-called physiological turn over, the cutis also frequently needs repair due to injuries. The cutaneous stem cell reservoir evoked the interest of basic biologists and dermatologists and there are several good reviews describing these stem cell pools (98; 229; 230). The epidermal stem cells seem to maintain the skin integrity under physiological turnover, whereas the bulge stem cells contribute more to the everyday repair (231). Krause showed the presence of male cells in the skin at a frequency of 1.2%–2.7% using fluorescence in situ hybridization analysis after she transplanted female mice with a single male hematopoietic stem cell (HSC) (154). Following wound healing the mouse BM-derived cells (BMDC) were reported to contribute to epidermal regeneration and differentiate into keratinocytes (195) as well as migrate into the hair follicle, the sebaceaous gland, and the epidermis, and their engraftment is significantly upregulated in wounded skin (Brittan, Braun et al. 2005). Due to the high cutaneous turnover rate, one wonders if these skin stem cell pools need renewal themselves, such as replacement by circulating cells. In the murine epithelium, the hematopoietic progenitor marker, CD34, is a specific marker of bulge cell keratinocytes (Trempus, Morris et al. 2003), but in the human, CD34 is present in stem cells of the outer root sheath (232-234). Since these cells lack CK15, a type I keratin, they were suggested to be transit amplifying cells, progeny of the bulge stem cells (233; 234). In a groundbreaking study, Deng et al. transplanted fluorescently

labeled mesenchymal stem cells from white male mice into lethally irradiated black female mice and observed that the recipient mice grew white hair all over the body. The authors concluded that BMDCs give rise to functional skin cells in an injury setting (235). In a human study, Korbling identified donor cells in the skin of female recipients of male HSC stem cells using a combination of immunohistochemistry for specific epithelial antigens and fluorescence in situ hybridization (236) and found 2%–7% chimerism in the biopsies. Similar numbers (1.6%–7.8% of donor-derived keratinocytes) were reported by Murata et al. in patients with acute graft versus host disease (GVHD) who previously received HSC transplant from male donors (198), in addition to 2.2%–9.4% chimeric endothelial cells, which were also shown in human samples by Suzuki (237). On the contrary, Hematti et al., who used similar samples but when performed polymerase chain reaction on cultured keratinocytes, could not detect any donor-derived cells (204). Using fingernail shavings from gender mismatched transplant patients, 2 independent publications demonstrated high chimerism (9%–73%) using short tandem repeat polymerase chain reaction (238; 239). These data suggest that—especially in injury settings—hematopoietic progenitors do indeed contribute to skin regeneration in both mice and humans.

Our group of patients (aged between 27 and 48 years) included 6 women, all of whom underwent BM transplantation due to malignant hematological diseases. All of them received BM (as opposed to granulocyte colony-stimulating factor [GCSF]-induced peripheral blood stem cells) and the skin biopsies were taken between 12 and 16 years after transplant. The treatment protocols before BM suppression and following the transplant were almost identical in all of them. Five patients received transplants from siblings, whereas one had a matching unrelated donor. This 1 patient had the highest level of chimerism in the scalp, which might be a simple coincidence given the restricted number of samples studied. It has been established that the epidermis contains 2 major types of stem cells: the epidermal proliferative unit and the stem cell niche in the bulge region. The observed patchy distribution of the donor-derived cells in our patients suggests that a circulating cell might have become an epidermal proliferative unit stem cell and gave rise to a lineage of keratinocytes in that area. A theoretical possibility should also be mentioned that the circulating BMDCs might become TA cells without becoming stem cells first, as already suggested in mouse

studies (202). This would explain that although there are columns of Y-positive cells, these do not spread over time, that is, the TA cells, unlike the mother stem cells, lose their ability to generate new stem cells. It seems that the majority of the donor-derived keratinocytes have a limited proliferation capacity based on the colocalization of Ki67, an accepted marker of proliferation, with the Y chromosome and CK.

The observations in this study strongly support the idea that circulating cells after BM transplantation can arrive at the skin and participate in its regeneration by establishing themselves as skin stem cells. The toxicity of chemotherapy resulting in hair loss constitutes an injury-induced need to regenerate hair. In Hematti's earlier negative study (204), all patients received blood progenitors collected from the peripheral blood after GCSF induction. In our study all patients received BM. A possible explanation of the discrepancy might be that other than hematopoietic cells in the BM participate in the process we observed. These other cells could be mesenchymal cells, or a unique as yet unknown skin progenitor that lives in the BM. The possibility of donor and recipient cell fusion seems to be very unlikely; for example, in his mouse studies Brittan excluded fusion as a cause for donor-derived epithelial cells by in situ hybridization for both Y and X chromosomes (201). In addition, in our previous study in cheek epithelial cells from gender-mismatched BMT patients, we also demonstrated the lack of fusion by X and Y chromosomal hybridization, as well as the donor origin of some (187). It is theoretically possible that keratinocytes might phagocytose Y chromosome-positive circulating blood cells, and then appear to be Y chromosome-positive themselves. Although keratinocytes have been shown to phagocytose pathogens and melanosomes (240; 241), there is no evidence that they take up blood cells, and this seems unlikely to have occurred.

Our results suggest that, indeed, there is a contribution of circulating BMDCs to the skin stem cell population. This contribution is modest, but might be boosted by danger signals, when regeneration is needed quickly and in large quantities, like in case of trauma or burn disease. Another possible group of diseases where transdifferentiation potential of bone marrow hematopoetic stem cells could be exploited is the genodermatoses, in other words inhereated skin disorders (242). In one of these dermatologic conditions (epidermolysis bullosa or EB in short) patients lack keratinocyte derived intra- or extracellular proteins responsible for the structural

integrity of the epidermis and the underlying basal membrane (243). Depending on the exact subtype of the disease this results in limited or generalized loss of the epidermis, leading to uncontrollable water, electrolyte and heat loss and serious infections. If allogeneic bone marrow (from a donor with normal genotype) is transplanted into a patient with EB, donor hematopoietic stem cells can potentially transdifferentiate into epidermal cells and replace abnormal keratinocytes. Once there is enough number of healthy keratinocytes, this process can normalize the skin homeostasis.

Although experimental data are promising, much work needs to be done to determine what patient population could benefit from the transdifferentiation potential of bone marrow cells and how this potential could be raised to a therapeutic level.

6. Conclusion

In order to address contradicting literature data on transdifferentiation potential of bone marrow hematopoetic stem cells, we decided to investigate the contribution of these cells to highly proliferating epithelial tissues. Our data indicate that HSCs actively participate in the homeostasis of uterine epithelial cells and the skin epidermis.

1. By developing a new mouse model we were able to both avoid the potential technical pitfalls of bone marrow transplantation and for the first time pinpoint the role of hematopoetic stem cells in the cyclic regeneration of the murine endometrial epithelium. Our observation, that with age an increasing ratio of the uterus epithelial cells became bone marrow derived, and the fact that in pregnancy almost all uterus epithelial cells were of blood origin suggest that HSCs play an important role in repopulating epithelial stem cell niches and this contribution is heavily dependent on the age and turnover rate of the tissues examined. From a clinical prospective this phenomenon could offer an explanation for the pathomechanism of endometriosis, a devastating disease caused by the ectopic formation of endometrial tissue in different organs like the lung, colon or even the brain. Circulating HSCs and their progenies have access to all the organs via the bloodstream. This means that certain so far unidentified factors can recruit blood cells and initiate an endometrial differentiation program in them ultimately leading to the manifestation of endometriosis disease.

2. Our human data indicate that a small percentage of keratinocytes on the back as well as on the scalp are derived from hematopoetic stem cells. A consistently larger chimerism rate seen on the scalp suggests that actively growing hair recruits more bone marrow derived cells that can participate in the constant renewing process of the hair follicles as well as the interfollicular epidermis. This also means that injury settings, with an imminent need for reparation, could significantly boost transdifferentiation potential of HSCs. Thus transdifferentiation potential of HSCs could be utilized in clinical settings and form a basis for future cellular therapies to treat diseases where quick reparation of the skin is critical.

7. Summary

In the last couple of years it became evident that bone marrow hematopoetic stem cells - besides producing blood lineages- are also capable to differentiate into a large number of none-hematopoetic cell types. The rate at which this transdifferentiation occurs is still debated. The seemingly contradictory data most likely stem from technical issues and grant further investigation utilizing newer animal models and more complex analytic tools. To address this controversy we decided to assess the contribution of HSCs to two rapidly renewing epithelial tissues: the uterine epithelium in mice and the skin epidermis in human.

In order to circumvent technical issues presented by bone marrow transplantation we developed a mouse model that can aid transdifferentation studies focusing on the plasticity of hematopoietic stem cells. Utilizing the Cre/lox technology we generated double transgenic mice engineered to show permanent GFP expression in all cells that ever expressed CD45, an antigen found exclusively on hematopoietic cells (hematopoietic stem cells, progenitors and mature white blood cells). Evaluation of the mice showed strong expression of GFP in all blood leukocytes, and a time and use dependent expression in the uterine epithelium, one of the tissues with the highest turnover rate in mice. To compare this newly developed system with the traditional bone marrow transplantation model, we transplanted GFP expressing bone marrow cells into wild-type mice and found a similar GFP expression pattern in the uterus epithelium several months after the infusion of tagged donor cells. In conclusion: we created a functional animal model system that can serve as a new tool for bone marrow transdifferentiation studies without the need of in vitro manipulation of donor stem cells, or toxic preconditioning of the recipient organism. Since preconditioning regimens frequently cause permanent sterility in the recipient organism, avoidance of these agents enabled us to assess bone marrow contribution to the pregnant uterus. To our surprise we found that the overwhelming majority of the pregnant uterus epithelium was composed of bone marrow derived cells, which suggest that bone marrow stem cells play an important role in the development and maintenance of the pregnant uterine epithelium. Also, our data indirectly support the hypothesis that endometriosis (ectopic presence of endometrial tissue) stems from the opportunistic planting and

transdifferentiation of HSCs in various organs.

The epidermis stood in the center of our human studies. Transdifferentiation of bone marrow cells into skin keratinocytes has been proven and also disproven by several research groups. To address these seemingly controversial results we analyzed skin biopsy samples from female patients who underwent bone marrow transplantation and received donor marrow stem cells from a male donor. Using a combination of immunostaining and in situ hybridization we detected donor derived keratinocytes in the patients' epidermis in the non-hairy back skin as well as in the hairy occipital area. Our results show that bone marrow derived keratinocytes tend to be found in greater number in the scalp area as compared to the back skin, they tend to appear in patches which suggest clonal origin for their presence, and they are able to actively divide within the epidermis. These observations point to the clinical potential of HSCs in epidermal injury settings where enhanced regeneration is needed (using the patient's own HSCs as an autologous stem cells source), or in genetic skin diseases where missing epidermal proteins could be replaced by the HSC derived keratinocytes (using HSCs from third party donors as an allogeneic stem cell source).

8. Összefoglalás

Az utóbbi évtized kutatási eredményei azt sugallják, hogy a csontvelői hematopoetikus őssejtek az érett vérsejteken kívül más sejt (illetve szövet) - típusok kialakítására is képesek. Ennek a folyamatnak -röviden transzdifferenciációnak- a mértéke nagyban függ a vizsgált szövet homeosztázisától és aktuális regenerációs igényétől. A transzdifferenciáció pontos megítélését ugyanakkor jelentősen befolyásolják a jelenség tanulmányozásához használt kutatási módszerek is. Az alkalmazott mintagyűjtés, illetve a választott analitikus módszerek nem mindíg teszik lehetővé a szöveti kimérizmus mértékének pontos megállapítását. Ezzel magyarázható, hogy a tudományos irodalomban fellelhető kutatások sok esetben egymásnak ellentmondó következtetésre jutottak a transzdifferenciáció fiziológiás jelentőségét illetően. Kutatásunk célja az volt, hogy új állatmodellek fejlesztésével, illetve komplex szövettani analitikai módszereket alkalmazva megvizsgáljuk a csontvelői hematopoetikus ő ssejtek epitheliális (uterus epithelium illetve bőr epidermis) regenerációban betöltött szerepét.

A Cre/lox génmanipulációs rendszer segítségével létrehoztunk egy olyan egeret, amelynek minden olyan sejtje, amely valaha expresszálta a CD45 fehérjét (vagyis hematopoetikus eredetű) zölden fluoreszkál. Ez azt jelenti, hogy ha egy vérsejt transzdifferenciálódik, vagyis az eredeti differenciációs programjától eltér, a GFP expresszióját ettől függetlenül megtartja, vagyis az újonnan létrejött sejt (pl bőr keratinocita) hematopoetikus eredete bizonyítható. A transzdifferenciációs kutatásokban standard módszernek tekinthető csontvelőtranszplantációs modellel, valamint az általunk kifejlesztett új egér modell segítségével megvizsgáltuk, hogy a csontvelői hematopoetikus sejtek milyen mértékben képesek hozzájárulni az egér uterus epithelium regenerálódásához. Eredményeink azt mutatták, hogy az idő előrehaladtával - több degenerációs/regenerációs ciklust követően - az uterus epitheliumban egyre több a GFP expressziót mutató hámsejt. Ez a regenerációs igénytől függő transzdifferenciációs potenciál a terhes uterus vizsgálatakor vált legszembetűnőbbé. Meglepetésünkre, a vemhes egér méhnyálkahártya hámsejtjeinek túlnyomó többsége GFP pozitívnak, vagyis csontvelői eredetűnek bizonyúlt. Ez arra

utal, hogy a csontvelő képes alkalmazkodni az endotmetrium fokozott regenerációs igényéhez, és aktívan résztvesz a megnagyobbodó uterus hámjának felépítésében. Eredményeink egyben azt is bizonyitják, hogy az általunk kifejlesztett egérmodell alkalmas a csontvelői eredetű transzdifferenciáció tanulmányozására, és a jövőben kiválthatja a csontvelőtranszplantációs modellt, megszabadúlva annak minden ismert fent leírt hátrányától.

Humán kísérleteink középpontjában a bőr epidermis állt. Olyan, csontvelő transzplantáción legalább 10 éve átesett nőbetegekből gyűjtöttünk bőrmintákat, akik férfi donortól kaptak csontvelőt. Mivel nőkben normálisan nem találunk Y kromoszómát, a férfi donor hematopoetikus sejtjei viszont XY nemi kromoszómákkal rendelkeznek, minden Y kromoszóma tartalmú sejt, ami megjelenik a csontvelő recipiensben donor csontvelő eredetűnek tekinthető. Ezt a törvényszerűséget kihasználva, a csontvelőből származó keratinociták analíziséhez kombináltuk az Y kromoszóma specifikus in situ hibridizációt és a keratinocita specifikus immunfestést.

Összefoglalva elmondhatjuk, hogy több mint 10 évvel a csontvelőtranszplantáció után mind a hátbőrön, mind a hajas fejbőrön fellelhetőek csontvelő eredetű keratinociták, melyek az összes keratinocita körülbelűl 1%-át teszik ki. Ki-67 (egy sejtproliferációs antigén) specifikus immunfestésünk, valamint a donor sejtek epidermiszben több helyen megfigyelt oszlopszerű elhelyezkedése arra utal, hogy – hasonlóan az uterus epitheliumhoz - a beépülő csontvelői eredetű keratinociták klonálisak, és képesek osztódni.

9. Acknowledgement

First and foremost I would like to express my sincere gratitude to my mentors Drs. Sarolta Kárpáti and Éva Mezey for their continuous support and patience throughout my research studies. I would also like to express my deepest appreciation to Dr. Tamás Masszi for his time and help in recruiting patients for this study. I owe a special thank you to Dr. Miklós Sárdy who showed me the beauty of dermatology during the university years and encouraged me to take a path towards academic dermatology. Last but not least I would like to acknowledge Drs. András Bratincsák, Zsuzsanna Tóth, and Sharon Key for their ideas and hard work during these studies, and Drs. Gyula Bottlik, Gergely Szombath and Miklós Krepuska for their help in the collection of the skin specimens.

10. References

1. Kehrl, J.H. (1995). Hematopoietic lineage commitment: role of transcription factors. Stem Cells 13, 223-241.

2. Shizuru, J.A., Negrin, R.S., and Weissman, I.L. (2005). Hematopoietic stem and progenitor cells: clinical and preclinical regeneration of the hematolymphoid system. Annu Rev Med 56, 509-538.

3. Kondo, M., Wagers, A.J., Manz, M.G., Prohaska, S.S., Scherer, D.C., Beilhack, G.F., Shizuru, J.A., and Weissman, I.L. (2003). Biology of hematopoietic stem cells and progenitors: implications for clinical application. Annu Rev Immunol 21, 759-806.

4. Weissman, I.L., Anderson, D.J., and Gage, F. (2001). Stem and progenitor cells: origins, phenotypes, lineage commitments, and transdifferentiations. Annu Rev Cell Dev Biol 17, 387-403.

5. Hemmat, S., Lieberman, D.M., and Most, S.P. (2010). An introduction to stem cell biology. Facial Plast Surg 26, 343-349.

6. Diczfalusy, E. (1969). Steps in the reproductive process susceptible to regulation. Bull World Health Organ 40, 479-491.

7. Shi, L., and Wu, J. (2009). Epigenetic regulation in mammalian preimplantation embryo development. Reprod Biol Endocrinol 7, 59.

8. Edwards, R.G. (1989). Early embryos and the fundamentals of embryology. Prog Clin Biol Res 296, 7-10.

9. Pera, M.F., Reubinoff, B., and Trounson, A. (2000). Human embryonic stem cells. J Cell Sci 113 (Pt 1), 5-10.

10. Robertson, E.J. (1997). Derivation and maintenance of embryonic stem cell cultures. Methods Mol Biol 75, 173-184.

11. Lee, J.B., Kim, J.M., Kim, S.J., Park, J.H., Hong, S.H., Roh, S.I., Kim, M.K., and Yoon, H.S. (2005). Comparative characteristics of three human embryonic stem cell lines. Mol Cells 19, 31-38.

12. Wang, Z., and Jaenisch, R. (2004). At most three ES cells contribute to the somatic lineages of chimeric mice and of mice produced by ES-tetraploid complementation. Dev Biol 275, 192-201.

13. Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S., and Jones, J.M. (1998). Embryonic stem cell lines derived from human blastocysts. Science 282, 1145-1147.

14. O'Hare, M.J. (1978). Teratomas, neoplasia and differentiation: a biological overview. I. The natural history of teratomas. Invest Cell Pathol 1, 39-63.

15. Kurosawa, H. (2007). Methods for inducing embryoid body formation: in vitro differentiation system of embryonic stem cells. J Biosci Bioeng 103, 389-398.

16. Guo, T., and Hebrok, M. (2009). Stem cells to pancreatic beta-cells: new sources for diabetes cell therapy. Endocr Rev 30, 214-227.

17. Hwang, D.Y., Kim, D.S., and Kim, D.W. (2010). Human ES and iPS cells as cell sources for the treatment of Parkinson's disease: current state and problems. J Cell Biochem 109, 292-301.

18. Pal, R. (2009). Embryonic stem (ES) cell-derived cardiomyocytes: a good candidate for cell therapy applications. Cell Biol Int 33, 325-336.

19. van Dartel, D.A., Pennings, J.L., de la Fonteyne, L.J., Brauers, K.J., Claessen, S., van Delft, J.H., Kleinjans, J.C., and Piersma, A.H. (2011). Concentration-dependent gene expression responses to flusilazole in embryonic stem cell differentiation cultures. Toxicol Appl Pharmacol 251, 110-118.

20. Winkler, J., Sotiriadou, I., Chen, S., Hescheler, J., and Sachinidis, A. (2009). The potential of embryonic stem cells combined with -omics technologies as model systems for toxicology. Curr Med Chem 16, 4814-4827.

21. Peng, S., Lacerda, A.E., Kirsch, G.E., Brown, A.M., and Bruening-Wright, A. (2010). The action potential and comparative pharmacology of stem cell-derived human cardiomyocytes. J Pharmacol Toxicol Methods 61, 277-286.

22. Andersson, H., Steel, D., Asp, J., Dahlenborg, K., Jonsson, M., Jeppsson, A., Lindahl, A., Kagedal, B., Sartipy, P., and Mandenius, C.F. (2010). Assaying cardiac biomarkers for toxicity testing using biosensing and cardiomyocytes derived from human embryonic stem cells. J Biotechnol 150, 175-181.

23. Asp, J., Steel, D., Jonsson, M., Ameen, C., Dahlenborg, K., Jeppsson, A., Lindahl, A., and Sartipy, P. (2010). Cardiomyocyte clusters derived from human embryonic stem cells share similarities with human heart tissue. J Mol Cell Biol 2, 276-283.

24. Li, Z., Huang, H., Boland, P., Dominguez, M.G., Burfeind, P., Lai, K.M., Lin, H.C., Gale, N.W., Daly, C., Auerbach, W., *et al.* (2009). Embryonic stem cell tumor model reveals role of vascular endothelial receptor tyrosine phosphatase in regulating Tie2 pathway in tumor angiogenesis. Proc Natl Acad Sci U S A 106, 22399-22404.

25. Saric, T., Frenzel, L.P., and Hescheler, J. (2008). Immunological barriers to embryonic stem cell-derived therapies. Cells Tissues Organs 188, 78-90.

26. Swijnenburg, R.J., Schrepfer, S., Govaert, J.A., Cao, F., Ransohoff, K., Sheikh, A.Y., Haddad, M., Connolly, A.J., Davis, M.M., Robbins, R.C., *et al.* (2008).

Immunosuppressive therapy mitigates immunological rejection of human embryonic stem cell xenografts. Proc Natl Acad Sci U S A 105, 12991-12996.

27. Knoepfler, P.S. (2009). Deconstructing stem cell tumorigenicity: a roadmap to safe regenerative medicine. Stem Cells 27, 1050-1056.

28. Robertson, J.A. (2010). Embryo stem cell research: ten years of controversy. J Law Med Ethics 38, 191-203.

29. Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663-676.

30. Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., and Yamanaka, S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861-872.

31. Nakagawa, M., Koyanagi, M., Tanabe, K., Takahashi, K., Ichisaka, T., Aoi, T., Okita, K., Mochiduki, Y., Takizawa, N., and Yamanaka, S. (2008). Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nat Biotechnol 26, 101-106.

32. Lyssiotis, C.A., Foreman, R.K., Staerk, J., Garcia, M., Mathur, D., Markoulaki, S., Hanna, J., Lairson, L.L., Charette, B.D., Bouchez, L.C.*, et al.* (2009). Reprogramming of murine fibroblasts to induced pluripotent stem cells with chemical complementation of Klf4. Proc Natl Acad Sci U S A 106, 8912-8917.

33. Wei, Z., Yang, Y., Zhang, P., Andrianakos, R., Hasegawa, K., Lyu, J., Chen, X., Bai, G., Liu, C., Pera, M.*, et al.* (2009). Klf4 interacts directly with Oct4 and Sox2 to promote reprogramming. Stem Cells 27, 2969-2978.

34. Wang, X., and Dai, J. (2010). Concise review: isoforms of OCT4 contribute to the confusing diversity in stem cell biology. Stem Cells 28, 885-893.

35. Kiefer, J.C. (2007). Back to basics: Sox genes. Dev Dyn 236, 2356-2366.

36. Amabile, G., and Meissner, A. (2009). Induced pluripotent stem cells: current progress and potential for regenerative medicine. Trends Mol Med 15, 59-68.

37. Welstead, G.G., Brambrink, T., and Jaenisch, R. (2008). Generating iPS cells from MEFS through forced expression of Sox-2, Oct-4, c-Myc, and Klf4. J Vis Exp.

38. Yu, J., Vodyanik, M.A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J.L., Tian, S., Nie, J., Jonsdottir, G.A., Ruotti, V., Stewart, R.*, et al.* (2007). Induced pluripotent stem cell lines derived from human somatic cells. Science 318, 1917-1920.

39. Soucek, L., and Evan, G.I. (2010). The ups and downs of Myc biology. Curr Opin Genet Dev 20, 91-95.

40. Martinez-Fernandez, A., Nelson, T.J., Ikeda, Y., and Terzic, A. (2010). c-MYC independent nuclear reprogramming favors cardiogenic potential of induced pluripotent stem cells. J Cardiovasc Transl Res 3, 13-23.

41. Aoki, T., Ohnishi, H., Oda, Y., Tadokoro, M., Sasao, M., Kato, H., Hattori, K., and Ohgushi, H. (2010). Generation of induced pluripotent stem cells from human adipose-derived stem cells without c-MYC. Tissue Eng Part A 16, 2197-2206.

42. Leon, J., Ferrandiz, N., Acosta, J.C., and Delgado, M.D. (2009). Inhibition of cell differentiation: a critical mechanism for MYC-mediated carcinogenesis? Cell Cycle 8, 1148-1157.

43. Cavaleri, F., and Scholer, H.R. (2003). Nanog: a new recruit to the embryonic stem cell orchestra. Cell 113, 551-552.

44. Zhang, X., Neganova, I., Przyborski, S., Yang, C., Cooke, M., Atkinson, S.P., Anyfantis, G., Fenyk, S., Keith, W.N., Hoare, S.F., *et al.* (2009). A role for NANOG in G1 to S transition in human embryonic stem cells through direct binding of CDK6 and CDC25A. J Cell Biol 184, 67-82.

45. Hanley, J., Rastegarlari, G., and Nathwani, A.C. (2010). An introduction to induced pluripotent stem cells. Br J Haematol 151, 16-24.

46. Sullivan, G.J., Bai, Y., Fletcher, J., and Wilmut, I. (2010). Induced pluripotent stem cells: epigenetic memories and practical implications. Mol Hum Reprod 16, 880-885.

47. Pfannkuche, K., Hannes, T., Khalil, M., Noghabi, M.S., Morshedi, A., Hescheler, J., and Droge, P. (2010). Induced pluripotent stem cells: a new approach for physiological research. Cell Physiol Biochem 26, 105-124.

48. Lee, E.S., Bou-Gharios, G., Seppanen, E., Khosrotehrani, K., and Fisk, N.M. (2010). Fetal stem cell microchimerism: natural-born healers or killers? Mol Hum Reprod 16, 869-878.

49. Miki, T., and Strom, S.C. (2006). Amnion-derived pluripotent/multipotent stem cells. Stem Cell Rev 2, 133-142.

50. Lee, M.W., Jang, I.K., Yoo, K.H., Sung, K.W., and Koo, H.H. (2010). Stem and progenitor cells in human umbilical cord blood. Int J Hematol 92, 45-51.

51. Schofield, R. (1978). The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells 4, 7-25.

52. Ferraro, F., Celso, C.L., and Scadden, D. (2010). Adult stem cels and their niches. Adv Exp Med Biol 695, 155-168.

53. Wu, J.Y., Scadden, D.T., and Kronenberg, H.M. (2009). Role of the osteoblast lineage in the bone marrow hematopoietic niches. J Bone Miner Res 24, 759-764.

54. Christiano, A.M. (2004). Epithelial stem cells: stepping out of their niche. Cell 118, 530-532.

55. de Rooij, D.G. (2009). The spermatogonial stem cell niche. Microsc Res Tech 72, 580-585.

56. Arcangeli, A., and Becchetti, A. (2010). Integrin structure and functional relation with ion channels. Adv Exp Med Biol 674, 1-7.

57. Perdih, A., and Dolenc, M.S. (2010). Small molecule antagonists of integrin receptors. Curr Med Chem 17, 2371-2392.

58. Prowse, A.B., Chong, F., Gray, P.P., and Munro, T.P. (2011). Stem cell integrins: implications for ex-vivo culture and cellular therapies. Stem Cell Res 6, 1-12.

59. Chiquet-Ehrismann, R. (2004). Tenascins. Int J Biochem Cell Biol 36, 986-990.

60. Ekblom, P., and Aufderheide, E. (1989). Stimulation of tenascin expression in mesenchyme by epithelial-mesenchymal interactions. Int J Dev Biol 33, 71-79.

61. Chaerkady, R., Kerr, C.L., Marimuthu, A., Kelkar, D.S., Kashyap, M.K., Gucek, M., Gearhart, J.D., and Pandey, A. (2009). Temporal analysis of neural differentiation using quantitative proteomics. J Proteome Res 8, 1315-1326.

62. Garcion, E., Halilagic, A., Faissner, A., and ffrench-Constant, C. (2004). Generation of an environmental niche for neural stem cell development by the extracellular matrix molecule tenascin C. Development 131, 3423-3432.

63. Scadden, D.T. (2006). The stem-cell niche as an entity of action. Nature 441, 1075-1079.

64. von Holst, A. (2008). Tenascin C in stem cell niches: redundant, permissive or instructive? Cells Tissues Organs 188, 170-177.

65. Haylock, D.N., and Nilsson, S.K. (2006). Osteopontin: a bridge between bone and blood. Br J Haematol 134, 467-474.

66. Nilsson, S.K., Johnston, H.M., Whitty, G.A., Williams, B., Webb, R.J., Denhardt, D.T., Bertoncello, I., Bendall, L.J., Simmons, P.J., and Haylock, D.N. (2005). Osteopontin, a key component of the hematopoietic stem cell niche and regulator of primitive hematopoietic progenitor cells. Blood 106, 1232-1239.

67. Weber, G.F., Ashkar, S., Glimcher, M.J., and Cantor, H. (1996). Receptor-ligand interaction between CD44 and osteopontin (Eta-1). Science 271, 509-512.

68. Burglin, T.R. (2008). The Hedgehog protein family. Genome Biol 9, 241.

69. Levy, V., Lindon, C., Zheng, Y., Harfe, B.D., and Morgan, B.A. (2007). Epidermal stem cells arise from the hair follicle after wounding. Faseb J 21, 1358-1366.

70. Ishizuya-Oka, A., and Hasebe, T. (2008). Sonic hedgehog and bone morphogenetic protein-4 signaling pathway involved in epithelial cell renewal along the radial axis of the intestine. Digestion 77 Suppl 1, 42-47.

71. Pitulescu, M.E., and Adams, R.H. (2010). Eph/ephrin molecules--a hub for signaling and endocytosis. Genes Dev 24, 2480-2492.

72. Krull, I.S., Mills, K., Hoffman, G., and Fine, D.H. (1980). The analysis of N-Nitrosoatrazine and N-Nitrosocarbaryl in whole mice. J Anal Toxicol 4, 260-262.

73. Fuchs, E. (2009). The tortoise and the hair: slow-cycling cells in the stem cell race. Cell 137, 811-819.

74. Potten, C.S. (2004). Keratinocyte stem cells, label-retaining cells and possible genome protection mechanisms. J Investig Dermatol Symp Proc 9, 183-195.

75. Fernandez-Gonzalez, R., Illa-Bochaca, I., Shelton, D.N., Welm, B.E., Barcellos-Hoff, M.H., and Ortiz-de-Solorzano, C. (2010). In situ analysis of cell populations: long-term label-retaining cells. Methods Mol Biol 621, 1-28.

76. Bock, T.A. (1997). Assay systems for hematopoietic stem and progenitor cells. Stem Cells 15 Suppl 1, 185-195.

77. Morita, Y., Ema, H., and Nakauchi, H. (2010). Heterogeneity and hierarchy within the most primitive hematopoietic stem cell compartment. J Exp Med 207, 1173-1182.

78. Tarnok, A., Ulrich, H., and Bocsi, J. (2010). Phenotypes of stem cells from diverse origin. Cytometry A 77, 6-10.

79. Skalnikova, H., Vodicka, P., Gadher, S.J., and Kovarova, H. (2008). Proteomics of neural stem cells. Expert Rev Proteomics 5, 175-186.

80. Spangrude, G.J., Heimfeld, S., and Weissman, I.L. (1988). Purification and characterization of mouse hematopoietic stem cells. Science 241, 58-62.

81. Friedenstein, A.J. (1980). Stromal mechanisms of bone marrow: cloning in vitro and retransplantation in vivo. Haematol Blood Transfus 25, 19-29.

82. Owen, M., and Friedenstein, A.J. (1988). Stromal stem cells: marrow-derived osteogenic precursors. Ciba Found Symp 136, 42-60.

83. Tong, L., Ji, L., Wang, Z., Tong, X., Zhang, L., and Sun, X. (2010). Differentiation of neural stem cells into Schwann-like cells in vitro. Biochem Biophys Res Commun 401, 592-597.

84. Barkho, B.Z., and Zhao, X. (2011). Adult Neural Stem Cells: Response to Stroke Injury and Potential for Therapeutic Applications. Curr Stem Cell Res Ther.

85. Mercola, M., Ruiz-Lozano, P., and Schneider, M.D. (2011). Cardiac muscle regeneration: lessons from development. Genes Dev 25, 299-309.

86. Apati, A., Orban, T.I., Varga, N., Nemeth, A., Schamberger, A., Krizsik, V., Erdelyi-Belle, B., Homolya, L., Varady, G., Padanyi, R.*, et al.* (2008). High level functional expression of the ABCG2 multidrug transporter in undifferentiated human embryonic stem cells. Biochim Biophys Acta 1778, 2700-2709.

87. Wang, J., Wei, X., Ling, J., Huang, Y., Huo, Y., and Zhou, Y. (2010). The presence of a side population and its marker ABCG2 in human deciduous dental pulp cells. Biochem Biophys Res Commun 400, 334-339.

88. Raaijmakers, M.H. (2007). ATP-binding-cassette transporters in hematopoietic stem cells and their utility as therapeutical targets in acute and chronic myeloid leukemia. Leukemia 21, 2094-2102.

89. Pfister, O., Oikonomopoulos, A., Sereti, K.I., and Liao, R. (2010). Isolation of resident cardiac progenitor cells by Hoechst 33342 staining. Methods Mol Biol 660, 53-63.

90. Biressi, S., and Rando, T.A. (2010). Heterogeneity in the muscle satellite cell population. Semin Cell Dev Biol 21, 845-854.

91. Ohyama, M. (2007). Hair follicle bulge: a fascinating reservoir of epithelial stem cells. J Dermatol Sci 46, 81-89.

92. Chia, L.A., and Kuo, C.J. (2010). The intestinal stem cell. Prog Mol Biol Transl Sci 96, 157-173.

93. Russo, F.P., and Parola, M. (2011). Stem and progenitor cells in liver regeneration and repair. Cytotherapy 13, 135-144.

94. Bonner-Weir, S., and Sharma, A. (2002). Pancreatic stem cells. J Pathol 197, 519-526.

95. Seeberger, K.L., Eshpeter, A., and Korbutt, G.S. (2011). Isolation and Culture of Human Multipotent Stromal Cells from the Pancreas. Methods Mol Biol 698, 123-140.

96. Smukler, S.R., Arntfield, M.E., Razavi, R., Bikopoulos, G., Karpowicz, P., Seaberg, R., Dai, F., Lee, S., Ahrens, R., Fraser, P.E.*, et al.* (2011). The adult mouse

and human pancreas contain rare multipotent stem cells that express insulin. Cell Stem Cell 8, 281-293.

97. Lavker, R.M., Zheng, P.S., and Dong, G. (1986). Morphology of aged skin. Dermatol Clin 4, 379-389.

98. Abbas, O., and Mahalingam, M. (2009). Epidermal stem cells: practical perspectives and potential uses. Br J Dermatol 161, 228-236.

99. Kaur, P. (2006). Interfollicular epidermal stem cells: identification, challenges, potential. J Invest Dermatol 126, 1450-1458.

100. Myung, P., Andl, T., and Ito, M. (2009). Defining the hair follicle stem cell (Part I). J Cutan Pathol 36, 1031-1034.

101. Forsberg, E.C., Bhattacharya, D., and Weissman, I.L. (2006). Hematopoietic stem cells: expression profiling and beyond. Stem Cell Rev 2, 23-30.

102. Adams, G.B., and Scadden, D.T. (2006). The hematopoietic stem cell in its place. Nat Immunol 7, 333-337.

103. Akashi, K. (2007). Cartography of hematopoietic stem cell commitment dependent upon a reporter for transcription factor activation. Ann N Y Acad Sci 1106, 76-81.

104. Kim, Y.H., Wee, Y.M., Choi, M.Y., Lim, D.G., Kim, S.C., and Han, D.J. (2011). IL-10 induced by CD11b+cells and IL-10 activated regulatory T cells play a role in immune modulation of mesenchymal stem cells in rat islet allograft. Mol Med.

105. Nemeth, K., Keane-Myers, A., Brown, J.M., Metcalfe, D.D., Gorham, J.D., Bundoc, V.G., Hodges, M.G., Jelinek, I., Madala, S., Karpati, S.*, et al.* (2010). Bone marrow stromal cells use TGF-beta to suppress allergic responses in a mouse model of ragweed-induced asthma. Proc Natl Acad Sci U S A 107, 5652-5657.

106. Shen, B., Wei, A., Whittaker, S., Williams, L.A., Tao, H., Ma, D.D., and Diwan, A.D. (2010). The role of BMP-7 in chondrogenic and osteogenic differentiation of human bone marrow multipotent mesenchymal stromal cells in vitro. J Cell Biochem 109, 406-416.

107. Hackett, C.H., Flaminio, M.J., and Fortier, L.A. (2011). Analysis of CD14 Expression Levels in Putative Mesenchymal Progenitor Cells Isolated from Equine Bone Marrow. Stem Cells Dev 20, 721-735.

108. Schipani, E., and Kronenberg, H.M. (2008). Adult mesenchymal stem cells. In StemBook (Cambridge (MA)).

109. Khosla, S., Westendorf, J.J., and Modder, U.I. (2010). Concise review: Insights from normal bone remodeling and stem cell-based therapies for bone repair. Stem Cells 28, 2124-2128.

110. Maitra, B., Szekely, E., Gjini, K., Laughlin, M.J., Dennis, J., Haynesworth, S.E., and Koc, O.N. (2004). Human mesenchymal stem cells support unrelated donor hematopoietic stem cells and suppress T-cell activation. Bone Marrow Transplant 33, 597-604.

111. Keith L Moore, V.P. (2003). The developing human (Philadelphia, Pennsylvania, Saunders).

112. Lanza, R. (2004). Stem Cells (San Diego, California, Elsevier Academic Press).

113. Wagers, A.J., and Weissman, I.L. (2004). Plasticity of adult stem cells. Cell 116, 639-648.

114. Mezey, E., Chandross, K.J., Harta, G., Maki, R.A., and McKercher, S.R. (2000). Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. Science 290, 1779-1782.

115. Mezey, E., Key, S., Vogelsang, G., Szalayova, I., Lange, G.D., and Crain, B. (2003). Transplanted bone marrow generates new neurons in human brains. Proc Natl Acad Sci U S A 100, 1364-1369.

116. Tran, S.D., Redman, R.S., Barrett, A.J., Pavletic, S.Z., Key, S., Liu, Y., Carpenter, A., Nguyen, H.M., Sumita, Y., Baum, B.J.*, et al.* (2011). Microchimerism in salivary glands after blood- and marrow-derived stem cell transplantation. Biol Blood Marrow Transplant 17, 429-433.

117. Eisenberg, C.A., Burch, J.B., and Eisenberg, L.M. (2006). Bone marrow cells transdifferentiate to cardiomyocytes when introduced into the embryonic heart. Stem Cells 24, 1236-1245.

118. Franco, D., de Boer, P.A., de Gier-de Vries, C., Lamers, W.H., and Moorman, A.F. (2001). Methods on in situ hybridization, immunohistochemistry and beta-galactosidase reporter gene detection. Eur J Morphol 39, 3-25.

119. Gerdes, H.H., and Kaether, C. (1996). Green fluorescent protein: applications in cell biology. FEBS Lett 389, 44-47.

120. Toth, Z.E., Shahar, T., Leker, R., Szalayova, I., Bratincsak, A., Key, S., Lonyai, A., Nemeth, K., and Mezey, E. (2007). Sensitive detection of GFP utilizing tyramide signal amplification to overcome gene silencing. Exp Cell Res 313, 1943-1950.

121. Park, J.Y., Hwang, E.M., Park, N., Kim, E., Kim, D.G., Kang, D., Han, J., Choi, W.S., Ryu, P.D., and Hong, S.G. (2007). Gateway RFP-fusion vectors for high throughput functional analysis of genes. Mol Cells 23, 357-362.

122. Taghizadeh, R.R., and Sherley, J.L. (2008). CFP and YFP, but not GFP, provide stable fluorescent marking of rat hepatic adult stem cells. J Biomed Biotechnol 2008, 453590.

123. Reynolds, B.A., and Weiss, S. (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255, 1707-1710.

124. Gage, F.H. (2002). Neurogenesis in the adult brain. J Neurosci 22, 612-613.

125. Taupin, P., and Gage, F.H. (2002). Adult neurogenesis and neural stem cells of the central nervous system in mammals. J Neurosci Res 69, 745-749.

126. van Praag, H. (2009). Exercise and the brain: something to chew on. Trends Neurosci 32, 283-290.

127. Eglitis, M.A., and Mezey, E. (1997). Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. Proc Natl Acad Sci U S A 94, 4080-4085.

128. Brazelton, T.R., Rossi, F.M., Keshet, G.I., and Blau, H.M. (2000). From marrow to brain: expression of neuronal phenotypes in adult mice. Science 290, 1775-1779.

129. Hess, D.C., and Borlongan, C.V. (2008). Stem cells and neurological diseases. Cell proliferation 41 Suppl 1, 94-114.

130. Li, Y., and Chopp, M. (2009). Marrow stromal cell transplantation in stroke and traumatic brain injury. Neurosci Lett 456, 120-123.

131. Mezey, E. (2007). Bone marrow-derived stem cells in neurological diseases: stones or masons? Regen Med 2, 37-49.

132. Phinney, D.G., and Isakova, I. (2005). Plasticity and therapeutic potential of mesenchymal stem cells in the nervous system. Curr Pharm Des 11, 1255-1265.

133. Sadan, O., Melamed, E., and Offen, D. (2009). Bone-marrow-derived mesenchymal stem cell therapy for neurodegenerative diseases. Expert Opin Biol Ther 9, 1487-1497.

134. Weimann, J.M., Charlton, C.A., Brazelton, T.R., Hackman, R.C., and Blau, H.M. (2003). Contribution of transplanted bone marrow cells to Purkinje neurons in human adult brains. Proc Natl Acad Sci U S A 100, 2088-2093.

135. Cogle, C.R., Yachnis, A.T., Laywell, E.D., Zander, D.S., Wingard, J.R., Steindler, D.A., and Scott, E.W. (2004). Bone marrow transdifferentiation in brain after transplantation: a retrospective study. Lancet 363, 1432-1437.

136. Crain, B.J., Tran, S.D., and Mezey, E. (2005). Transplanted human bone marrow cells generate new brain cells. J Neurol Sci 233, 121-123.

137. Grompe, M. (2003). The role of bone marrow stem cells in liver regeneration. Semin Liver Dis 23, 363-372.

138. Popp, F.C., Piso, P., Schlitt, H.J., and Dahlke, M.H. (2006). Therapeutic potential of bone marrow stem cells for liver diseases. Curr Stem Cell Res Ther 1, 411-418.

139. Ying, Q.L., Nichols, J., Evans, E.P., and Smith, A.G. (2002). Changing potency by spontaneous fusion. Nature 416, 545-548.

140. Petersen, B.E., Bowen, W.C., Patrene, K.D., Mars, W.M., Sullivan, A.K., Murase, N., Boggs, S.S., Greenberger, J.S., and Goff, J.P. (1999). Bone marrow as a potential source of hepatic oval cells. Science 284, 1168-1170.

141. Theise, N.D., Nimmakayalu, M., Gardner, R., Illei, P.B., Morgan, G., Teperman, L., Henegariu, O., and Krause, D.S. (2000). Liver from bone marrow in humans. Hepatology 32, 11-16.

142. Lagasse, E., Connors, H., Al-Dhalimy, M., Reitsma, M., Dohse, M., Osborne, L., Wang, X., Finegold, M., Weissman, I.L., and Grompe, M. (2000). Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat Med 6, 1229-1234.

143. Vassilopoulos, G., Wang, P.R., and Russell, D.W. (2003). Transplanted bone marrow regenerates liver by cell fusion. Nature 422, 901-904.

144. Terada, N., Hamazaki, T., Oka, M., Hoki, M., Mastalerz, D.M., Nakano, Y., Meyer, E.M., Morel, L., Petersen, B.E., and Scott, E.W. (2002). Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature 416, 542-545.

145. Guidotti, J.E., Bregerie, O., Robert, A., Debey, P., Brechot, C., and Desdouets, C. (2003). Liver cell polyploidization: a pivotal role for binuclear hepatocytes. J Biol Chem 278, 19095-19101.

146. Wang, X., Willenbring, H., Akkari, Y., Torimaru, Y., Foster, M., Al-Dhalimy, M., Lagasse, E., Finegold, M., Olson, S., and Grompe, M. (2003). Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature 422, 897-901.

147. Austin, T.W., and Lagasse, E. (2003). Hepatic regeneration from hematopoietic stem cells. Mech Dev 120, 131-135.

148. Dorrell, C., and Grompe, M. (2005). Liver repair by intra- and extrahepatic progenitors. Stem Cell Rev 1, 61-64.

149. Kodama, S., Kuhtreiber, W., Fujimura, S., Dale, E.A., and Faustman, D.L. (2003). Islet regeneration during the reversal of autoimmune diabetes in NOD mice. Science 302, 1223-1227.

150. Chong, A.S., Shen, J., Tao, J., Yin, D., Kuznetsov, A., Hara, M., and Philipson, L.H. (2006). Reversal of diabetes in non-obese diabetic mice without spleen cell-derived beta cell regeneration. Science 311, 1774-1775.

151. Nishio, J., Gaglia, J.L., Turvey, S.E., Campbell, C., Benoist, C., and Mathis, D. (2006). Islet recovery and reversal of murine type 1 diabetes in the absence of any infused spleen cell contribution. Science 311, 1775-1778.

152. Suri, A., Calderon, B., Esparza, T.J., Frederick, K., Bittner, P., and Unanue, E.R. (2006). Immunological reversal of autoimmune diabetes without hematopoietic replacement of beta cells. Science 311, 1778-1780.

153. Ianus, A., Holz, G.G., Theise, N.D., and Hussain, M.A. (2003). In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. J Clin Invest 111, 843-850.

154. Krause, D.S., Theise, N.D., Collector, M.I., Henegariu, O., Hwang, S., Gardner, R., Neutzel, S., and Sharkis, S.J. (2001). Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 105, 369-377.

155. Suratt, B.T., Cool, C.D., Serls, A.E., Chen, L., Varella-Garcia, M., Shpall, E.J., Brown, K.K., and Worthen, G.S. (2003). Human pulmonary chimerism after hematopoietic stem cell transplantation. Am J Respir Crit Care Med 168, 318-322.

156. Mattsson, J., Jansson, M., Wernerson, A., and Hassan, M. (2004). Lung epithelial cells and type II pneumocytes of donor origin after allogeneic hematopoietic stem cell transplantation. Transplantation 78, 154-157.

157. Albera, C., Polak, J.M., Janes, S., Griffiths, M.J., Alison, M.R., Wright, N.A., Navaratnarasah, S., Poulsom, R., Jeffery, R., Fisher, C.*, et al.* (2005). Repopulation of human pulmonary epithelium by bone marrow cells: a potential means to promote repair. Tissue Eng 11, 1115-1121.

158. Krause, D.S. (2008). Bone marrow-derived cells and stem cells in lung repair. Proc Am Thorac Soc 5, 323-327.

159. Sinclair, R.A. (1972). Origin of endothelium in human renal allografts. Br Med J 4, 15-16.

160. Poulsom, R., Forbes, S.J., Hodivala-Dilke, K., Ryan, E., Wyles, S., Navaratnarasah, S., Jeffery, R., Hunt, T., Alison, M., Cook, T.*, et al.* (2001). Bone marrow contributes to renal parenchymal turnover and regeneration. J Pathol 195, 229-235.

161. Gupta, S., Verfaillie, C., Chmielewski, D., Kim, Y., and Rosenberg, M.E. (2002). A role for extrarenal cells in the regeneration following acute renal failure. Kidney Int 62, 1285-1290.

162. Imasawa, T., Utsunomiya, Y., Kawamura, T., Zhong, Y., Nagasawa, R., Okabe, M., Maruyama, N., Hosoya, T., and Ohno, T. (2001). The potential of bone marrow-derived cells to differentiate to glomerular mesangial cells. J Am Soc Nephrol 12, 1401-1409.

163. Ito, T., Suzuki, A., Imai, E., Okabe, M., and Hori, M. (2001). Bone marrow is a reservoir of repopulating mesangial cells during glomerular remodeling. J Am Soc Nephrol 12, 2625-2635.

164. Sugimoto, H., Mundel, T.M., Sund, M., Xie, L., Cosgrove, D., and Kalluri, R. (2006). Bone-marrow-derived stem cells repair basement membrane collagen defects and reverse genetic kidney disease. Proc Natl Acad Sci U S A 103, 7321-7326.

165. Gupta, S., and Rosenberg, M.E. (2008). Do stem cells exist in the adult kidney? Am J Nephrol 28, 607-613.

166. Krause, D., and Cantley, L.G. (2005). Bone marrow plasticity revisited: protection or differentiation in the kidney tubule? J Clin Invest 115, 1705-1708.

167. Baer, P.C., and Geiger, H. (2010). Mesenchymal stem cell interactions with growth factors on kidney repair. Curr Opin Nephrol Hypertens 19, 1-6.

168. Camussi, G., Deregibus, M.C., and Tetta, C. (2010). Paracrine/endocrine mechanism of stem cells on kidney repair: role of microvesicle-mediated transfer of genetic information. Curr Opin Nephrol Hypertens 19, 7-12.

169. Ferrari, G., Cusella-De Angelis, G., Coletta, M., Paolucci, E., Stornaiuolo, A., Cossu, G., and Mavilio, F. (1998). Muscle regeneration by bone marrow-derived myogenic progenitors. Science 279, 1528-1530.

170. Gussoni, E., Soneoka, Y., Strickland, C.D., Buzney, E.A., Khan, M.K., Flint, A.F., Kunkel, L.M., and Mulligan, R.C. (1999). Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature 401, 390-394.

171. Gussoni, E., Bennett, R.R., Muskiewicz, K.R., Meyerrose, T., Nolta, J.A., Gilgoff, I., Stein, J., Chan, Y.M., Lidov, H.G., Bonnemann, C.G., *et al.* (2002). Long-term persistence of donor nuclei in a Duchenne muscular dystrophy patient receiving bone marrow transplantation. J Clin Invest 110, 807-814.

172. Corbel, S.Y., Lee, A., Yi, L., Duenas, J., Brazelton, T.R., Blau, H.M., and Rossi, F.M. (2003). Contribution of hematopoietic stem cells to skeletal muscle. Nat Med 9, 1528-1532.

173. Abedi, M., Foster, B.M., Wood, K.D., Colvin, G.A., McLean, S.D., Johnson, K.W., and Greer, D.A. (2007). Haematopoietic stem cells participate in muscle regeneration. Br J Haematol 138, 792-801.

174. Orlic, D., Kajstura, J., Chimenti, S., Jakoniuk, I., Anderson, S.M., Li, B., Pickel, J., McKay, R., Nadal-Ginard, B., Bodine, D.M., *et al.* (2001). Bone marrow cells regenerate infarcted myocardium. Nature 410, 701-705.

175. Orlic, D., Kajstura, J., Chimenti, S., Limana, F., Jakoniuk, I., Quaini, F., Nadal-Ginard, B., Bodine, D.M., Leri, A., and Anversa, P. (2001). Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc Natl Acad Sci U S A 98, 10344-10349.

176. Nygren, J.M., Jovinge, S., Breitbach, M., Sawen, P., Roll, W., Hescheler, J., Taneera, J., Fleischmann, B.K., and Jacobsen, S.E. (2004). Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. Nat Med 10, 494-501.

177. Makino, S., Fukuda, K., Miyoshi, S., Konishi, F., Kodama, H., Pan, J., Sano, M., Takahashi, T., Hori, S., Abe, H., *et al.* (1999). Cardiomyocytes can be generated from marrow stromal cells in vitro. J Clin Invest 103, 697-705.

178. Tomita, S., Li, R.K., Weisel, R.D., Mickle, D.A., Kim, E.J., Sakai, T., and Jia, Z.Q. (1999). Autologous transplantation of bone marrow cells improves damaged heart function. Circulation 100, II247-256.

179. Doyonnas, R., LaBarge, M.A., Sacco, A., Charlton, C., and Blau, H.M. (2004). Hematopoietic contribution to skeletal muscle regeneration by myelomonocytic precursors. Proc Natl Acad Sci U S A 101, 13507-13512.

180. Rota, M., Kajstura, J., Hosoda, T., Bearzi, C., Vitale, S., Esposito, G., Iaffaldano, G., Padin-Iruegas, M.E., Gonzalez, A., Rizzi, R., *et al.* (2007). Bone marrow cells adopt the cardiomyogenic fate in vivo. Proc Natl Acad Sci U S A 104, 17783-17788.

181. Balsam, L.B., Wagers, A.J., Christensen, J.L., Kofidis, T., Weissman, I.L., and Robbins, R.C. (2004). Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. Nature 428, 668-673.

182. Murry, C.E., Soonpaa, M.H., Reinecke, H., Nakajima, H., Nakajima, H.O., Rubart, M., Pasumarthi, K.B., Virag, J.I., Bartelmez, S.H., Poppa, V., *et al.* (2004). Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 428, 664-668.

183. Thiele, J., Varus, E., Wickenhauser, C., Kvasnicka, H.M., Metz, K.A., and Beelen, D.W. (2004). Regeneration of heart muscle tissue: quantification of chimeric cardiomyocytes and endothelial cells following transplantation. Histol Histopathol 19, 201-209.

184. Assmus, B., Fischer-Rasokat, U., Honold, J., Seeger, F.H., Fichtlscherer, S., Tonn, T., Seifried, E., Schachinger, V., Dimmeler, S., and Zeiher, A.M. (2007). Transcoronary transplantation of functionally competent BMCs is associated with a decrease in natriuretic peptide serum levels and improved survival of patients with

chronic postinfarction heart failure: results of the TOPCARE-CHD Registry. Circ Res 100, 1234-1241.

185. Gersh, B.J., Simari, R.D., Behfar, A., Terzic, C.M., and Terzic, A. (2009). Cardiac cell repair therapy: a clinical perspective. Mayo Clin Proc 84, 876-892.

186. Endler, G., Greinix, H., Winkler, K., Mitterbauer, G., and Mannhalter, C. (1999). Genetic fingerprinting in mouthwashes of patients after allogeneic bone marrow transplantation. Bone Marrow Transplant 24, 95-98.

187. Tran, S.D., Pillemer, S.R., Dutra, A., Barrett, A.J., Brownstein, M.J., Key, S., Pak, E., Leakan, R.A., Kingman, A., Yamada, K.M*., et al.* (2003). Differentiation of human bone marrow-derived cells into buccal epithelial cells in vivo: a molecular analytical study. Lancet 361, 1084-1088.

188. Brittan, M., Hunt, T., Jeffery, R., Poulsom, R., Forbes, S.J., Hodivala-Dilke, K., Goldman, J., Alison, M.R., and Wright, N.A. (2002). Bone marrow derivation of pericryptal myofibroblasts in the mouse and human small intestine and colon. Gut 50, 752-757.

189. Okamoto, R., Yajima, T., Yamazaki, M., Kanai, T., Mukai, M., Okamoto, S., Ikeda, Y., Hibi, T., Inazawa, J., and Watanabe, M. (2002). Damaged epithelia regenerated by bone marrow-derived cells in the human gastrointestinal tract. Nat Med 8, 1011-1017.

190. Meignin, V., Soulier, J., Brau, F., Lemann, M., Gluckman, E., Janin, A., and Socie, G. (2004). Little evidence of donor-derived epithelial cells in early digestive acute graft-versus-host disease. Blood 103, 360-362.

191. Spyridonidis, A., Schmitt-Graff, A., Tomann, T., Dwenger, A., Follo, M., Behringer, D., and Finke, J. (2004). Epithelial tissue chimerism after human hematopoietic cell transplantation is a real phenomenon. Am J Pathol 164, 1147-1155.

192. Jabbour, H.N., Kelly, R.W., Fraser, H.M., and Critchley, H.O. (2006). Endocrine regulation of menstruation. Endocr Rev 27, 17-46.

193. Gargett, C.E., Chan, R.W., and Schwab, K.E. (2007). Endometrial stem cells. Curr Opin Obstet Gynecol 19, 377-383.

194. Taylor, H.S. (2004). Endometrial cells derived from donor stem cells in bone marrow transplant recipients. Jama 292, 81-85.

195. Sasaki, M., Abe, R., Fujita, Y., Ando, S., Inokuma, D., and Shimizu, H. (2008). Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type. J Immunol 180, 2581-2587.

196. Ando, S., Abe, R., Sasaki, M., Murata, J., Inokuma, D., and Shimizu, H. (2009). Bone marrow-derived cells are not the origin of the cancer stem cells in ultraviolet-induced skin cancer. Am J Pathol 174, 595-601.

197. Wu, Y., Chen, L., Scott, P.G., and Tredget, E.E. (2007). Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells 25, 2648-2659.

198. Murata, H., Janin, A., Leboeuf, C., Soulier, J., Gluckman, E., Meignin, V., and Socie, G. (2007). Donor-derived cells and human graft-versus-host disease of the skin. Blood 109, 2663-2665.

199. Fan, Q., Yee, C.L., Ohyama, M., Tock, C., Zhang, G., Darling, T.N., and Vogel, J.C. (2006). Bone marrow-derived keratinocytes are not detected in normal skin and only rarely detected in wounded skin in two different murine models. Exp Hematol 34, 672-679.

200. Niino, D., Nakashima, M., Kondo, H., Ito, M., Matsuu, M., Shichijo, K., Nakayama, T., Tomonaga, M., and Sekine, I. (2005). Correlation of donor-derived keratinocytes and severity of graft-versus-host disease (GVHD) in epidermis. Pathol Res Pract 200, 775-781.

201. Brittan, M., Braun, K.M., Reynolds, L.E., Conti, F.J., Reynolds, A.R., Poulsom, R., Alison, M.R., Wright, N.A., and Hodivala-Dilke, K.M. (2005). Bone marrow cells engraft within the epidermis and proliferate in vivo with no evidence of cell fusion. J Pathol 205, 1-13.

202. Borue, X., Lee, S., Grove, J., Herzog, E.L., Harris, R., Diflo, T., Glusac, E., Hyman, K., Theise, N.D., and Krause, D.S. (2004). Bone marrow-derived cells contribute to epithelial engraftment during wound healing. Am J Pathol 165, 1767-1772.

203. Harris, R.G., Herzog, E.L., Bruscia, E.M., Grove, J.E., Van Arnam, J.S., and Krause, D.S. (2004). Lack of a fusion requirement for development of bone marrow-derived epithelia. Science 305, 90-93.

204. Hematti, P., Sloand, E.M., Carvallo, C.A., Albert, M.R., Yee, C.L., Fuehrer, M.M., Blancato, J.K., Kearns, W.G., Barrett, J.A., Childs, R.W.*, et al.* (2002). Absence of donor-derived keratinocyte stem cells in skin tissues cultured from patients after mobilized peripheral blood hematopoietic stem cell transplantation. Exp Hematol 30, 943-949.

205. Medina, R.J., Kataoka, K., Miyazaki, M., and Huh, N.H. (2006). Efficient differentiation into skin cells of bone marrow cells recovered in a pellet after density gradient fractionation. Int J Mol Med 17, 721-727.

206. Jacewicz, R., Lewandowski, K., Rupa-Matysek, J., Jedrzejczyk, M., Brzezinski, P.M., Dobosz, T., Jonkisz, A., Szram, S., Komarnicki, M., and Berent, J. (2010). Donor-derived DNA in hair follicles of recipients after allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 45, 1638-1644.

207. Parkes (1926). On the occurrence of the oestrous cycle after X-ray sterilisation. Part I. Irradiation of mice at three weeks old. Proceedings of the royal society of london series b-containing papers of a biological character 100, 172-199

208. Walter, I., Klein, M., Handler, J., Aurich, J.E., Reifinger, M., and Aurich, C. (2001). Lectin binding patterns of uterine glands in mares with chronic endometrial degeneration. Am J Vet Res 62, 840-845.

209. Tabibzadeh, S. (1990). Proliferative activity of lymphoid cells in human endometrium throughout the menstrual cycle. J Clin Endocrinol Metab 70, 437-443.

210. Allen, E. (1922). The oestrus cycle in the mouse. American Journal of Anatomy, 297–371.

211. Dharma, S.J., Kholkute, S.D., and Nandedkar, T.D. (2001). Apoptosis in endometrium of mouse during estrous cycle. Indian J Exp Biol 39, 218-222.

212. Hermiston, M.L., Xu, Z., and Weiss, A. (2003). CD45: a critical regulator of signaling thresholds in immune cells. Annu Rev Immunol 21, 107-137.

213. McKinney-Freeman, S.L., Jackson, K.A., Camargo, F.D., Ferrari, G., Mavilio, F., and Goodell, M.A. (2002). Muscle-derived hematopoietic stem cells are hematopoietic in origin. Proc Natl Acad Sci U S A 99, 1341-1346.

214. Chan, R.W., Schwab, K.E., and Gargett, C.E. (2004). Clonogenicity of human endometrial epithelial and stromal cells. Biol Reprod 70, 1738-1750.

215. Cervello, I., Martinez-Conejero, J.A., Horcajadas, J.A., Pellicer, A., and Simon, C. (2007). Identification, characterization and co-localization of label-retaining cell population in mouse endometrium with typical undifferentiated markers. Hum Reprod 22, 45-51.

216. Chan, R.W., and Gargett, C.E. (2006). Identification of label-retaining cells in mouse endometrium. Stem Cells 24, 1529-1538.

217. Trowbridge, I.S., and Thomas, M.L. (1994). CD45: an emerging role as a protein tyrosine phosphatase required for lymphocyte activation and development. Annu Rev Immunol 12, 85-116.

218. Nakahara, J., Seiwa, C., Tan-Takeuchi, K., Gotoh, M., Kishihara, K., Ogawa, M., Asou, H., and Aiso, S. (2005). Involvement of CD45 in central nervous system myelination. Neurosci Lett 379, 116-121.

219. Aliotta, J.M., Keaney, P., Passero, M., Dooner, M.S., Pimentel, J., Greer, D., Demers, D., Foster, B., Peterson, A., Dooner, G., *et al.* (2006). Bone marrow production of lung cells: the impact of G-CSF, cardiotoxin, graded doses of irradiation, and subpopulation phenotype. Exp Hematol 34, 230-241.

220. Kotton, D.N., Ma, B.Y., Cardoso, W.V., Sanderson, E.A., Summer, R.S., Williams, M.C., and Fine, A. (2001). Bone marrow-derived cells as progenitors of lung alveolar epithelium. Development 128, 5181-5188.

221. Theise, N.D., Henegariu, O., Grove, J., Jagirdar, J., Kao, P.N., Crawford, J.M., Badve, S., Saxena, R., and Krause, D.S. (2002). Radiation pneumonitis in mice: a severe injury model for pneumocyte engraftment from bone marrow. Exp Hematol 30, 1333-1338.

222. Moayeri, S. (2007). The Endometrium: Molecular, Cellular and Clinical Perspectives (London, Informa).

223. Valeri, R.M., Ibrahim, N., and Sheaff, M.T. (2002). Extramedullary hematopoiesis in the endometrium. Int J Gynecol Pathol 21, 178-181.

224. Gargett, C. (2006). Identification and characterisation of human endometrial stem/progenitor cells. Aust N Z J Obstet Gynaecol, 250-253.

225. Du, H., and Taylor, H.S. (2007). Contribution of bone marrow-derived stem cells to endometrium and endometriosis. Stem Cells 25, 2082-2086.

226. Rier, S.E., Martin, D.C., Bowman, R.E., Dmowski, W.P., and Becker, J.L. (1993). Endometriosis in rhesus monkeys (Macaca mulatta) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Fundam Appl Toxicol 21, 433-441.

227. Starzinski-Powitz, A., Zeitvogel, A., Schreiner, A., and Baumann, R. (2003). [Endometriosis--a stem cell disease?]. Zentralbl Gynakol 125, 235-238.

228. Martin, J.D., Jr., and Hauck, A.E. (1985). Endometriosis in the male. Am Surg 51, 426-430.

229. Blanpain, C., and Fuchs, E. (2009). Epidermal homeostasis: a balancing act of stem cells in the skin. Nat Rev Mol Cell Biol 10, 207-217.

230. Fuchs, E. (2009). Finding one's niche in the skin. Cell Stem Cell 4, 499-502.

231. Verstappen, J., Katsaros, C., Torensma, R., and Von den Hoff, J.W. (2009). A functional model for adult stem cells in epithelial tissues. Wound Repair Regen 17, 296-305.

232. Kloepper, J.E., Tiede, S., Brinckmann, J., Reinhardt, D.P., Meyer, W., Faessler, R., and Paus, R. (2008). Immunophenotyping of the human bulge region: the quest to define useful in situ markers for human epithelial hair follicle stem cells and their niche. Exp Dermatol 17, 592-609.

233. Hoang, M.P., Keady, M., and Mahalingam, M. (2009). Stem cell markers (cytokeratin 15, CD34 and nestin) in primary scarring and nonscarring alopecia. Br J Dermatol 160, 609-615.

234. Poblet, E., Jimenez, F., Godinez, J.M., Pascual-Martin, A., and Izeta, A. (2006). The immunohistochemical expression of CD34 in human hair follicles: a comparative study with the bulge marker CK15. Clin Exp Dermatol 31, 807-812.

235. Deng, W., Han, Q., Liao, L., Li, C., Ge, W., Zhao, Z., You, S., Deng, H., Murad, F., and Zhao, R.C. (2005). Engrafted bone marrow-derived flk-(1+) mesenchymal stem cells regenerate skin tissue. Tissue Eng 11, 110-119.

236. Korbling, M., Katz, R.L., Khanna, A., Ruifrok, A.C., Rondon, G., Albitar, M., Champlin, R.E., and Estrov, Z. (2002). Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. N Engl J Med 346, 738-746.

237. Suzuki, T., Nishida, M., Futami, S., Fukino, K., Amaki, T., Aizawa, K., Chiba, S., Hirai, H., Maekawa, K., and Nagai, R. (2003). Neoendothelialization after peripheral blood stem cell transplantation in humans: a case report of a Tokaimura nuclear accident victim. Cardiovasc Res 58, 487-492.

238. Imanishi, D., Miyazaki, Y., Yamasaki, R., Sawayama, Y., Taguchi, J., Tsushima, H., Fukushima, T., Yoshida, S., Sasaki, H., Hata, T*., et al.* (2007). Donor-derived DNA in fingernails among recipients of allogeneic hematopoietic stem-cell transplants. Blood 110, 2231-2234.

239. Pearce, L., Lim, Z.Y., Usai, M., Ho, A.Y., Mufti, G.J., and Pagliuca, A. (2008). Mixed donor chimaerism in recipient fingernails following reduced-intensity conditioning haematopoietic SCT. Bone Marrow Transplant 42, 361-362.

240. Belleudi, F., Purpura, V., Scrofani, C., Persechino, F., Leone, L., and Torrisi, M.R. (2011). Expression and signaling of the tyrosine kinase FGFR2b/KGFR regulates phagocytosis and melanosome uptake in human keratinocytes. Faseb J 25, 170-181.

241. Sharlow, E.R., Paine, C.S., Babiarz, L., Eisinger, M., Shapiro, S., and Seiberg, M. (2000). The protease-activated receptor-2 upregulates keratinocyte phagocytosis. J Cell Sci 113 (Pt 17), 3093-3101.

242. Spitz, J. (2005). Genodermatoses: a clinical guide to genetic skin disorders (New York, Lippincott Williams & Wilkins).

243. Fine, J.D. (2010). Inherited epidermolysis bullosa: past, present, and future. Ann N Y Acad Sci 1194, 213-222.

11. Publication list

Publications related to the present thesis

Nemeth K, Key S, Bottlik G, Masszi T, Mezey E, Karpati S.
Analyses of donor derived keratinocytes in hairy and non-hairy skin biopsies of female patients following allogeneic male bone marrow transplantation.
Stem Cells Dev. 2011 Feb 2. [Epub ahead of print]

IF: 4,14

Bratincsák A, Brownstein MJ, Cassiani-Ingoni R, Pastorino S, Szalayova I, Tóth ZE, Key S, **Németh K**, Pickel J, Mezey E. CD45-positive blood cells give rise to uterine epithelial cells in mice.
Stem Cells. 2007 Nov;25(11):2820-6.

IF: 7,53

Publications not related to the thesis

Brown JM, **Nemeth K**, Kushnir-Sukhov NM, Metcalfe DD, Mezey E.
Bone marrow stromal cells inhibit mast cell functions via a COX2 dependent mechanism
Clin Exp Allergy. 2011Jan 24. [Epub ahead of print]

IF: 4,08

Németh K. , Keane-Myers A. , Brown JM, Metcalfe DD, Gorham, J.D., Bundoc VG, Hodges MG, Jelinek I, Madala S, Karpati S and Mezey E
Bone marrow stromal cells use TGF-β to suppress allergic responses in a mouse model of ragweed-induced asthma
Proc Natl Acad Sci U S A. 2010 Mar 23;107(12):5652-7.

IF: 9,43

Németh K, Mayer B, Mezey E,
Modulation of bone marrow stromal cell functions in infectious diseases by toll-like receptor ligands.
J Mol Med 2010 Jan;88(1):5-10. Epub 2009 Sep 13

IF: 5,00

Németh K, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, Robey PG, Leelahavanichkul K, Koller BH, Brown JM, Hu X, Jelinek I, Star RA, Mezey E.
Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production.
Nature Medicine. 2009 Jan;15(1):42-9.

IF: 27,55

Mezey E, Mayer B, **Németh K**.
Unexpected roles for bone marrow stromal cells (or MSCs): a real promise for cellular, but not replacement, therapy**.**
Oral Dis. 2009 Jul 27. [Epub ahead of print]

IF: 1,92

Toth ZE, Leker RR, Shahar T, Pastorino S, Szalayova I, Asemenew B, Key S, Parmelee A, Mayer B, **Nemeth K**, Bratincsák A, Mezey E.
The combination of granulocyte colony-stimulating factor and stem cell factor significantly increases the number of bone marrow-derived endothelial cells in brains of mice following cerebral ischemia.
Blood. 2008 Jun 15;111(12):5544-52.

IF: 10,43

Centlow M, Carninci P, **Nemeth K**, Mezey E, Brownstein M, Hansson SR.
Placental expression profiling in preeclampsia: local overproduction of hemoglobin may drive pathological changes.
Fertility and Sterility. 2008 Nov;90(5):1834-43.

IF: 4,16

Toth ZE, Shahar T, Leker R, Szalayova I, Bratincsák A, Key S, Lonyai A, **Németh K**, Mezey E.
Sensitive detection of GFP utilizing tyramide signal amplification to overcome gene silencing.
Exp Cell Res. 2007 May 15;313(9):1943-50. Epub 2007 Mar 12

IF: 3,69