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

Introduction

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.

Transdifferentation to 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. 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.

It has been long wondered whether circulating cells contributed to this process in humans. A study group 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 and it was concluded that non-uterine cells contribute to endometrial regeneration. In another study conducted by a different research group very low level of chimerism (<0.01%) was detected 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.

Transdifferentiation to 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.

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. 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.

Further research applying new techniques could help better assess contribution rate of hematopoetic stem cells to both the uterine epithelium and the epidermis.

Specific aims

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.

Methods

Mouse experiments

Bone Marrow Transplantation Studies

Allogenic bone marrow transplantation was used to detect progeny of GFP+ BM-derived cells. Briefly, C57BL/6J mice (n=6) received whole-body irradiation (900 rad) and were transplanted intravenously 2 hours after irradiation with one million bone marrow cells harvested from a GFP+ mouse (C57BL/6-Tg [ACTB-EGFP] 1Osb/J, 003291; Jackson Laboratory, Bar Harbor, ME, http://www. jax.org). At later time points, transplanted animals were sacrificed and tissues harvested as described above.

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. 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, 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 (unpublished observations).

Production of Double-Transgenic Mice

Recombined BAC DNA was microinjected into C57BL/6J zygotes that were implanted into estrogen-primed foster mothers, and trans- genic 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). 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. 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.

Immunohistochemistry

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 Laborato- ries, 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. 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.

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 Maxiscript transcription kit 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).

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 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.

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).

Human Studies

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. 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).

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).

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.

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.

Results

Contribution of bone marrow to the uterus epithelium

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, and the progeny of CD45+cells, such as Kupfer cells and microglia 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 of the CD45-positive white blood cells of double-transgenic mice expressed GFP.

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. 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. These bone marrow-derived GFP+ epithelial cells bound L. tetragonolobus lectin, a marker with affinity for glycoprotein on the luminal surface of epithelial cells. The GFP+ epithelial (i.e., immunopositive for L. tetragonolobus) cells did not express the common leukocyte

antigen CD45, but numerous CD45+/GFP+ cells were detected in the uterine stroma, which is known to harbor CD45+lymphoid cells. Immunostaining using a pan-cytokeratin (cytokeratins [CKs] 8, 18, and 19) antibody also showed colocalization with GFP, further confirming the epithelial character of the GFP- positive cells.

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, and indeed, we saw many CD45/GFP+ cells in the stroma of the transplanted mice and of the CD45/Cre-Z/EG mice. We also found GFP+ cells in the epithelial layer of the endometrium. Although these GFP+cells could be stained with L. tetragonolobus lectin and with anti- keratin-8, -18, and 19 antibody, another marker for epithelial cells, they could not be stained with anti-CD45 antibody. Expression of GFP mRNA in these cells was further confirmed by in situ hybridization histochemistry. Thus, CD45+ cells appear to give rise to epithelial cells in the uterus of nonirradiated transgenic animals.

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. In contrast to humans, the epithelium does not shed, but it exhibits continuous degeneration and regeneration. In the course of the cycle, epithelial cells undergo vacuolar degeneration and apoptosis and are replaced by newly generated cells. 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 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, 0.5% of the epithelial cells in 12-week-old mice and 6% in a 20-week-old animal were GFP+. 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.

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+. 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.

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) and the specificity of the probe is 100% (i.e. no positive nuclei were found in the female patient samples).

In all samples studied, we observed Y chromosome positive nuclei in epithelial layers and hair follicles 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. When Ki67 was added as a nuclear proliferation marker, we observed rare occasional cells that were triple positive (Y chromosome, cytokeratin and Ki67). 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) in all samples examined, 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). 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.

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.

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.

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.

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 can 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).

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, JD,
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