

P280**Progressive decrease in number and change in niche preference of the ABCB5+ mesenchymal stem cell subset in the skin during aging**

B. Meier¹, Y. Ziouta², A. Basu¹, A. Sindrilaru¹, A. Hainzl¹, P. Maity¹, M. Wlaschek¹, C. Ganss², M. H. Frank³, N. Y. Frank^{3,4} and K. Scharffetter-Kochanek¹ ¹Department of Dermatology and Allergic Diseases, University of Ulm, Ulm, Germany; ²TICEBA GmbH, Heidelberg, Germany; ³Children's Hospital Boston, Transplantation Research Center, Boston, USA; ⁴Harvard Medical School, Division of Genetics, Brigham and Women's Hospital, Harvard, USA

Recently, the expression of ABCB5, a novel P-glycoprotein of the ABC superfamily of active transporters, was found in a newly defined mesenchymal stem cell subpopulation in the skin. Even though its decrease in number and/or function may result in impaired regenerative capacity and aging, robust *in vivo* data are currently not available. Here we studied the cell surface expression pattern, the cell number and the specific localization of ABCB5-positive mesenchymal stem cells in human skin in young (0–20 years), middle aged (21–70 years) and old healthy individuals (>71 years). Dermal ABCB5+ cells showed variability for expression of the hematopoietic progenitor cell antigen CD34 or for CD133, indicating that they represent a distinct stem cell subset in human skin. In contrast, ABCB5+ cells expressed a panel of previously established mesenchymal stem cell markers, including CD29, CD90, CD59 and CD44. The specificity of the anti-ABCB5 mAb was confirmed using a competitive 16-mer peptide. Notably, a significant decline of the percentage of ABCB5+ cells was found in the old age group compared to the younger age groups ($P < 0.0001$), while the total number of resident cells in the dermis per high power field remained identical in all age groups. In a first attempt to understand the mechanisms underlying the ABCB5+ stem cell decline in old individuals, we studied the expression of γ H2AX, a phosphorylated histone protein which detects DNA double strand breaks and initiates a DNA damage response with the induction of cell cycle inhibitors like p16. Preliminary data indicate that by contrast to age-dependent increase in γ H2AX and p16 in dermal fibroblast, no consistent age-dependent increase in γ H2AX and p16 was found in ABCB5+ mesenchymal stem cells. Interestingly, ABCB5+ mesenchymal stem cells were found in close association of CD31+ vessels in younger individuals, while this perivascular localisation was lost in the old age group. Collectively, a robust decrease in ABCB5+ mesenchymal stem cells with changes in niche preference may contribute to a reduced regenerative capacity in skin aging.

Progressive Decrease in Number and Change in Niche Preference of the ABCB5⁺ Mesenchymal Stem Cell Subset in the Skin during Aging

Barbara Meier¹, Yvonne Ziouta², Lea Sante¹, A. Basu¹, A. Hainzl¹, A. Sindriluru¹, P. Maity¹, M. Wlaschek¹, Ch. Ganss², J. Fiedler³, G. Ravalli³, M.F. Frank⁴, N.Y. Frank^{4,5} and Karin Scharffetter-Kochanek¹

¹ Department of Dermatology and Allergic Diseases, Ulm University, Ulm, Germany

² TICEBA GmbH, Heidelberg, Germany

³ Department of Orthopedics, University of Ulm, Germany

⁴ Transplant Research Center, Children's Hospital Boston, Harvard Medical School, Boston, USA

⁵ Division of Genetics, Brigham and Women's Hospital, Harvard Medical School, Boston, USA

Background

Recently, the expression of ABCB5, a novel P-glycoprotein of the ABC superfamily of active transporters, was found in a newly defined mesenchymal stem cell (MSC) subpopulation in the skin. Even though this subpopulation is preferentially found in the skin, it occurs also in all connective tissue containing organs. ABCB5⁺ MSCs share many but not all markers of conventional MSCs (see Fig. 1), reveal plastic adherence and differentiation to adipogenic and osteogenic lineages (see Fig. 1). ABCB5 belongs to a drug persistence transporter family, regulates the membrane potential and prevents cell fusion (Frank et al., 2003). ABCB5⁺ malignant melanoma initiating cells have immunomodulatory properties as they express the negative co-stimulator PD-1 and suppress T cell activation and stimulate Foxp3⁺ regulatory T cells (Schatten and Frank, 2009). Interestingly, ABCB5 was identified as a marker of melanoma stem cells (Frank et al., 2005). The decrease in number and/or function of the newly identified ABCB5⁺ MSC subpopulation may result in impaired regenerative capacity and aging. However, robust *in vivo* data are currently not available. Here we studied the cell surface expression pattern, the expression of aging markers, the cell number and specific localization of ABCB5⁺ MSCs in human skin in young (0-20 years), middle-aged (21-70 years) and old healthy individuals (>71 years).

Results

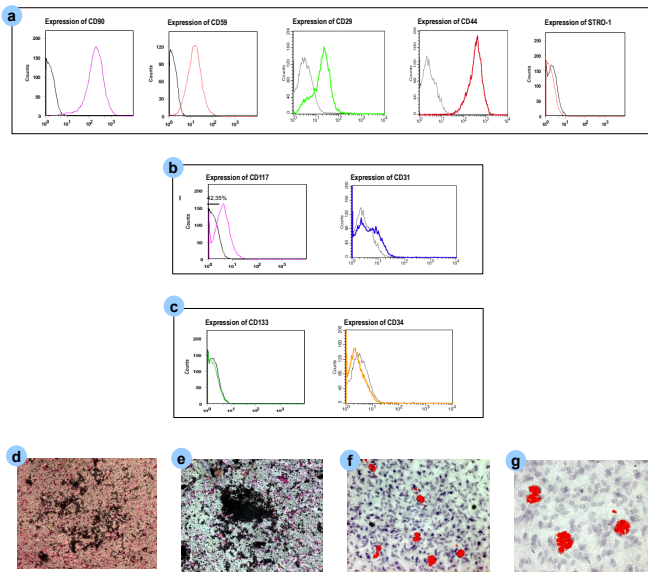


Fig. 1 ABCB5⁺ cells isolated from human skin show characteristics of mesenchymal stem cells
a. Using FACS analysis, markers present on ABCB5⁺ cells (CD90, CD59, CD29, CD44, STRO-1) have earlier been found also on bone marrow derived mesenchymal stem cells (BM-MSCs). b. In addition, ABCB5⁺ cells express CD117 and a very low level of CD31, which are variably expressed on BM-MSCs. c. CD133 and CD34, markers for hematopoietic stem cells, are absent on ABCB5⁺ cells and also on BM-MSCs. d. Following culture of skin-derived ABCB5⁺ cells in an osteoblast-differentiation medium (DMEM) supplemented with 10%FCS, 1% glutamine, ascorbic acid at a concentration of 50µg/ml DMEM, β-glycerolphosphat at a concentration of 0.216 µg/ml DMEM, 100nM Dexamethason, and penicillin and streptomycin at routine concentrations for 4 weeks resulted in osteogenic differentiation. Osteogenic differentiation was studied using von Kossa staining. e. Higher magnification of d. (20x) f. Culture in an adipogenic differentiation medium DMEM supplemented with 0.5 µM dexamethason, 0.5 µM hydrocortisone, 0.5µM lobulylmethylxanthine, 60 µM indomethacin for 6 weeks resulted in adipogenic differentiation. Adipogenic differentiation was monitored by the enrichment of Oil Red staining of lipids in adipocytes. g. Higher magnification of f. (20x).

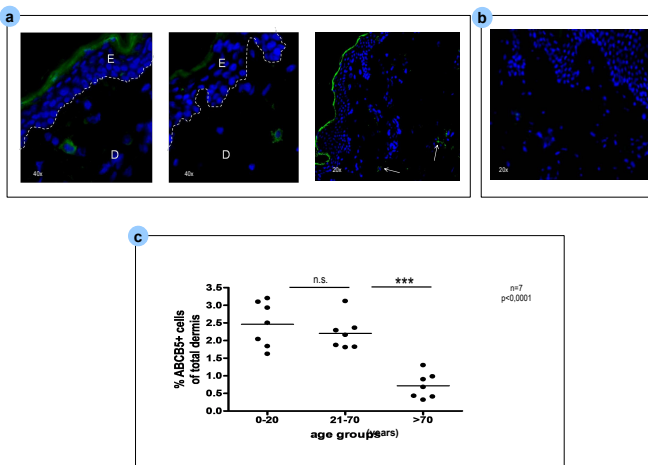


Fig. 2 Significant decrease in number of dermal ABCB5⁺ stem cell subset during aging
a. Immunofluorescence staining of ABCB5⁺ MSCs in the dermal compartment of human skin of a 13 years old individual. b. Using a peptide competition assay (16-mer peptide RFGAYLQAGRMTPG) the immunofluorescence staining was completely inhibited indicating the specificity of the staining. c. Immunofluorescence stainings with an anti-ABCB5 antibody were performed on skin sections of individuals at different age groups (0-20, 21-70, >70 years). ABCB5⁺ cells were counted per total number of dermal cells on 100 high power fields per skin donor for seven individuals per age group in the dermal compartment of the skin. Statistical significance was assessed using the t-test.

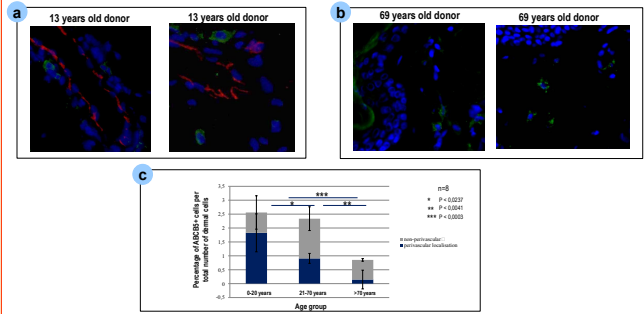


Fig. 3 Change of niche preference of ABCB5⁺ mesenchymal stem cells during life-time
a. A perivascular localization was predominantly found in skin sections of young individuals. Immunofluorescence double staining for ABCB5 (green) and CD31 (red) as an endothelial marker on skin sections of a 13 years old individual. b. Double staining as outlined in a shows that ABCB5⁺ rather occur independent of vessels in a 69 years old individual. ABCB5⁺ cells (green). No red fluorescence (CD31) was found in this skin section. c. The perivascular and non-perivascular localization of skin sections of individuals at different age groups (0-20, 21-70, >70 years) was assessed using double immunofluorescence staining. ABCB5⁺ cells (green) were counted in co-localization to blood vessels (CD31-red immunofluorescence). The percentage of ABCB5⁺ cells per total number of dermal cells were assessed in perivascular and non-perivascular localization. Statistical analysis was done by t-test.

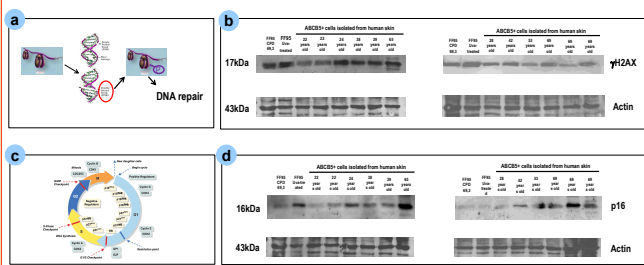


Fig. 4 No consistent age-dependant increase in γH2AX and p16 was found in ABCB5⁺ mesenchymal stem cells.
a. γH2AX represents a phosphorylated histone protein which is a solid *in vivo* aging marker (Keogh et al. 2005)⁴ and is recruited upon phosphorylation to DNA double-strand breaks where it initiates DNA repair. b. ABCB5⁺ mesenchymal stem cells were isolated from the skin of individuals at different ages and Western blot analysis was performed using lysates from ABCB5⁺ cells. γH2AX protein expression was assessed using an antibody against γH2AX. Human dermal fibroblasts (FF95) served as controls. c. p16 induces a permanent growth arrest as it prevents cells from entering the S-phase by inhibition of the cell cycle-dependent kinases CDK4 and 6. p16 has earlier been identified as a solid *in vivo* marker for skin aging (Ressler et al 2005). d. p16 expression in skin-derived ABCB5⁺ mesenchymal stem cells from individuals at different age was assessed by Western blot analysis.

Gene	P-value	Fold up- or down-regulation (69/24 years)	Signaling pathway
Glycosylphosphatidylinositol anchored molecule like protein	0.000050	201.7638	Apoptosis
Tumor protein p73	0.001811	7.5815	Apoptosis
Growth arrest and DNA-damage-inducible gene gamma	0.000791	5.45855	Apoptosis

Table 1. ABCB5⁺ MSCs from old individuals are prone to apoptosis compared to ABCB5⁺ MSCs from young individuals
A DNA Damage PCR Array was performed. cDNA of ABCB5⁺ cells of a 69 and a 24 years old individual was analysed for DNA damage specific genes, including genes for apoptosis, cell cycle, and DNA repair. cDNA of three genes coding for apoptosis were highly increased suggesting that apoptosis of ABCB5⁺ MSCs in older individuals may account for the decrease in ABCB5⁺ cell numbers.

Summary

Dermal ABCB5⁺ mesenchymal stem cells express a panel of previously established mesenchymal stem cell markers, including CD29, CD90, CD59 and CD44, while hematopoietic stem cell markers like CD34 and CD133 are not expressed (Fig. 1). Dermal ABCB5⁺ MSC reveal the capacity to differentiate into adipocytes and osteocytes and reveal plastic adherence. Notably, a significant decline of the percentage of ABCB5⁺ cells was found in the old age group compared to the younger age groups (p<0.0001), while the total number of resident cells in the dermis per high power field remained identical in all age groups. No consistent age-dependant increase in two robust biomarkers for *in vivo* aging γH2AX and p16 was found in ABCB5⁺ MSC, indicating that the DNA damage response pathway with activation of the cell cycle inhibitor p16 is not involved in the reduced numbers of ABCB5⁺ MSC. Microarray PCR analysis identified mainly proteins involved in apoptosis (Glycosylphosphatidylinositol anchored molecule like protein, p73 and Growth arrest and DNA-damage-inducible gene gamma). ABCB5⁺ MSCs were found in close association of CD31⁺ vessels in younger individuals, while this perivascular localization was lost in the old age group.

Conclusions

A progressive decrease in number and change in niche preference of the ABCB5⁺ mesenchymal stem cell subset was observed in skin aging. This reduction in ABCB5⁺ mesenchymal stem cells may rather be due to enhanced apoptosis and not to the DNA damage response pathway which activates the cell cycle inhibitor p16 and results in irreversible growth arrest.

Perspectives

Longterm, the regenerative capacities of ABCB5⁺ mesenchymal stem cells may be successfully exploited for tissue regeneration and difficult-to-treat wounds in the elderly.

References

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