Arabidopsis Oil Body-Expressed Oleosin-rhFGF5 Inhibits Hair Growth in Mouse

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Research Article

Keywords: Oil body, Oleosin-rhFGF5, Arabidopsis, Hair growth

Posted Date: February 8th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1280906/v1

Abstract

As a member of the fibroblast growth factor family, FGF5 can regulate hair growth, promote injury repair and the development of the body. The expression of exogenous recombinant human FGF5 protein in Arabidopsis by transgenic technology is of great significance to explore its potential mechanism of action and clinical application in the future. We constructed the pOTB-rhFGF5 oil body fusion expression vector and transformed into Arabidopsis. The transgenic Arabidopsis was identified by PCR, SDS-PAGE and Western blot. The pharmacological activity of the recombinant oleosin-rhFGF5 protein in hair growth inhibition was tested *in vitro* as well as *in vivo*. PCR assay, SDS-PAGE and western blot showed that the recombinant human *FGF5* gene was stably inherited and expressed in Arabidopsis, and the target protein size was 47 kDa. Moreover, our experiments showed that the 12.5 ng/mL oleosin-rhFGF5 began to inhibit the proliferation of hair follicle epithelial cell, the inhibition effect was proportional to the dose. *In vivo* activity studies also showed that the oleosin-rhFGF5 had the activity of inhibiting hair regeneration. We successfully transformed the recombinant human *FGF5* gene into Arabidopsis and expressed the target protein olesoin-rhFGF5 in Arabidopsis. The recombinant protein olesoin-rhFGF5 has hair regeneration inhibition activity both *in vivo* and *vitro*.

1. Introduction

Fibroblast growth factor 5 (FGF5) is a member of the fibroblast growth factor family. Its expression in various tissues can lead to different biological effects on cells[1]. In the early stage of embryonic development, FGF5 expression promotes embryonic development, but with the growth and development of the body, FGF5 expression gradually decreases and only expresses in specific tissues[2]. FGF5 can promote the regeneration of endothelial cells and accelerate the repair of vascular injury; overexpression of FGF5 may raise blood pressure, but its mechanism is not clear[3]. In breast cancer patients, FGF5 overexpression in cancer cells can promote the proliferation of cancer cells and aggravate the disease[4]. FGF5 is also an important hair growth regulator. In the process of hair growth cycle, FGF5 can promote the growth of hair from the growth stage to the regression stage and inhibit the growth of hair[5]. FGF5 knockout mice show excessive growth of hair[6]. Therefore, FGF5 has the biological functions of embryo development, cell proliferation, tissue repair, hair growth inhibition and so on. A safe and efficient method is to produce recombinant human FGF5 protein in vitro, which is the great significance to study the function of FGF5.

The plant oil body fusion expression system is quite efficient for expressing foreign protein. The coding gene of the target protein is linked downstream of the oleosin gene, and oleosin promoter drives the expression of the target protein gene together with oleosin gene in the oil body surface of the transgenic plant[7]. Comparing with the prokaryotic expression system, the oil body fusion expression system has the characteristics of high expression and easy separation, it can reduce the cost of protein separation and purification. Moreover, as a higher eukaryote, the plant has a complete protein expression and modification system, which makes the expressed protein further to modify in the cell. Thus, the plant expressed protein structure is closer to the nature protein and the protein could retain better biological

activity[8]. The specific expression of oleosin-rhFGF10 protein in safflower can not only promote the transdermal absorption of protein drugs, but also significantly improve the efficacy of the interest protein[9]. Recombinant oleosin-aFGF proteins expression *Arabidopsis thaliana* was found to heal the wound better than the aFGF expression in prokaryote[10]. Therefore, this study will use the plant oil body fusion expression system to express the recombinant human FGF5 protein and explore the biological activity and related advantages of the fusion protein through *in vitro* and *in vivo* experiments.

2. Materials And Methods

2.1 pOTB-rhFGF5 expression vector

The cDNA gene sequence of human FGF5 was retrieved from NCBI (Gene ID: 2250). Sangon Biological Company optimized the sequence and synthesized the target gene, adding Nco I and Hind III to its ends. The target gene was digested by Nco I and Hind III to obtain the fragment with sticky end. The target gene ligated with pOTB using T_4 ligase 4℃. Finally, the pOTB-rhFGF5 expression vector was successfully constructed (Figure 1). The expression cassette pOTB-rhFGF5 was transformed into E. coli *DH5α* strain. After sequence validation it was transformed from *DH5α* into *Agrobacterium tumefacians* EHA105 cells, and positive clones were screened using PCR.

2.2 Agrobacterium-mediated Arabidopsis transformation

Arabidopsis plants were transformed using floral dip method. In brief, EHA105 cells were cultured in 1L YEP medium, which was then centrifuged at 5000 rpm to precipitate cells. The cells were dissolved in floral dip solution to make the OD 0.8 at 600 nm. Arabidopsis inflorescences were soaked in the infection solution for 5 min and the treated plants were bagged and cultured in wet and dark conditions for 24 hours. The plants were then uncovered and allowed to grow until seed set. The seeds were collected to get T2 and then T3 plants.

2.3 Screening and identification of transgenic Arabidopsis

T1 Arabidopsis seeds were sown to get 4-6 leaves seedlings, which were sprayed with 0.1% glyphosate solution for preliminary screening of transgenic ones. Few leaves from glyphosate-survived Arabidopsis were used to extract genomic DNA which was used as template with forward primer: CATATGCACGGGGAGA and reserve primer: AAGCTTATCCAAAGCG for PCR verification of transgenic lines. Finally, the seeds of transgenic Arabidopsis were harvested. To get oil bodies, 0.05 g of transgenic Arabidopsis seeds were grinded in 1 mL PBS. The solution was centrifuged at 12000 rpm for 5 min to suspended Arabidopsis oil body protein. After heat denaturation of protein, Western blot was used to identify the expression of the target protein. Thus, the T2 transgenic Arabidopsis was determined at gene as well as protein level. The above steps were repeated to obtain T3 generation transgenic Arabidopsis seeds, which were used for studying oleosin-rhFGF5 protein activity.

2.4 Cell proliferation experiment

The skin of tentacles were obtained from C57BL/6 suckling mice under aseptic condition, and the hair follicles of growing period were extracted from the dermis. The hair follicle tissue was digested by 0.1% type I collagenase at 37℃ and 5% CO_2 for 15 min. Subsequently, the tissue was digested by 0.25% trypsin for 15 min after the removal of type I collagenase, and the digestion was terminated by adding an appropriate amount of high sugar DMEM medium containing 10% FBS. The cells were filtered through a 100-mesh cell sieve and centrifuged at 1000 r/min for 10 min. 5×10^4 cells/mL were inoculated into the culture dishes pretreated with type I collagen, and the K-SFM medium containing 10% FBS, 100 U/mL ampicillin and 100 U/mL streptomycin. The cells were subcultured for later experimental study. The cells were cultured up to 6 generations, and randomly divided into control, WT, rhFGF5 and oleosin-rhFGF5 groups. The number of cells in each group was 2×10^3 cells/well, and each group was repeated three times. The cells were starved in K-SFM culture medium containing 0.1% FBS for 24 hours. On the second day, the control group was treated with 0.01 M PBS, while the WT, rhFGF5 and oleosin-rhFGF5 groups were treated with 0, 6.25, 12.5, 25, 50 and 100 ng/mL wild-type oil body protein, rhFGF5 protein and oleosin-rhFGF5 protein, respectively. They were cultured for 12 h at 37℃, 5% CO_2, and 20 μL (5 mg/L) MTT solution was added into each well, incubated for 4 h at 37℃, and the medium and MTT solution were discarded, followed by adding 100 μL DMSO solution and incubation at room temperature for 10 min. The absorption value was measured at 490 nm wavelength.

2.5 Effect of oleosin-rhFGF5 on hair growth of mice

C57BL/6 male mice aged 6 weeks and weighing about 20 g were randomly divided into 5 groups as follow: control group, WT group, oleosin-rhFGF5 group, rhFGF5 group and Hair removal cream group, with 6 mice in each group. Before the experiment, mice were anesthetized with isoflurane, back hair shaved, evenly applied hair removal cream on the back, and depilated hair to form a 2 cm×3cm administration area, resulting in back hair loss model. The control group was treated with 200 μL 0.01 M PBS, the WT group with 200 μL wild type Arabidopsis oil body protein containing 3.1 mg oleosin, the hair removal cream group was treated with hair removal cream, the rhFGF5 group was treated with 200 μL solution containing 5 μg rhFGF5 protein, the oleosin-rhFGF5 group was treated with 200 μL 3.1 mg transgenic Arabidopsis oil body protein, and the mixed oil body protein contained 5 μg oleosin-rhFGF5 protein. The frequency of administration was once a day, and the treatment period was 15 days. At the end of the treatment, the mice were killed by dislocation of spine, and the back skin of the mice was analyzed by histopathology.

2.6 H&E staining analysis

The back skin of mice was fixed with 4% paraformaldehyde overnight. 5 μm thick sections of dehydrated transparent skin were paraffin embedded for pathological analysis. After the sections were dewaxed and hydrated, they were stained with H&E staining kit. The shape, number and hair regeneration of hair follicles were observed under optical microscope.

2.7 Immunohistochemical staining

The sections were soaked into the sodium citrate solution of 0.01 M with pH 6.0 and heated under high temperature and pressure for 5 min to repair the tissue antigens. The solution containing 3% H_2O_2 and 80% methanol added to the sections for 15 min to inactivate endogenous horseradish peroxidase (HRP). The sections were then treated with 1% Triton in PBS. At room temperature, 5% BSA was used to block the sections for 1 h and incubated overnight at 4 ℃with polyclonal rabbit anti-cytokeratin 14 (Bioss, China, bsm-52054R, 1:200). The goat anti rabbit HRP was incubated at room temperature for 1 h. DAB kit was used to stain the positive protein, and the sections were observed under optical microscope.

2.8 Western blot experiment

Total protein from the transgenic Arabidopsis and skin tissues were extracted, followed by quantification by BCA protein quantitative method. The sample amount of each protein was 30 μg. The protein was separated by 12% SDS-PAGE, transferred to PVDF membrane and blocked by 5% skimmed milk powder. The first antibody rabbit polyclonal anti β-actin (Bioss, China, bs-0061R, 1:5000), rabbit polyclonal anti FGF5 (Bioss, China, bs-1257R, 1:1000), rabbit polyclonal cytokeratin 14 (Bioss, China, bsm-52054, 1:1000) were incubated overnight at 4℃ followed by incubation with the second antibody goat anti rabbit at room temperature for 2 h. The target bands were visualized using electrochemiluminescence, and the relative protein expression was quantitatively analyzed by Image J software.

3. Result

3.1 Recombinant oleosin-rhFGF5 was successfully expressed in Arabidopsis

The 0.1% glyphosate screening initially confirmed that the foreign gene may be successfully transferred into Arabidopsis. Moreover, a gene-specific PCR further verified transgenic Arabidopsis by showing the target band of expected length of about 500bp, for which WT Arabidopsis was negative (Figure 2a). The oil body protein was extracted from Transgenic Arabidopsis seeds and an SDS-PAGE and Western blot analysis also proved that the target protein was about 47kDa, matching the expected size (Figure 2b, 2c). Therefore, through the identification of genome and protein of transgenic Arabidopsis, we finally obtained T3 transgenic Arabidopsis.

3.2 Recombinant oleosin-rhFGF5 showed inhibitory effect on the proliferation of hair follicle epithelial cells in vitro

The growth cycle of hair can be divided into growth period, regression period and rest period. When hair enters the growth stage, hair follicle epithelial cells proliferate and differentiate rapidly under the regulation of various growth factors to form hair fibers, which is the basis of hair regeneration. Our experiment showed that there was no significant change in the number of hair follicle epithelial cells in four groups before administration of 6.25 ng/mL protein. However, in rhFGF-5 and oleosin-rhFGF5 groups, the administration of 12.50 ng/mL protein inhibited the proliferation of hair follicle epithelial cells exhibiting significant difference as compared with the control and WT groups. The inhibition of hair

follicle epithelial cells proliferation gradually increased with the increase of protein dose, which was in direct proportion to the dose. Moreover, the inhibition of hair follicle epithelial cells in oleosin-rhFGF5 group was weaker than the rhFGF5 group (Figure 3). The above study confirmed that oleosin-rhFGF5 can inhibit the proliferation of hair follicle epithelial cells *in vitro*.

3.3 Oleosin-rhFGF5 inhibits hair regeneration in mice in vivo

The *in vivo* activity analysis of oleosin-rhFGF5 revealed that in the control and WT groups treated with PBS and wild-type Arabidopsis oil body protein, the hair on the back of the mice began to regenerate, which gradually turned black in 10 days, indicating that the subcutaneous tissue had formed new hair. Until day 15, the black area on the back of the mice increased, and a little new hair appeared, however, the length and density of regeneration hair were lower than before. Moreover, we found that WT group had better effect on promoting hair regeneration than control group. In hair removal cream group, rhFGF5 group and oleosin-rhFGF5 group, the hair regeneration on the back of mice was significantly inhibited. On the 10th day, the hair regeneration on the back of mice in hair removal cream and rhFGF5 groups was negligible, however, the hair growth inhibition in oleosin-rhFGF5 group was weaker as the back was slightly blackened. On the 15th day of administration, the rhFGF5 group had no obvious hair regeneration and thus no blackness on the mice back. However, the hair removal cream group and oleosin-rhFGF5 group, we observed slight blackening on mice back, indicating some hair regeneration under subcutaneous, though the hair regeneration in these two groups was weaker than control group and WT group (Figure 4a).

In addition, we studied histopathology of the hair growth inhibition activity of oleosin-rhFGF5. H&E staining analysis showed that on the 10th day, there were more hair follicles in the subcutaneous tissue of control and WT groups, and the black dots existed in the hair follicles, indicating that there was a small amount of hair regeneration in the hair follicles. On the contrary, in rhFGF5, oleosin-rhFGF5 and hair removal cream groups, the hair regeneration was weaker, lacking black dots in the subcutaneous hair follicles, and the number of subcutaneous hair follicles in these three groups were decreased. Meanwhile, compared with the control and WT groups, the hair follicle volume showed a significant trend of atrophy in rhFGF5 and hair removal cream group, but the atrophy was not obvious in the oleosin-rhFGF5 group. On the 15th day of the treatment, new hair in the subcutaneous hair follicles of the control and WT groups were further elongated, and the volume of the hair follicles was increased compared with the 10th day, especially in the WT group, completely new hair under the skin could be observed. In rhFGF5, the oleosin-rhFGF5 and the hair removal cream groups, the hair growth was still inhibited, and there was no obvious new hair in the hair follicles, especially in the hair removal cream group. The atrophy of hair follicles in hair removal cream group was more serious than rhFGF5 and oleosin-rhFGF5 groups. However, oleosin-rhFGF5 group had more hair follicles and larger hair follicle volume than rhFGF5 group (Figure 4b). Our *in vivo* study showed that oleosin-rhFGF5 had the activity of inhibiting hair growth, however, weaker than rhFGF5.

3.4 The effect of oleosin-rhFGF5 on the expression of cytokeratin 14

The expression of cytokeratin 14 is an important index of hair regeneration. Immunohistochemistry analysis showed that on the 10th day (Figure 5a), the positive staining rate of cytokeratin 14 in control and WT groups was significantly higher than rhFGF5, oleosin-rhFGF5 and hair removal cream groups. The protein was mainly expressed near the hair follicle, the expression of cytokeratin 14 in WT group was higher than control group. Similarly, the expression of cytokeratin 14 in oleosin-rhFGF5 group was higher than rhFGF5 and hair removal cream groups. On the 15th day of administration, there was no significant difference in the expression of cytokeratin 14 between the control and WT groups, but was higher than rhFGF5, oleosin-rhFGF5 and hair removal cream groups. Meanwhile, the expression of cytokeratin 14 in rhFGF5, oleosin-rhFGF5 and hair retardant cream groups on 15th day was higher than the 10th day, but it was highest in oleosin-rhFGF5 among these three groups. Quantitative analysis of cytokeratin 14 expression by Western blot assay showed that it had the same trends as observed in immunohistochemistry (Figure 5b, 5d). The above results proved that oleosin-rhFGF5 can inhibit hair growth from the perspective of molecular pharmacology.

4. Discussion

In this study, oleosin-rhFGF5 protein was successfully expressed in Arabidopsis seeds by oil body fusion expression system, and it's *in vitro* and *in vivo* activities were explored. The results showed that oleosin-rhFGF5 could inhibit hair follicle cell proliferation and reduce hair regeneration, which fully confirmed the possibility of using plant bioreactor to express active oleosin-rhFGF5 protein. The growth factor protein itself has the disadvantages of poor stability and easy to be hydrolyzed by the related protease *in vivo*. Thus, when the growth factor proteins were produced, they must be stored at low temperature to slow down the degradation of protein. Therefore, it also needs some economic investment in the subsequent storage of protein. However, the fusion protein is expressed in plant seeds by oil body fusion expression technology, as the plant seeds lose a lot of water when matured, this can weaken the hydrolysis of oil body protein by related enzymes in the seeds. At the same time, the *in vitro* stability research report of the fusion protein proves that the fusion protein has better thermal stability and anti-protease hydrolysis ability. Thus, thus the fusion protein was easier to store for a long time[9]. In addition, prokaryotic expression of the target protein may lead to a certain amount of endotoxin in the purified protein, which is an important factor affecting the safety of protein drugs[11], but the expression in plants can well avoid the hidden danger of endotoxin caused by prokaryotic expression. However, plants are eukaryote with a higher protein post-processing and modification system, it can further modify protein structure, affecting the efficacy of protein drugs. On the other hand, the oleosin-rhFGF5 protein expressed by plant oil body fusion was easier to be separated and purified than the prokaryotic expression protein. Under the influence of the hydrophobic structure of oil body, only the broken seeds need to be separated by simple centrifugation, and the mixture containing oleosin protein and oleosin-rhFGF5 protein can be obtained.

In this study, we have confirmed that the mixture can inhibit hair regeneration through *in vivo* and *in vitro*. However, in the *in vivo* study, we found that oleosin-rhFGF5 treatment group was weaker than rhFGF5 group in inhibiting hair growth, just as on the 10th day of hair regeneration, oleosin-rhFGF5 group showed a small range of blackening phenomenon on the back of mice, while WT group was also better than

control group, so we speculated that WT type oil body and oil body protein mixture had a certain degree of hair growth stimulating effect. In addition, the oleosin-rhFGF5 showed weak inhibition of hair regeneration. Interestingly, through the analysis of tissue H&E staining, we observed that the hair follicles atrophied with the prolongation of the administration until to the15th day in the rhFGF5 and hair removal cream groups. On the other hand, in the oleosin-rhFGF5 group, not only the growth of hair was inhibited, but also the damage of hair follicles was significantly weaker, as the volume of hair follicle in subcutaneous tissue did not shrink significantly on the 15th day. This might be an advantage of rhFGF5 genetically modified oil body protein, for example, some head surgeries need to temporarily inhibit the hair growth on the diseased skin, in parallel with avoiding affecting hair recovery of the treated site after surgery. Thus the application of genetically modified oil body protein may be a good choice.

However, as an exogenous protein production plant, plant bioreactor itself has some shortcomings. Although the recombinant oleosin-rhFGF5 protein was successfully expressed in Arabidopsis, however, through the quantitative analysis of ELISA experiment we found that the expression of the target protein was only about 0.19%, and we still need to obtain a higher expression level of transgenic Arabidopsis through continuous strain screening. On the other hand, Arabidopsis itself has less oil body protein than other oil crops, so it is more suitable to be a tool to study the expression of exogenous protein in plants. Perhaps the expression of the target protein in plants with higher seed oil content can increase the yield of the target protein. Therefore, this study generally confirmed the possibility of rhFGF5 gene expression in plants and proved the hair regeneration inhibition activity of oleosin-rhFGF5 protein *in vitro* and *in vivo*.

Abbreviations

fibroblast growth factor, FGF; recombinant human Fibroblast growth factor, rhFGF5; polymerase chain reaction, PCR; sodium dodecyl sulfate polyacrylamide gel electrophoresis, SDS-PAGE; acid fibroblast growth factor, aFGF; fetal bovine serum, FBS; phosphate buffered saline, PBS; hematoxylin and eosin, H&E; horseradish peroxidase, HRP; 3,3N-Diaminobenzidine Tertrahydrochloride, DAB; wild type, WT; enzyme linked immunosorbent assay, ELISA.

Declarations

Author contribution

Hongyu Wang mainly carried out the experimental work and written the paper. Muhammad Noman contributed to the English editor of the article.

Funding

This work was supported by State Key Research and Development Project (2017YFD0501000).

Conflict of interest

The authors declare that they have no conflict of interests.

Ethics approval

All procedures performed in studies involving animal participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The study approved by the Laboratory Animal Welfare and Ethics Committee of Jilin Agricultural University (No.20201204001).

Informed consent

The authors declare that this research does not involve any surveys or participants in any capacity.

References

1. Allerstorfer, S, G Sonvilla, H Fischer, S Spiegl-Kreinecker, C Gauglhofer, U Setinek, T Czech, C Marosi, J BuchroithnerJ Pichler (2008) FGF5 as an oncogenic factor in human glioblastoma multiforme: autocrine and paracrine activities. Oncogene, 27: 4180-4190. https://10.1038/onc.2008.61.
2. Clase, K L, P J Mitchell, P J Ward, C M Dorman, S E JohnsonK Hannon (2000) FGF5 stimulates expansion of connective tissue fibroblasts and inhibits skeletal muscle development in the limb. Developmental dynamics: an official publication of the American Association of Anatomists, 219: 368-380. https://10.1002/1097-0177(2000)9999:9999<::AID-DVDY1056>3.0.CO;2-8.
3. Ren, Y, X JiaoL Zhang (2018) Expression level of fibroblast growth factor 5 (FGF5) in the peripheral blood of primary hypertension and its clinical significance. Saudi journal of biological sciences, 25: 469-473. https://10.1016/j.sjbs.2017.11.043.
4. Huang, Y, H WangY Yang (2018) Expression of fibroblast growth factor 5 (FGF5) and its influence on survival of breast cancer patients. Medical science monitor: international medical journal of experimental and clinical research, 24: 3524-3530. https://10.12659/MSM.907798.
5. Higgins, C A, L Petukhova, S Harel, Y Y Ho, E Drill, L Shapiro, M WajidA M Christiano (2014) FGF5 is a crucial regulator of hair length in humans. Proceedings of the National Academy of Sciences, 111: 10648-10653. https://10.1073/pnas.1402862111.
6. Hébert, J M, T Rosenquist, J GötzG R Martin (1994) FGF5 as a regulator of the hair growth cycle: evidence from targeted and spontaneous mutations. Cell, 78: 1017-1025. https://10.1016/0092-8674(94)90276-3.
7. Liu, X, Y Chen, X Wu, H Li, C Jiang, H Tian, L Tang, D Wang, T YuX Li (2012) SUMO fusion system facilitates soluble expression and high production of bioactive human fibroblast growth factor 23 (FGF23). Applied microbiology and biotechnology, 96: 103-111. https://10.1007/s00253-011-3864-4.

8. Ling, H (2007) Oleosin fusion expression systems for the production of recombinant proteins. Biologia, 62: 119-123. https://10.2478/s11756-007-0041-4.
9. Li, W, Y Jing, J Cai, H Wang, H Tian, H Jian, W Qiang, L Zhang, H LiX Li (2017) Oil Body-Bound Oleosin-rhFGF-10: A Novel Drug Delivery System that Improves Skin Penetration to Accelerate Wound Healing and Hair Growth in Mice. International Journal of Molecular Sciences, 18: 2177-2195. https://10.3390/ijms18102177.
10. Yang, J, L Guan, Y Guo, L Du, F Wang, Y Wang, L Zhen, Q Wang, D ZouW Chen (2015) Expression of biologically recombinant human acidic fibroblast growth factor in Arabidopsis thaliana seeds via oleosin fusion technology. Gene, 566: 89-94. https://10.1016/j.gene.2015.04.036.
11. Levarski, Z, A eoltysová, J Krahulec, S StuchlíkJ Turňa (2014) High-level expression and purification of recombinant human growth hormone produced in soluble form in Escherichia coli. Protein Expression & Purification, 100: 40-47. https://10.1016/j.pep.2014.05.003.

Figures

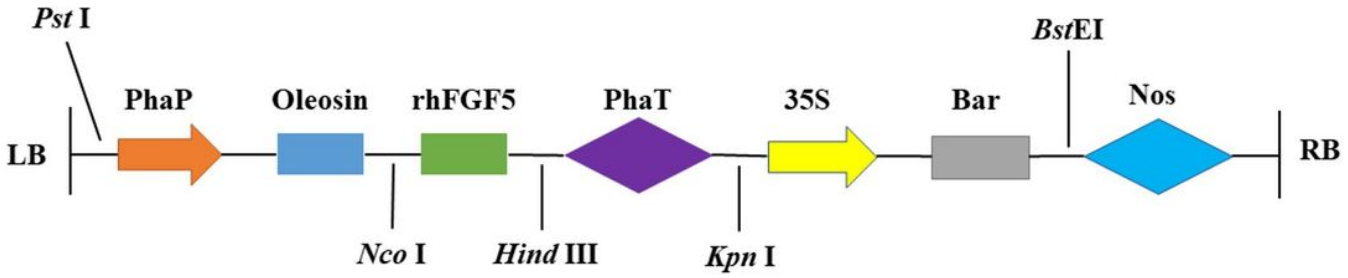

Figure 1

pOTB-rhFGF5 expression vector. The pOTB-rhFGF5 vector included a phaseolin promoter/terminator, an Arabidopsis thaliana oleosin gene, rhFGF5 gene, the 35S promoter, the bar gene and Nos terminator. PhaP: phaseolin promoter, Oleosin: Arabidopsis thaliana oleosin gene, rhFGF5: recombinant human fibroblast growth factor 5, PhaT: phaseolin terminator, 35S: CaMV35S promoter, Bar: the glufosinate resistance gene, Nos: Nopaline synthase terminator.

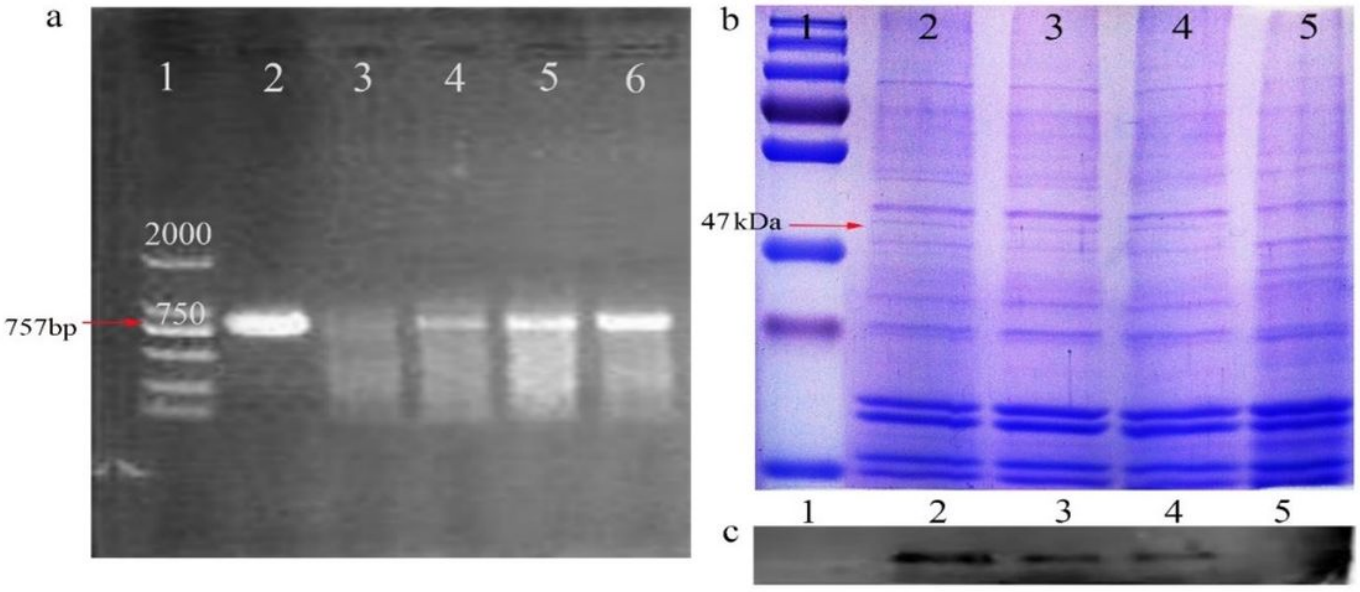

Figure 2

Gene and protein level identification of transgenic Arabidopsis. (a) Genome PCR assay identification, 1 present DNA mark, 2 present positive control group, 3 wild type Arabidopsis genome, 4-6 present transgenic Arabidopsis genome; (b) SDS-PAGE identified the oleosin-rhFGF5, 1 present protein mark, 2-4 present transgenic Arabidopsis, 5 present wild type Arabidopsis; (c) Western blotting identified the interest protein. 1 present protein mark, 2-4 present transgenic Arabidopsis, 5 present wild type Arabidopsis, the interest protein molecular was 47kDa.

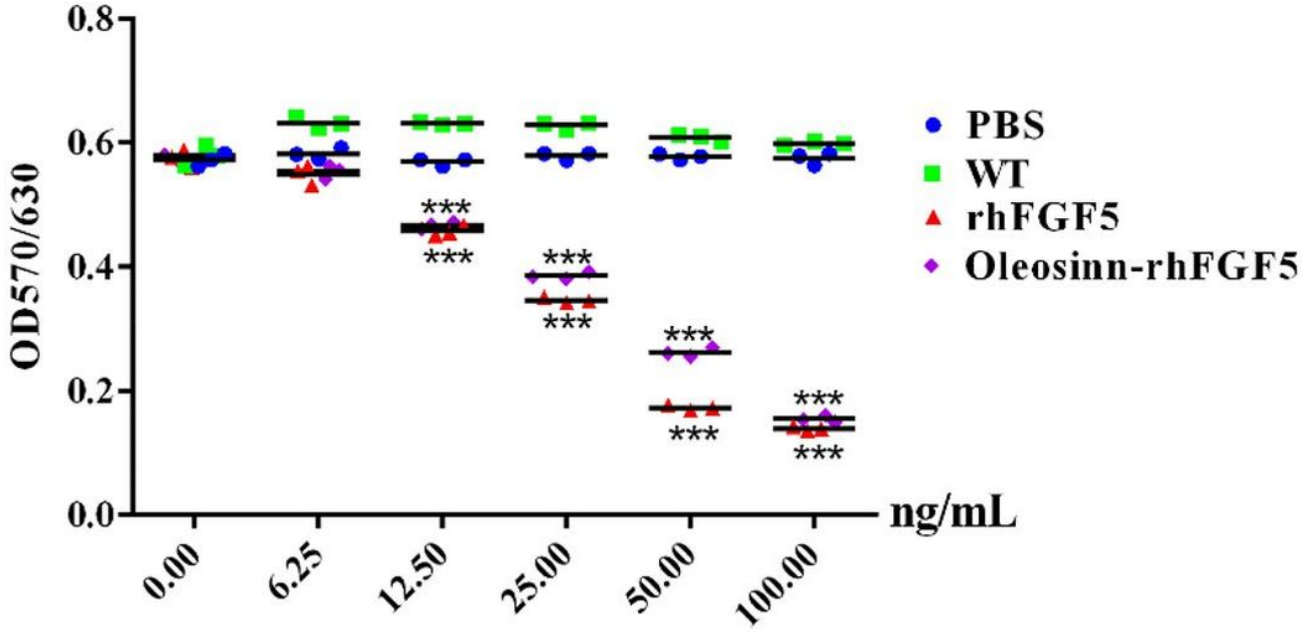

Figure 3

Effects of different concentrations of oleosin-rhFGF5 on the inhibition of hair follicle epithelial proliferation. PBS present the cell was treated with 0.01 M PBS, WT present the cell was treated with different concentration of wide type oil body, rhFGF5 present the cell was treated with different concentration of rhFGF5 protein, oleosin-rhFGF5 present the cell was treated with different concentration of oleosin-rhFGF5 protein, repeat 3 times for each group, and the results are expressed in mean value, t-student test was used to analysis the statistical difference, ***P<0.001.

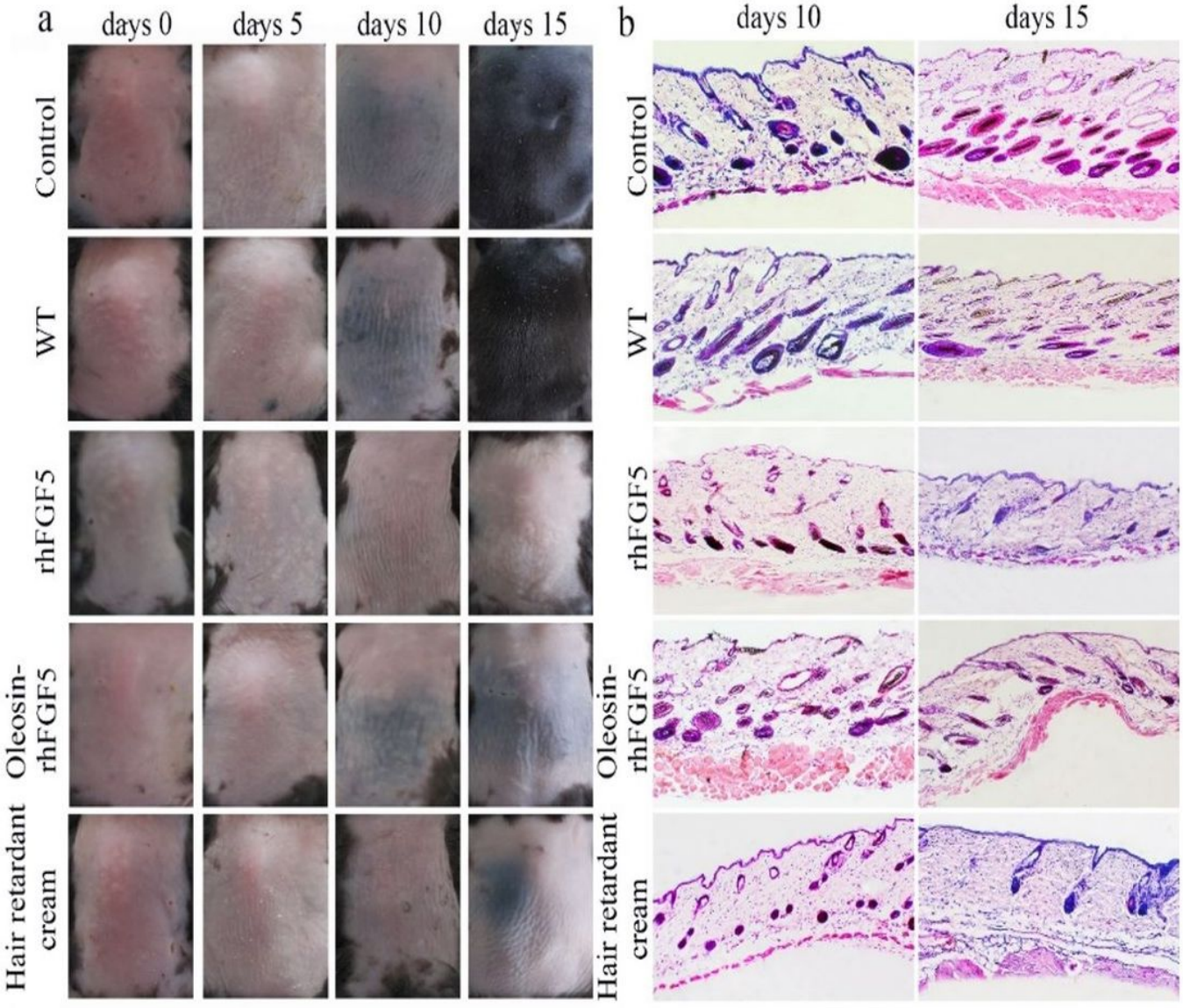

Figure 4

In vivo study of oleosin-rhFGF5 on inhibiting hair growth. (a) Hair regeneration on the back of mice in different days after administration; (b) Pathological analysis of the skin of mice after H&E staining on days 10 and days 15, the magnification is 100 times, n=6.

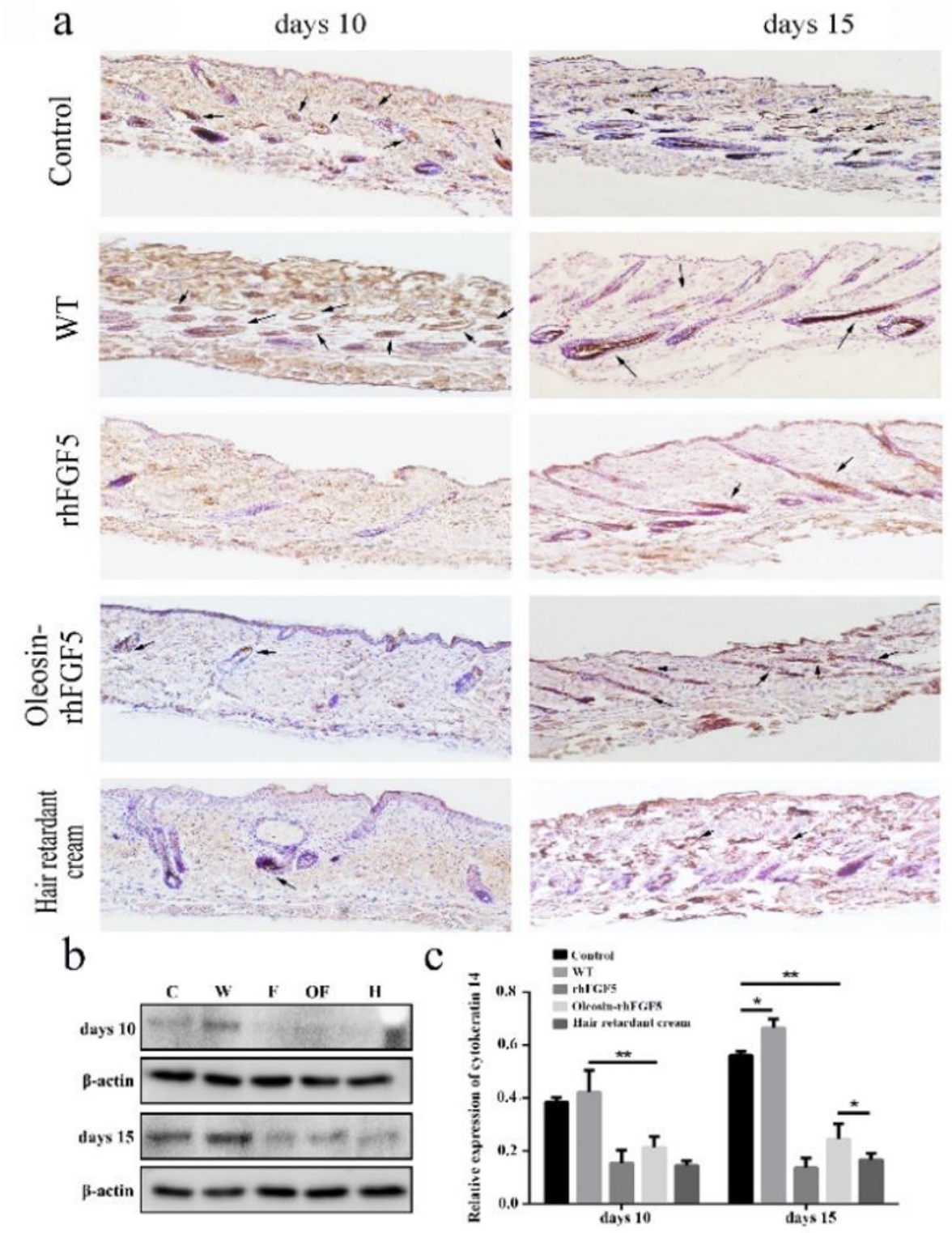

Figure 5

Immunohistochemistry staining and western blotting to analysis the cytokeratin 14 expression in vivo. (a) Immunohistochemistry staining of cytokeratin 14, (b) Western blot analysis the cytokeratin 14 expression in skin, (c) Statistical analysis of the expressed protein in b. t-student test was used to analysis the statistical difference, *P<0.05, **P<0.01, n=6.