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SONIC HEDGEHOG STIMULATES THE RECRUITMENT OF ENDOTHELIAL PROGENITOR CELLS*

SONIC HEDGEHOG STYMULUJE REKRUTACJĘ ENDOTELIALNYCH KOMÓREK PROGENITOROWYCH

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Abstract

Aim: *The present work focused on the Sonic hedgehog (SHH) gene that stimulates angiogenesis in the skin. It is postulated that endothelial progenitor cells (EPCs) are responsible for skin angiogenesis. The recruitment of endothelial progenitor cells was verified in the mouse skin transfected with the pSHH gene construct using the quantitative PCR method.*

Material and methods: *The tests were performed on male Balb/c mice. The SHH gene preparation was administered intradermally and/or intramuscularly. The assessment of the expression of EPCs angiogenic genes was performed using the qPCR method. The statistical analysis of the selected results was performed using the t-Student test. Differences were considered statistically significant when $p<0.05$.*

Results: *Studies indicate that the SHH gene administered to mouse skin as pSHH, pSHH/PEI/NaCl and pSHH/PEI/H_20 formulations recruits endothelial progenitor cells to the sites of injection. The increased expression of genes specific to endothelial progenitor cells as CD34, CD44, CD133, KDR and others was observed.*

Conclusions: *The sonic hedgehog gene stimulates the recruitment to the skin the cells expressing EPCs markers. The mechanism of proangiogenic activity of SHH is linked to EPCs mobilization. SHH gene seems to be a potential gene-candidate to proangiogenic gene therapy applications.*

Key words: sonic hedgehog, angiogenesis, gene therapy

Streszczenie

Cel: *W pracy zwrócono uwagę na rolę genu Sonic Hodgehog (SHH) w przebiegu angiogenezy skórnej. Zakłada się, iż kluczową rolę w angiogenezie skórnej odgrywają endotelialne komórki progenitorowe (EPCs). W pracy oceniono wpływ genu SHH na mobilizację EPCs.*

Materiał i metody: *Badania wykonano na myszach Balb/c transfekowanych śródskórnie i domięśniowo preparatami pSHH, pSHH/PEI/NaCl and pSHH/PEI/H_20. Ocenę rekrutacji EPCs do miejsc wstrzyknięcia dokonano metodą ilościowego PCR. Wykonano analizę statystyczna wyników. Różnice znamienne statystycznie oznaczano przy $p<0.05$.*

Wyniki: *Badania wykazały, iż zastosowane preparaty SHH przyczyniają się do mobilizacji komórek eksprymujących charakterystyczne dla EPCs markery. Wykazano podwyższoną ekspresję genów CD34, CD44, CD133, KDR.*

Wnioski: *Sonic Hedgehog stymuluje rekrutację EPCs. Mechanizm proangiogennych właściwości SHH związany jest z EPCs. Wydaje się, iż SHH jest genem-kandydatem dla proangiogennej terapii genowej.*

Słowa kluczowe: sonic hedgehog, angiogenesis, gene therapy

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INTRODUCTION

Sonic hedgehog (*SHH*) is a crucial gene in the morphogenesis of various organs such as the limbs, skeleton, lungs, eyes, teeth, skin and hair [1-3]. Preliminary studies also suggest the *SHH* participates in new vessel formation and the mobilization of endothelial progenitor cells (EPCs) from bone marrow to wound healing [5-7]. So far, the well characterized homologue of Hh proteins is SHH. So far SHH is the well- characterized homologue of Hh proteins. It occurs as a precursor protein (45 kD) that cleaves to N-terminal (20 kDa) and C-terminal (25 kDa) fragments. The receptor for SHH is located on targeted cells and is composed of two transmembrane proteins Ptc1 and Smo encoded by Patched and Smoothened genes respectively [8]. The interaction of Hh with Ptc1-Smo results in the Gli family protein activation: Gli1, Gli2, GliI3 (transcriptional Hedgehog signaling mediators) and induces the transcription of Hedgehog target genes [10]. Apart from being activators, Gli2 and Gli3 proteins also act transcription inhibitors.

Hedgehog proteins play a critical role in organ development in embriogenesis and regulate epithelial mesenchymal interactions. It is also known that SHH stimulates direct (interaction with SHH receptors) and indirect mobilization of endothelial progenitor cells to the new vessel formation sites [7]. The indirect effect of SHH is linked to the regulation of proliferation, differentiation, cell migration and intercellular interaction by the expression of proangiogenic genes, such as *VEGF, ANG1, ANG2.*

The PI3K/Akt signaling pathway which is characteristic for endothelial cells is crucial in the recrutment and differentiation of EPCs toward endothelial cells. This pathway is induced by VEGF (the vascular endothelial growth factor) and SDF (the stromal derived factor) [11,12]. It is known that VEGF and SDF interacts with EPCs cell mobilization through activating MMP-9 [15]. Moreover, VEGF is able to regulate the expression of *SDF* and its receptor *CXCR4* [16].

Some studies suggest that the endogenous SHH pathway is physiologically involved in the skin wound healing process. This hypothesis has been approved by *Asai* et al [7] in the wound model of individuals with diabetes when angiogenesis is known to be deficient. *Asahara* et al [17] demonstrated that endothelial progenitor cells derived from bone marrow get to the sites of injury and differentiate into endothelial cells. These cells have a characteristic ability to migrate from bone marrow to peripheral blood and to differentiate into endothelial cells of blood vessels. The presence of surface markers such as CD133, CD34 and KDR is typical only for newborn cells. *Sieveking* et al [18] found that early EPCs are unable to form blood vessels *de novo*, and may not join the newly formed vessels but affect the promotion of angiogenesis by secreting factors that stimulate endothelial cells. The surface marker profile depends on the stage of cell differentiation. The late EPCs show a reduced level of CD34 and the absence of CD133 and are involved in the formation of blood vessels directly through the incorporation. This mechanism is quite complex and consists of several stages: chemotaxis, adhesion, transmigration, invasion and differentiation in endothelial cells [15, 18]. Additionally, the late EPCs acquire the capacity for endocytosis of acLDL as well as CD31 and VE-Kadherin expression on their surface. Thanks to this surface phenotype it is possible to isolate EPCs *in vitro* [19, 20]. It is worth mentioning that the number EPCs depends on age, gender and the health status of the individual and represents a very small percentage of peripheral blood cells – 0.01% down to 0.0001% of blood cells [19, 21].

MATERIAL AND METHODS

DNA plasmid encoding human sonic hedgehogs (pSHH) were constructed by prof. Maciej Małecki from the Cell Biology Department of the Cancer Center in Warsaw. Before that *in vivo* use plasmid was complexed with polyethyleneimine (PEI25 kDa) and prepared in aqueous (pSHH/PEI/H_2O) and sodium chloride (pSHH/PEI/NaCl) solutions. The tests were performed on male Balb/c mice. The gene preparation was administrated intradermally (20-60 μg) and/or intramuscularly (20 μg). All the experiments involved in the project were carried out in accordance with the principles for the care and use of research animals and approved by the Local Ethic Committee for Animal Experiments (Medical University of Warsaw, No. 27/04/08). The statistical analysis of selected results was performed using the *t-Student* test. Differences were considered statistically significant when $p<0.05$.

DNA and RNA isolation, cDNA synthesis

Skin samples were harvested 3, 10 and 20 days after intradermal injection of plasmid pSHH and pulverized in liquid nitrogen (B. Braun Biotech International, Melsungen AG). Total DNA was isolated using the Qiamp DNA mini kit (Qiagen) according to the manufacturer's instructions. Total RNA was extracted by Chomczynski and Sacchi's method and reverse-transcribed with the use of Advantege® RT-for-PCR Kit (Clontech Laboratories). The quality and quantity of nucleic acids (NanoDrop 1000) were determined by optical density measurements at 260 and 280 nm using a spectrophotometer.

The real-time polymerase chain reaction analysis

To determine the number of plasmid copies in the DNA isolated from the skin tissue, the quantitative real time PCR was performed using primers within the *cmv* promoter sequence. The standard curve was derived from the serial 10-fold dilutions of the control plasmid vector containing the *cmv* promoter. The curve was calculated by plotting logarithms (base 10) of concentrations of the control vector against the crossing points. The specificity of qPCR products was confirmed using the melting curve analysis and agarose gel electrophoresis.

The relative level of expression of the selected genes: *SHH, Ang1, Ang2, VEGF, CD133, CD34, CD44, KDR, FLT1, TIE1, TIE2, SDF1, PTCH1* was calculated employing the comparative threshold cycle (C_T) method ($\Delta\Delta C_T$). β-actin was used as the endogenous control gene for the target C_T normalization. The differences of C_T values (ΔC_T) were calculated by taking the mean C_T value from

duplicate reactions and subtracting the mean C_T value of β-actin from duplicate reactions. The fold change in the gene expression in the skin harvested from mice after the pSHH injection relative to control (untreated mice) was calculated using the formula: $2^{-\Delta\Delta CT}$.

Reactions were performed in duplicate on the ABI Prism® 7000 Sequence Detection System (Version 1.1, Applied Biosystems) using the SYBR® Green PCR Master Mix (Applied Biosystems) with the appropriate primers under the following conditions: 10 min at 95°C followed by 40 cycles of amplification (15 s at 95°C and 60 s at 60°C). The optimization of the real-time PCR reaction conditions was performed according to the manufacturer's instructions (PE Applied-Biosystems, User Bulletin 2 applied to the SYBR-Green I core reagent protocol) but scaled down to 25 µl per reaction. Threshold cycle (Ct) values were determined by software (ABI Prism 7000 SDS Software, Version 1.1, Applied Biosystems). Analyses were carried out using standard procedures as described in Applied Biosystems User Bulletin 2.

Standard PCR

The polymerase chain reaction (PCR) was performed with the appropriate primers (under the following conditions: 5 min at 94°C (initial denaturation step) followed by 35 cycles (30 s at 94°C, 30 s at temperature (T) specific for the primers used, 40 s at 72°C), 10 minutes at 72°C (final extension).

RESULTS

The results indicate that both pSHH/NaCl and pSHH/PEI/NaCl injections induce increased EPCs recruitment. The plasmid complexed with PEI significantly enhanced the relative expression of KDR, CD34,CD133 in the skin and muscles as compared with pDNA (Fig. 1, 2). The level of the CD34 marker in skin increased fourfold after the injection of pSHH/PEI/NaCl while the effect of other markers was comparable.

Next, the influence of the pSHH/PEI/$H_2$0 complex on the expression of proangiogenic factors, as well as the mobilization and differentiation of endothelial progenitor cells was investigated. As shown on figure 3 after 3,10 and 20 days of intradermal administration of pSHH/PEI/H_2O the increase of the expression of all endothelial markers has been observed. The expression of CD34 and CD44 maintained at a high level throughout the duration of the experiment in contrast to CD133 and KDR. The expression of CD133 and KDR was comparable after 10 and 20 days.

The copy number of plasmid DNA (pSHH) per 1mg of total DNA in the transfected skin (Fig. 4) was determined as well. A significant increase was observed in the skin from the 3rd day following the injection. Our data suggest that the intradermal route of administration is effective and guarantees a high level of gene expression. Further results (Fig. 5) demonstrate the increase in the expression of all proangiogenic factors and their receptors as compared to control samples. The expression levels of *VEGF, FLT-1 and SDF-1* were comparable 3 days after the injection and reached their maximum after 10 days.

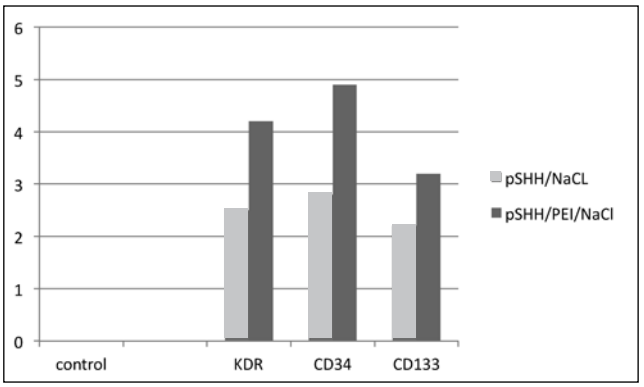

Fig. 2. The comparison of the relative level of EPCs marker (KDR, CD34, CD133) expression in muscle after pSHH/NaCl and pSHH/PEI/NaCl injection.

Ryc. 2. Porównanie poziomu markerów EPCs (KDR, CD34, CD133) w mięśniach po iniekcji pSHH/NaCl i pSHH/PEI/NaCl.

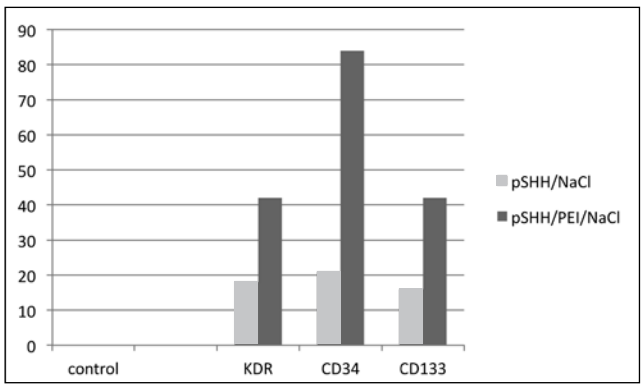

Fig. 1. The comparison of the relative level of EPCs marker (KDR, CD34, CD133) expression in skin after pSHH/NaCl and pSHH/PEI/NaCl injection.

Ryc. 1. Porównanie poziomu markerów EPCs (KDR, CD34, CD133) w skórze po iniekcji pSHH/NaCl i pSHH/PEI/NaCl.

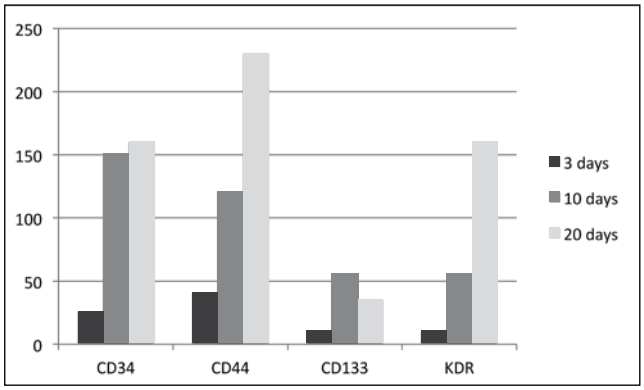

Fig. 3. The comparison of the relative level of EPCs marker (CD34, CD44, CD133, KDR) expression in skin after pSHH/PEI/H2O intradermal injection. Analyses were performed after 3, 10 and 20 days.

Ryc. 3. Porównanie poziomu markerów EPCs (CD34, CD44, CD133, KDR) w skórze po iniekcji pSHH/PEI/H2O. Badania wykonano po 3, 10 i 20 dniach.

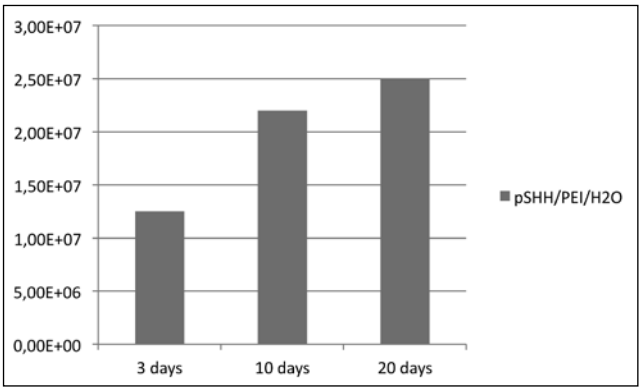

Fig. 4. The copy number of plasmid pSHH in the skin after pSHH/PEI/H2O administration.

Ryc. 4. Liczba kopii plazmidu pSHH w skórze transfekowanej preparatem pSHH/PEI/H2O.

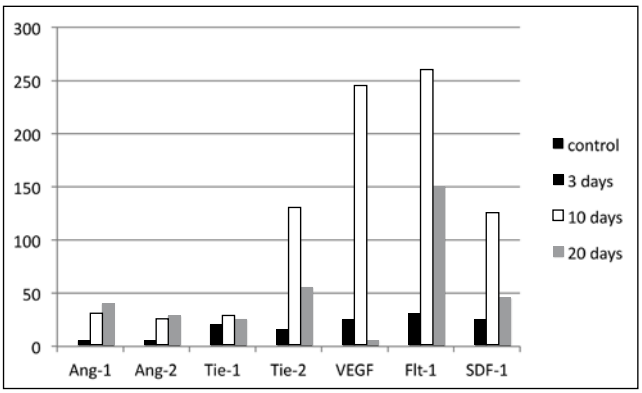

Fig. 5. Mean fold change in proangiogenic cytokine gene expression in mouse skin after intradermal pSHH/PEI/H20 administration.

Ryc. 5. Ekspresja genów cytokin proangiogennych w skórze transfekowanej preparatem pSHH/PEI/H20.

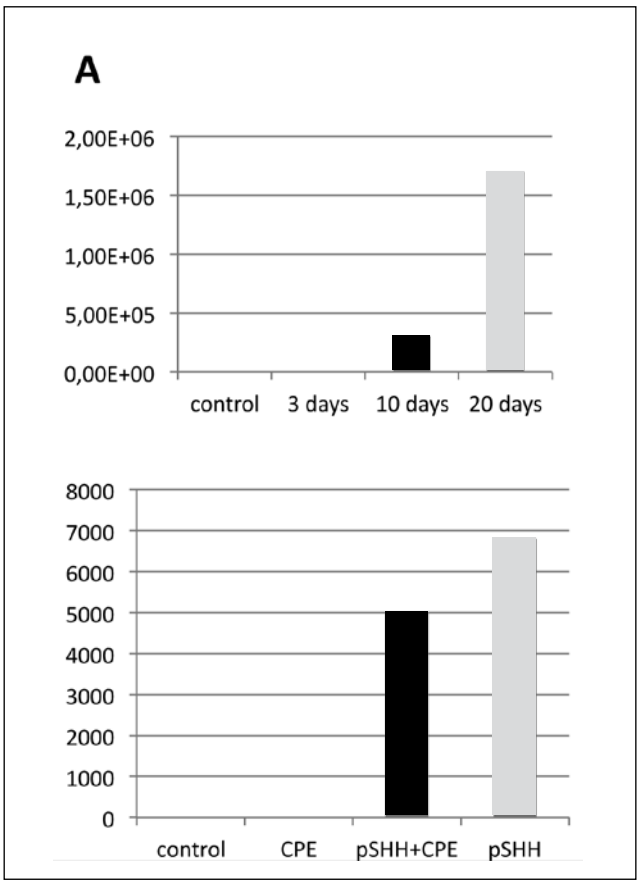

Fig. 6. Mean fold change in *SHH* gene expression 3, 10 and 20 days after intradermal administration of pSHH/PEI/H2O (A) and 3 days after administration of pSHH/PEI/H2O with cyklopamine (CPE) (B).

Ryc. 6. Ekspresja genu SHH w skórze transfekowanej preparatem pSHH/PEI/H2O po 3, 10 i 20 dniach od transfekcji (A) i po 3 dniach pod transfekcji pSHH/PEI/H2O z cyklopaminą (CPE) (B).

The signaling pathway genes, such as SHH, GLI-1 and Ptc-1, play a crucial role in angiogenesis.The literature and experimental data suggest that the expression level of these factors increases following the administration of proangiogenic genes and is decreased by cyclopamine (CPE); CPE inhibits the activity of sonic hedgehog protein. Our experiments confirmed this hypothesis (Fig. 6-8); demonstrating that the pSSH/PEI/H20 complex induces the expression of both *SHH, Gli-1 and Ptc-1*. A significant increase of SHH and Gli-1 expression was observed 20 days post injection. In both cases the CPE aplication reduced the mRNA expression level as soon as day 3.

DISCUSSION AND CONCLUSIONS

Neovascularization is the formation of functional microvascular networks with red blood cell perfusion and is one of the most important processes in the body. It is important to understand this process especially when gene therapy is going to be applied. There are two forms of gene therapy related to angiogenesis: angiogenic and antiangiogenic gene therapy. The former is associated with gene transfer determining the formation of blood vessels, the latter acts via vascularization inhibition or the existing blood vessels inactivation [22]. It is known that EPCs stimulation results in the formation of new blood vessels. *In vitro* studies show that the number of EPCs depends on proangiogenic factors such as VEGF and SHH.

Our preliminary results suggest that Sonic Hedgehog may affect the endothelial progenitor cell mobilization from bone marrow to muscles and skin in Balb/c mice. The intradermal and intramuscular injection of plasmid encoding the *SHH* gene (pSHH) confirmed this hypothesis. The pSHH expression in the skin results in a statistically significant increase of EPCs expression markers as compared with control - from 13 (CD133) up to 23 (CD34) times.

The complexation of DNA with polyethyleneimine (pDNA/PEI) has contributed to increasing the efficiency of gene transfer and a fourfold increase of EPCs as compared to the pDNA. After SHH administration a 2.5-fold increase of EPCs in muscles has been also noted. Our results suggests that the pSHH/PEI complex almost doubles the mobilization efficiency. The proangiogenic properties of SHH have been proved by Asai et al. who discovered the

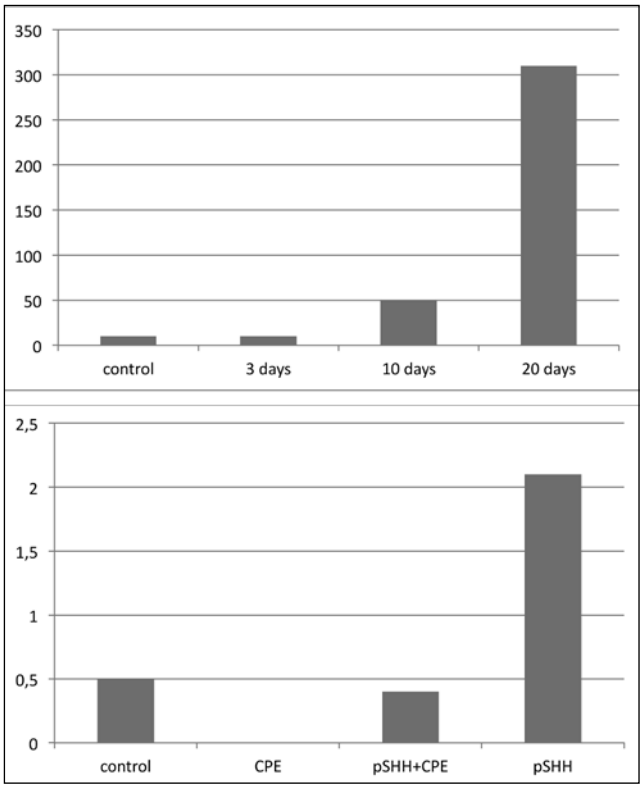

Fig. 7. Mean fold change in *Gli-1* gene expression 3,10 and 20 days after intradermal administration of pSHH/PEI/H2O (A) and 3 days after administration of pSHH/PEI/H2O with cyklopamine (CPE) (B).

Ryc. 7. Ekspresja genu Gli-1 w skórze transfekowanej pSHH/PEI/H2O po 3,10 I 20 dnaich od transfekcji (A) i po 3 dniach od podania pSHH/PEI/H2O z cyklopaminą (B).

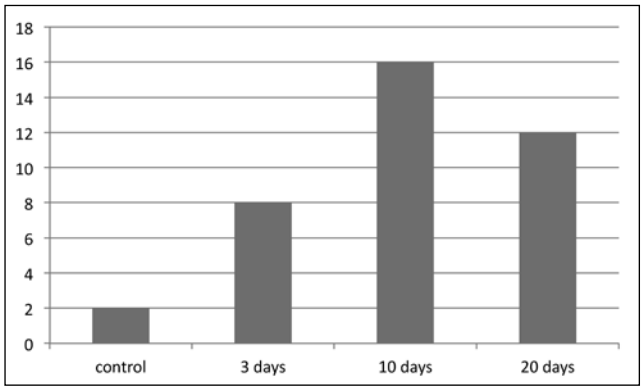

Fig. 8. Mean fold change in *Ptc-1* gene expression 3, 10 and 20 days after intradermal administration of pSHH/PEI/H2O.

Ryc. 8. Ekspresja genu Ptc-1 w skórze transfekowanej pSHH/PEI/H2O po 3, 10 i 20 dniach od podania preparatu.

Shh receptor- PTCH-1 on the EPCs surface and confirmed the Shh effect on EPCs migration and proliferation. Additionally, they demonstrated that Sonic Hedgehog increases the SDF-1 alfa expression and consequently is able to stimulate the hematopoetic cells. [23].

Our study suggests that both intradermal and intramuscular plasmid administration of pSHH and pSHH/PEI contribute to a relative increase in *KDR, CD34, CD133* expression. Moreover, the pSHH/PEI complex shows higher relative expression levels than pSHH in muscles and skin. Additionally, pSHH/PEI demonstrates the highest recruitment of EPCs in the skin tissue. This may be caused by enhanced blood circulation in skin folds which enables faster and more efficient migration of endothelial progenitor cells from blood into the tissue. It is also possible that residual cells present in the tissue contribute to increased number of EPCs in the skin. Data suggest that intradermal pSHH/PEI injection is more suitable than intramuscularadministration, although this route leads to higher Shh protein level.

Both plasmids pSHH/PEI/NaCl and pSHH/PEI/H20 increased the proangiogenic gene expression and level of EPCs markers. Our results show effective skin cell transfection in the mouse model and the potential participation of EPCs in angiogenesis. Furthermore, intradermal SHH administration caused an increase of microvascular networks. Our previous study confirmed this hypothesis [24]. The amount of blood vessels and expression of the *SHH* gene were high following SHH/PEI/H20 injection. The expression of other genes reamined at a comparable level. Moreover, our results confirmed the study of Yamazaki. It was shown that blocking the SSH pathway by the cyclopamine - signaling pathway inhibitor, inhibits the effect of SHH on endothelial progenitor cells [25]. We were able to obtain complete or partial down-regulation of selected gene expression after cyclopamine injection.

REFERENCES

1. *Sanchez-Camacho C., Rodriguez J., Ruiz J.M.*: Trousse F, Bovolenta P. Morphogens as growth cone signaling molecules. Brain Res. Rev., 2005, 49, 242-252.
2. *Nagase T., Nagase M., Machida M., Yamagishi M.*: Hedgehog Signaling. A Biophysical or Biomechanical Modulator in Embryonic Development. Ann. N.Y. Acad. Sci. 2007, 1101, 412-438.
3. *Nanba D., Nakanishi Y., Hieda Y.*: Role of Sonic hedgehog signaling in epithelial and mesenchymal development of hair follicles in an organ culture of embryonic mouse skin. Develop. Growth Differ. 2003, 45, 231-239.
4. *Geng L., Cuneo K.C., Cooper M.K., Wang H., Sekhar K,. Fu A., Hallahan D.E.*: Hedgehog signaling in the murine melanoma microenvironment. Angiogenesis. 2007, 10, 259-267.
5. *Pola R., Ling L.E., Silver M., Corbley M.J., Kearney M., Blake Pepinsky R., Shapiro R., Taylor F.R., Baker D.P., Asahara T., Isner J.M.*: The morphogen Sonic hedgehog is an indirect angiogenic agent upregulating two families of angiogenic growth factors. Nat Med. 2001, Jun; 7 (6), 706-711.
6. *Kusano K.F., Pola R., Murayama T., Curry C., Kawamoto A., Iwakura A., Shintani S., Ii M., Asai J., Tkebuchava T., Thorne T., Takenaka H., Aikawa R., Goukassian D., von Samson P., Hamada H., Yoon Y.S., Silver M., Eaton E., Ma H., Heyd L., Kearney M., Munger W., Porter J.A., Kishore R., Losordo D.W.*: Sonic hedgehog myocardial gene therapy: tissue repair through transient reconstitution of embryonic signaling. Nat Med. 2005, 11 (11), 1197-1204.

7. *Asai J., Takenaka H., Kusano K.F., Ii M., Luedemann C., Curry C., Eaton E., Iwakura A., Tsutsumi Y., Hamada H., Kishimoto S., Thorne T., Kishore R., Losordo D.W.*: Topical sonic hedgehog gene therapy accelerates wound healing in diabetes by enhancing endothelial progenitor cell-mediated microvascular remodeling. Circulation. 2006, 113 (20), 2413-2424.
8. *Chuang P., McMahon A.P.*: Vertebrate hedgehog signaling modulated by induction of a hedgehog-binding protein. Nature 1999, 397, 617-621.
9. *Athar M., Tang X., Lee J.L., Kopelovich L., Kim A.L.*: Hedgehog signaling in skin development and cancer Exp. Dermatol., 2006 Sep; 15(9), 667-677.
10. *Tabin C.J., McMahon A.P.*: Recent advances in hedgehog signaling. Trends Cell Biol. 1997, 7, 442-445.
11. *Tang J., Wang J., Kong X., Yang J., Guo L., Zheng F., Zhang L., Huang Y., Wan Y.*: Vascular endothelial growth factor promotes cardiac stem cell migration via the PI3K/Akt pathway. Exp. Cell Res., 2009, 315 (20), 3521-3531.
12. *Zheng H., Fu G., Dai T., Huang H.*: Migration of endothelial progenitor cells mediated by stromal cell-derived factor-1alpha/CXCR4 via PI3K/Akt/eNOS signal transduction pathway. J. Cardiovasc. Pharmacol., 2007, 50 (3), 274-280.
13. *Fu J.R., Liu W.L., Zhou J.F., Sun H.Y., Xu H.Z., Luo L., Zhang H., Zhou Y.F.*: Sonic hedgehog protein promotes bone marrow-derived endothelial progenitor cell proliferation, migration and VEGF production via PI 3-kinase/Akt signaling pathways. Acta Pharmacol. Sin., 2006, 27 (6), 685-693.
14. *Xuan Y.H., Jung H.S., Choi Y.L. et al.*: Enhanced expession of hedgehog signaling molecules in squamosus carcinoma of uterine cervix and its precursor lesions. Modern. Pathol., 2006, 19, 1139-1147.
15. *Dome B., Dobos J., Tovari J., Paku S., Kovacs G., Ostoros G. et al.*: Circulating bone marrow-derived endothelial progenitor cells: characterization, mobilization, and therapeutic considerations in malignant disease. Cytometry A 2008, 73(3), 186-193.
16. Kryczek I., Lange A., Mottram P., Alvarez X., Cheng P., Hogan M. et al.: CXCL12 and vascular endothelial growth factor synergistically induce neoangiogenesis in human ovarian cancers. Cancer Res., 2005, 65(2), 465-472.
17. *Asahara T., Murohara T., Sullivan A., Silver M., van der Zee R., Li T. et al.*: Isolation of putative progenitor endothelial cells for angiogenesis. Science, 1997, 275(5302), 964-967.
18. *Sieveking D.P., Buckle A., Celermajer D.S., Ng M.K.*: Strikingly different angiogenic properties of endothelial progenitor cell subpopulations: insights from a novel human angiogenesis assay. J. Am. Coll. Cardiol., 2008, 51(6), 660-668.
19. *Gross P., Herbrig K.*: Role of endothelial progenitor cells in cardiovascular pathology. Rocz. Akad. Med. Bialymst 2004, 49, 174-177.
20. *Loomans C.J., Wan H., de Crom R., van Haperen R., de Boer H.C., Leenen P.J. et al.*: Angiogenic murine endothelial progenitor cells are derived from a myeloid bone marrow fraction and can be identified by endothelial NO synthase expression. Arterioscler. Thromb. Vasc. Biol., 2006, 26(8), 1760-1767.
21. *Ensley A.E.*: Functional evaluation of circulating endothelial progenitor cells for vascular tissue engineering. Doctor thesis, Georgia Institute of Technology, 2006.
22. *Malecki M., Kolsut P., Proczka R.*: Angiogenic and antiangiogenic gene therapy. Gene Ther., 2005, 12 Suppl 1, S159-169.
23. *Asai J., Takenaka H., Kusano K.F., Ii M., Luedemann C., Curry C. et al.*: Topical sonic hedgehog gene therapy accelerates wound healing in diabetes by enhancing endothelial progenitor cell-mediated microvascular remodeling. Circulation 2006, 113(20), 2413-2424.
24. *Małecki M., Podolska K.*: Preparaty genowe kodujące czynniki angiogenne efektywnie stymulują neowaskularyzację w skórze oraz wzrost włosów. Postępy dermatologii i alergologii, 2008. 25(5), 199-205.
25. *Yamazaki M., Nakamura K., Mizukami Y., Ii M., Sasajima J., Sugiyama Y.*: Sonic hedgehog derived from human pancreatic cancer cells augments angiogenic function of endothelial progenitor cells. Cancer Sci., 2008, 99(6), 1131-1138.

Authors' contributions/Wkład Autorów
According to the order of the Authorship/Według kolejności

Conflicts of interest/Konflikt interesu
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