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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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ALOPECIA AREATA PROFILING SHOWS LNCRNAS REGULATE THE SUPPRESSED EXPRESSION OF KERATIN

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Abstract.

Background: Alopecia areata (AA) is one of the most common autoimmune hair diseases. Long non-coding RNAs (lncRNAs) have been shown to be involved in the pathophysiological progression of a variety of diseases; however, how lncRNAs are involved in the pathogenesis of alopecia areata is not fully understood.

Objective: In order to study the differential expression profiles of mRNA and lncRNA in patients with alopecia areata and provide experimental basis for the diagnosis and treatment of alopecia areata.

Method: We collected skin tissues from the normal and bald areas of the scalp of five patients with alopecia areata. We used RNA sequencing to detect mRNA and lncRNA expression profiles in the skin, screen for differentially expressed genes, and then perform enrichment analyses to determine the functions of the differentially expressed genes and construct a lncRNA-mRNA interaction network.

Results: Our results show that normal and bald areas of the scalp in patients with alopecia areata have different mRNA and lncRNA expression profiles, with a total of 344 mRNAs and 116 lncRNAs differentially expressed. Functional enrichment analysis of these co-expressed the differentially expressed genes (DEGs) was enriched for biological processes such as intermediate filament organization, keratinization, and epithelial cell differentiation and so on. Based on the co-expression of differential lncRNAs and DEGs obtained in this project, further overlap analysis of 100 kb of DEGs upstream and downstream of the lncRNAs ultimately revealed that 11 lncRNAs cis-regulate 15 target mRNAs.

Conclusion: The pathogenesis of alopecia areata is closely related to multiple genes and multiple pathways, in which keratin family genes may play a key role. In conclusion, this study provides new and promising biomarkers for the diagnosis of alopecia areata.

Key words. Alopecia areata, bioinformatics, Keratin genes, differentially expressed genes, co-expression network.

Introduction.

Alopecia areata is one of the most common autoimmune diseases, with an incidence rate of about 2.1% [1]. It is characterized by hair follicle immune attack mediated by T cells leading to alopecia [2]. Alopecia areata is not life-threatening, but because the lesions mostly appear in the exposed parts such as the head and face, it has a direct impact on the appearance of patients and a great burden on their spirit and psychology. Therefore, it is particularly important to solve the onset and recurrence of alopecia areata. However, the specific pathological mechanism is not completely clear, and there is no unified cognition. Among the many factors in the study of AA pathogenesis, immune system disorder and heredity have always been the main areas of concentration [3], and there are other possible factors, including infection factors, cytokines, emotional stress, abnormal melanocytes or keratinocytes and nervous system factors [4].

Long non-coding RNA (lncRNA) itself does not encode protein but forms multi-level regulation gene expression in the form of RNA [5,6]. Studies have shown that lncRNA plays an important role in many cellular processes, including cell cycle, differentiation, metabolism and disease [7]. Some studies have found that lncRNA are differentially expressed in dermal papilla cells during hair follicle development, however, the specific lncRNA and its related regulatory mechanism in alopecia areata are largely unknown. The existing literature reports that the function of lncRNA in alopecia areata mainly focuses on the competing endogenous RNA (ceRNA), and some only focus on a single lncRNA [8-10]. At present, there are still few studies on the regulatory network of lncRNA-DEG and its possible functions in alopecia areata through the direction of whole genome lncRNAs. We hypothesized that after alopecia areata, differentially expressed lncRNAs participated in transcription regulation, which led to the differential expression of mRNAs, and the regulatory axis of lncRNA-DEG played a molecular regulatory role in alopecia areata.

In this project, RNA-seq of tissue samples from alopecia areata disease group and normal control group were systematically analyzed, and lncRNAs and mRNAs with differential expression were obtained. Combined with co-expression analysis, the regulatory network of lncRNA-DEG in the whole genome was explored, and valuable results were obtained, which provided theoretical basis for the prevention and treatment of alopecia areata.

Materials and Methods.

RNA extraction and sequencing:

The scalp tissues from the normal and bald areas of the five patients with alopecia areata were collected. Total RNA was treated with RQ1 DNase (Promega) to remove DNA. The quality and quantity of the purified RNA were determined by measuring the absorbance at 260nm/280nm (A260/A280) using smartspec plus (BioRad). RNA integrity was further verified by 1.5% agarose gel electrophoresis.

For each sample, 1 μg of total RNA was treated with RQ1 DNase (Promega) to remove DNA before used for directional RNA-seq library preparation by VAHTS® Universal V8 RNA-seq Library Prep Kit for Illumina (N605) for RNA-seq library preparation. mRNAs were captured by VAHTS mRNA capture Beads (Vazyme, N401). Fragmented mRNAs were converted

into double strand cDNA. Following end repair and A tailing, the DNAs were ligated to VAHTS RNA Multiplex Oligos Set 1 for Illumina (N323), the ligated products were amplified, purified, quantified and stored at -80°C before sequencing. The strand marked with dUTP (the 2nd cDNA strand) is not amplified, allowing strand-specific sequencing.

For high-throughput sequencing, the libraries were prepared following the manufacturer's instructions and applied to Illumina Novaseq 6000 system for 150 nt paired-end sequencing.

RNA-Seq Raw Data Clean and Alignment:

Raw reads containing more than 2-N bases were first discarded. Then adaptors and low-quality bases were trimmed from raw sequencing reads using FASTX-Toolkit (Version 0.0.13). The short reads less than 16nt were also dropped. After that, clean reads were aligned to the human GRCh38 genome by HISAT2 [11,12] allowing 4 mismatches. gene counts were generated using HTSeq package v2.0.2, and then to quantify gene expressions in terms of fragments per kilobase of transcript per million mapped reads (FPKM) estimates [13].

New transcripts predict assembly:

Group the RNASeq data, use stringtie to assemble the data of each group and predict the transcripts, screen the expression of the predicted transcripts of each group, eliminate the transcripts with FPKM< 1, and then use stringtie [14] to combine them into one transcript (GTF file).

lncRNA prediction:

In order to predict credible lncRNA, we used four software to predict lncRNA: CPC2 [15], LGC [16], CNCI [17] and CPAT [18]. We counted the non - coding transcripts identified by the above four analysis software. After the above steps, we successively removed the transcripts that overlap with the known coding genes, are less than 200bp in length, have potential coding ability, and are less than 1000bp away from the nearest gene from the assembly results, obtained the prediction results of new lncRNA, and used the intersection of the four software for subsequent analysis and processing.

Prediction of cis regulatory target:

Set the threshold of co-location as 100kb upstream and downstream of lncRNA in the trans regulatory relationship pair [19], and then calculate the Pearson correlation coefficient between lncRNA and mRNA of co-location for co expression analysis to screen the lncRNA target relationship pairs that meet the absolute value of correlation number greater than 0.6 and P value ≤ 0.01, Then take the intersection of the two data sets of co-location and co expression to obtain the cis target of lncRNA.

Differentially Expressed Genes (DEG) analysis:

The R Bioconductor package DESeq2 [20] was utilized to screen out the differentially expressed genes (DEGs). The P value <0.01 and fold change>2 or < 0.5 were set as the cut-off criteria for identifying DEGs.

Functional enrichment analysis:

To sort out functional categories of DEGs, Gene Ontology (GO) terms were identified using KOBAS 2.0 server [21]. Hyper geometric test and Benjamini-Hochberg FDR controlling procedure were used to define the enrichment of each term.

Results.

RNA-seq analysis of mRNAs in scalp tissue from patients with alopecia areata:

We identified 359 significantly differentially expressed mRNA in alopecia areata patients' alopecia areata zones, of which 300 genes occurred in down-regulation, and GO analysis showed that the down-regulated genes were mainly enriched in intermediate filamentous tissue, keratinization and epithelial cell differentiation (Figure 1A-1D). Among the differentially expressed down-regulated genes, KRT31, KRT85, KRT35, KRT16, KRT6A, KRT6B, KRT32, KRT75, KRT82, KRT73, KRT72, KRT36, KRT87P, KRT84, and KRT73-AS1 are all keratin family genes (Figure 1E, Figure S1).

Distinct lncRNA expression profiles in scalp tissue from patients with alopecia areata:

Using total RNA-Seq data, we further analyzed the lncRNA expression profiles of alopecia areata patients' alopecia areata zones. After quality control and data normalization, a total of 116 DE- lncRNAs were screened according to the criteria of FC ≥2 or ≤0.5.

Compared with normal scalp tissues, 105 down-regulated lncRNAs and 11 up-regulated lncRNAs were identified (Figure 2), and the DE- lncRNAs were mainly concentrated in the down-regulated genes, which was consistent with the trend of differentially expressed genes. Among them, several RP11 family members such as RP11-1020M18.10, RP11-399K19.1, RP11-315F22.1, RP11-963H4.7, RP11-107M16.2, RP11-190A12.10, RP11-845M18.6, RP11-143E21.3, RP11-462G2.1 genes were differentially expressed in down-regulated lncRNAs. We therefore speculate that RP11 family members may play a regulatory role in AA disease (Figure 2).

Construction of a lncRNA and mRNA interaction network:

We correlated the expression of all differential lncRNAs and DEGs genes in this study and found that 116 lncRNAs positively regulated 336 DEGs and 83 lncRNAs negatively regulated 234 DEGs. GO functional enrichment analysis of these co-expressed DEGs enriched for biological processes such as intermediate filament organization, keratinization, and epithelial cell differentiation. Combined with relevant literature reports, we focused the differential genes on keratin family genes, the bar graph shows the number of lncRNAs targeted by keratin genes (Figure 4A), and the hierarchical clustering heatmap shows the expression levels of the top 10 lncRNAs sorted by the number of targeted keratin genes (Figure 4B). And the co-expression network composed of differentially expressed lncRNAs and keratin genes was mapped (Figure 4C). The list of trans target genes was opened, and the target genes were arranged in NC-1 descending order, and the top three were KRT31, KRT85, and KRTAP11-1 (Figure 3E), which were also of interest to us in the previous DEG analyses, and the corresponding lncRNAs upstream of these three genes were found to be RP11-190A12.10 and RP11- 1020M18.10 (Figure 3D).

In order to accurately find the cis-target of lncRNAs, we performed overlap analysis of 100 kb DEGs upstream and downstream of lncRNAs, and finally found that 11 lncRNAs cis-regulate 15 target mRNAs. among them, RP11-845M18.6

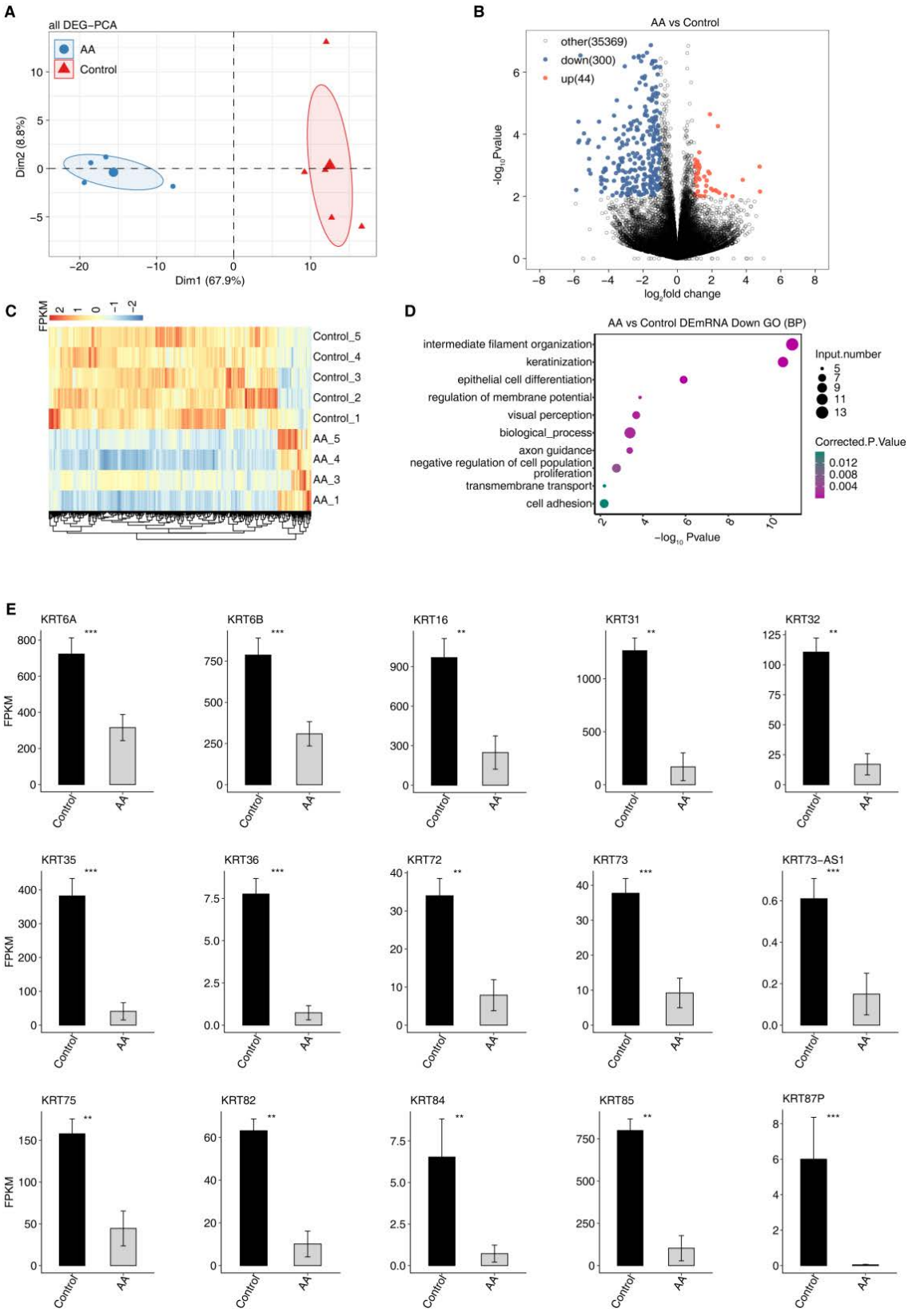

Figure 1. *Alopecia areata disease regulates gene expression in skin tissue.*
(A) PCA base on FPKM value of all differentially expressed gene (DEG)in skin tissue. The ellipse for each group is the confidence ellipse.
(B) Volcano plot showing all DEG between skin tissue of alopecia areata disease patient (AA) and biological normal samples (Control).
(C) Hierarchical clustering heat map showing expression levels of all DEGs.
(D) The Scatter plot exhibiting the most enriched GO biological process results of the down-regulated DEGs.
(E) Bar plot showing the expression pattern and statistical difference of DEGs for some important genes. Error bars represent mean ± SEM.
****P-value < 0.001; **P-value < 0.01; *P-value < 0.05.*

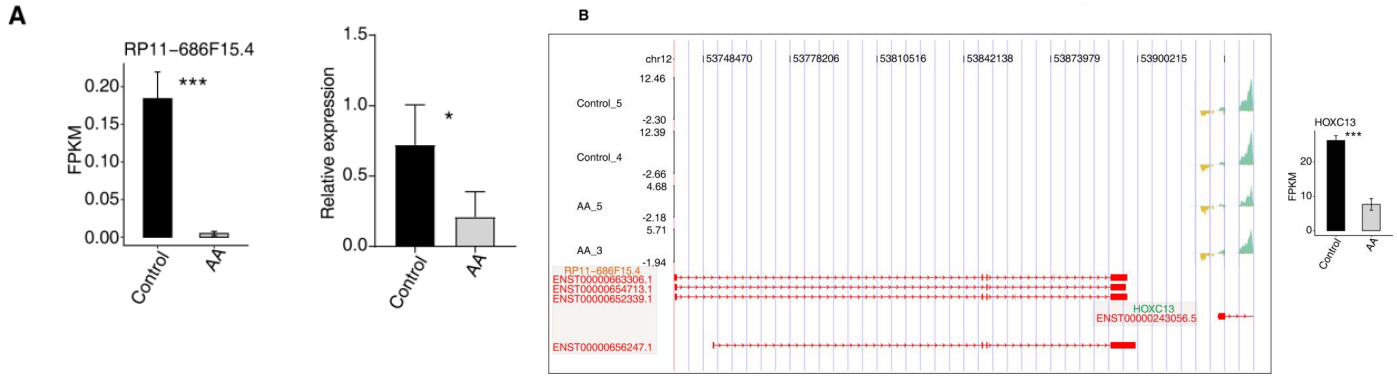

Figure S1. *lncRNA regulates keratin genes expression in skin tissue.*
*(A) Bar plot showing the FPKM and qPCR result ofRP11-686F15.4. Error bars represent mean ± SEM. ***P-value < 0.001; **P-value < 0.01; *P-value < 0.05.*
*(B) IGV-sashimi plot showing the reads distribution of genes in the up panel and the transcripts of each gene are shown below(Left). Bar plot showing the FPKMofHOXC13. Error bars represent mean ± SEM. ***P-value < 0.001; **P-value < 0.01; *P-value < 0.05.*

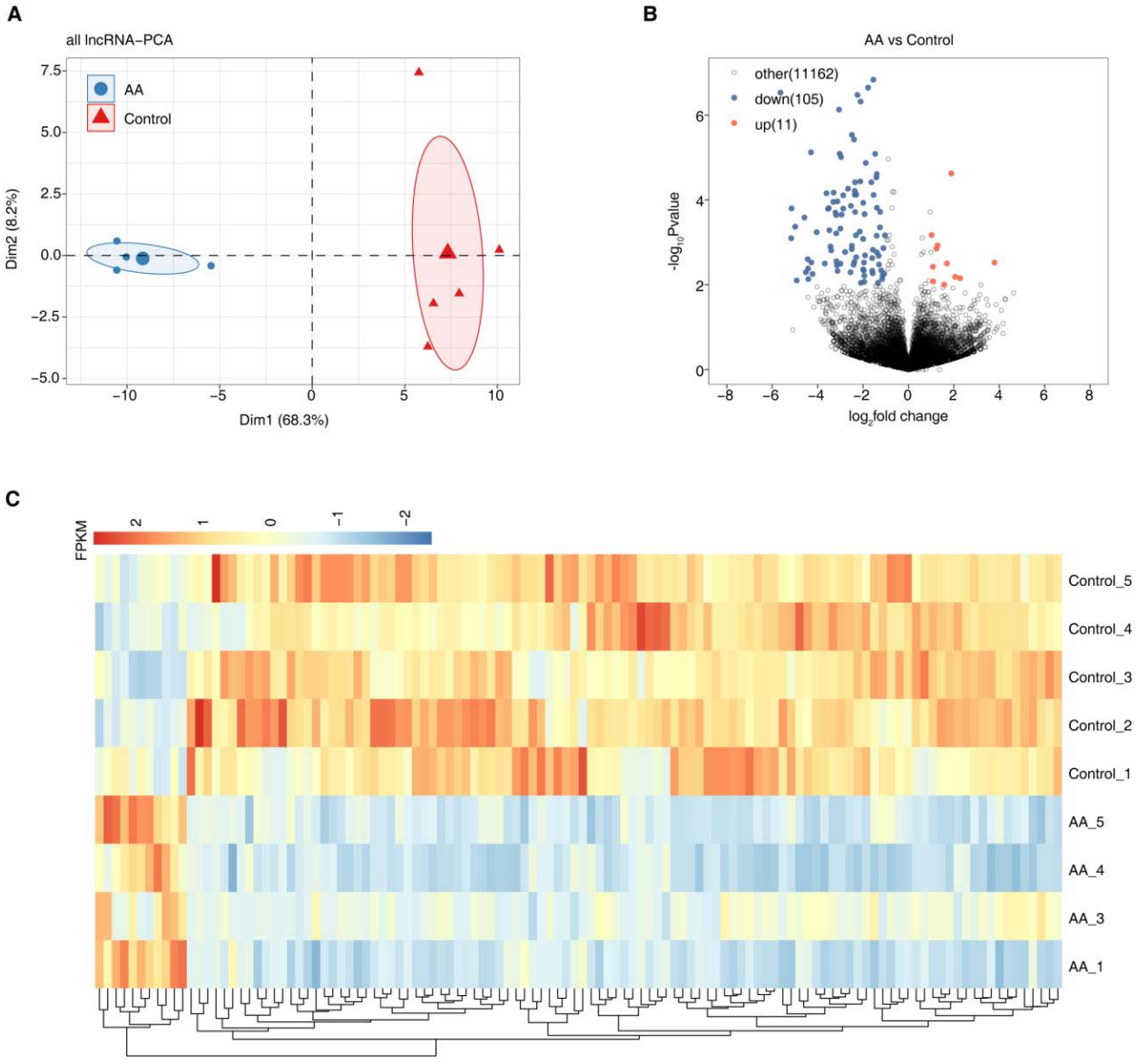

Figure 2. *Alopecia areata disease regulates lncRNA expression in skin tissue.*
(A) PCA base on FPKM value of all detected lncRNAs in skin tissue. The ellipse for each group is the confidence ellipse.
(B) Volcano plot showing all differentially expressed lncRNAs (DElncRNAs) between skin tissue of AA and Control.
(C) Hierarchical clustering heat map showing expression levels of all DElncRNA.

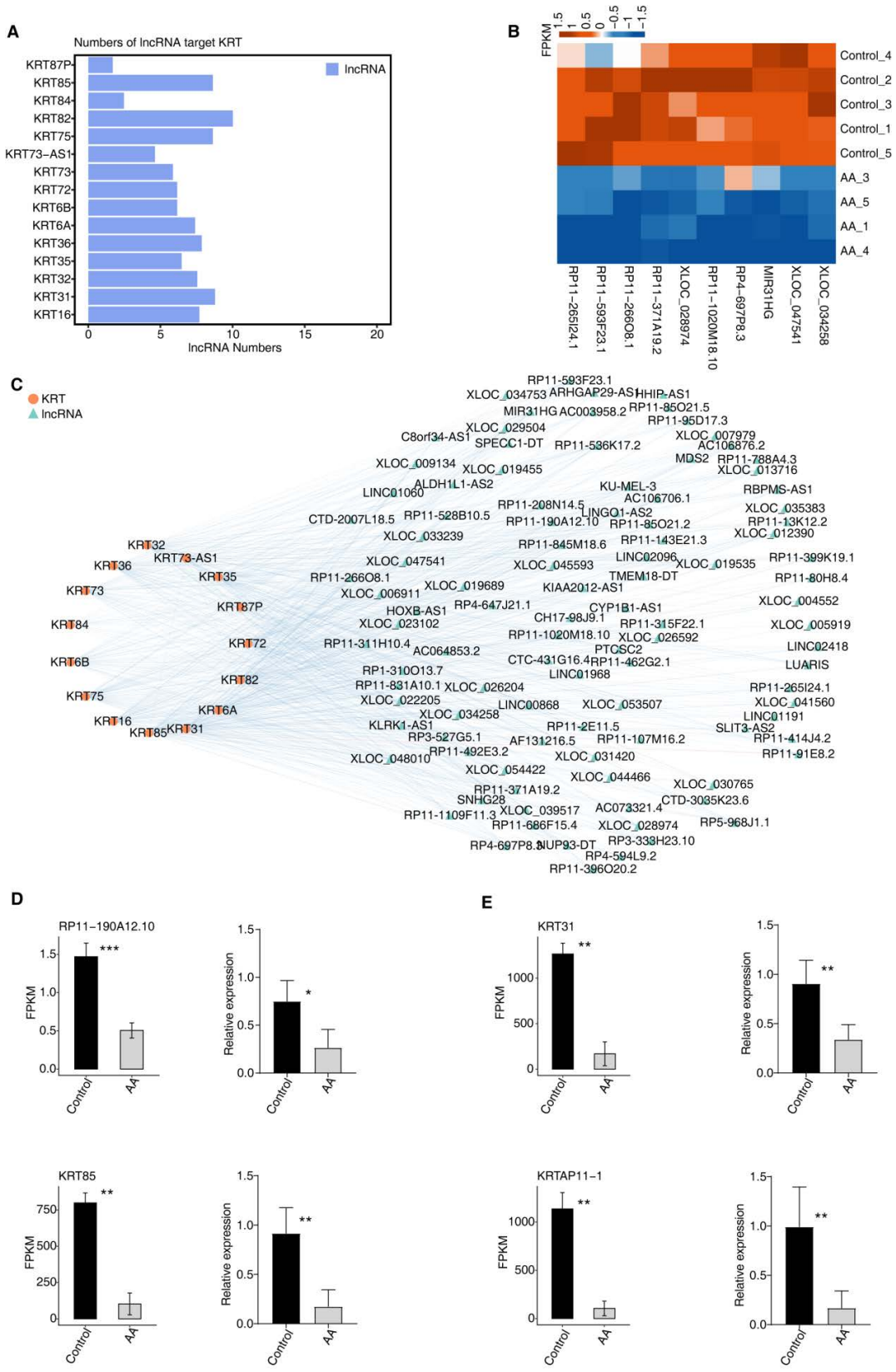

Figure 3. *DElncRNA selectively targets the keratin genes in skin tissue.*
(A) Bar plot showing the numbers of lncRNA targeted by differentially down regulated keratin genes.
(B) Hierarchical clustering heat map showing expression levels of top 10 lncRNAs sorted by numbers targeted thekeratin genes.
(C) Diagram of network shows the co-expression network comprising differentially expressed lncRNA and differentially down regulated keratin genes. Cutoffs of P value ≤ 0.01 and Pearson coefficient ≥ 0.6 or ≤ -0.6 were applied to identify the co-expression pairs.
(D) Box plot showing the expression levels and qPCR result of RP11-190A12.10.
(E) Box plot showing the expression levels and qPCR results of KRT31, KRT85 and KRTAP11-A.

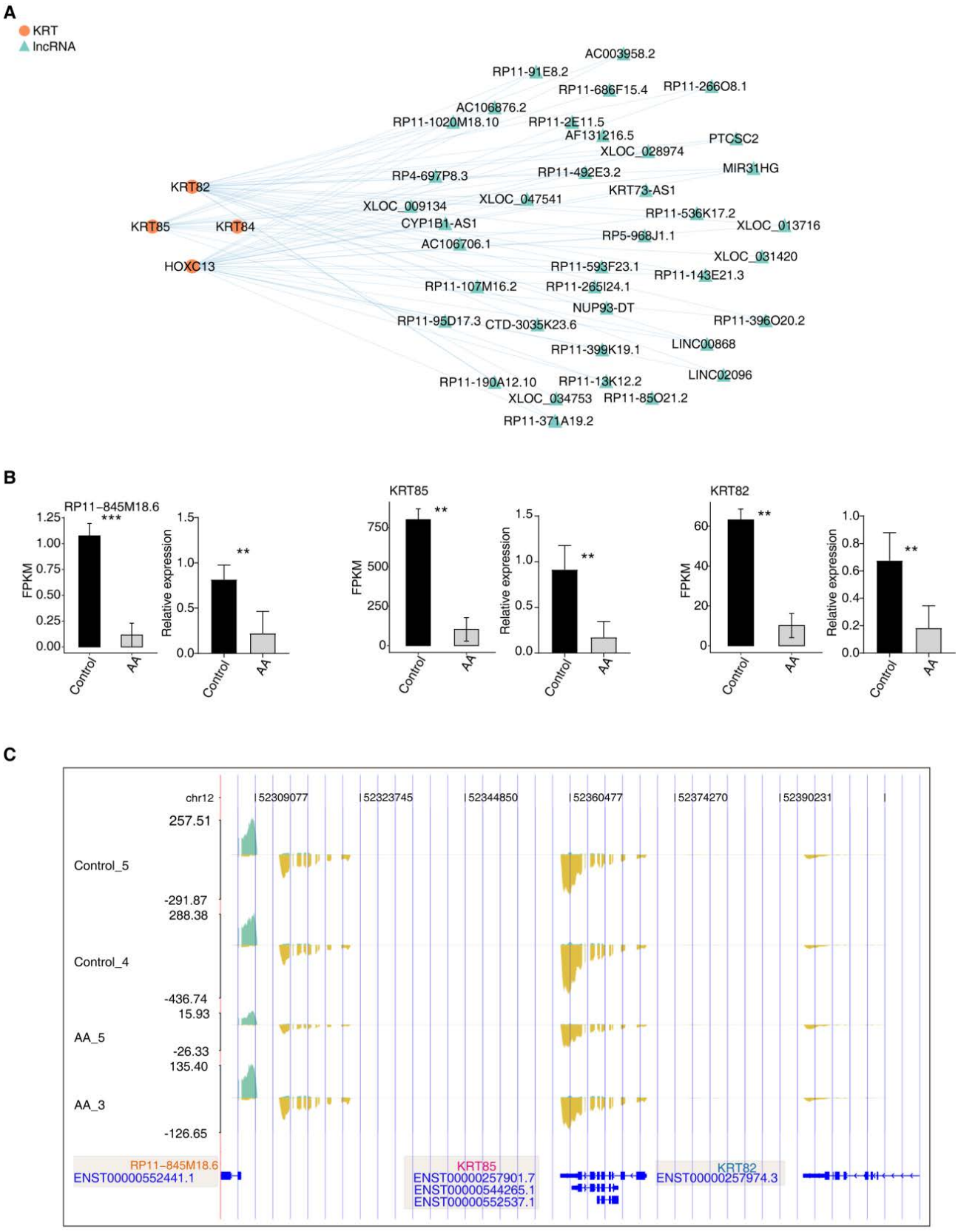

Figure 4. *lncRNA regulates keratin genes expression in skin tissue.*
(A) Diagram of network shows the co-expression network comprising differentially expressed lncRNA and differentially three important keratin genes. Cutoffs of P value ≤ 0.01 and Pearson coefficient ≥ 0.6 or ≤ -0.6 were applied to identify the co-expression pairs.
*(B) Bar plot showing the FPKM and qPCR result ofRP11-845M18.6, KRT85 and KRT82. Error bars represent mean ± SEM. ***P-value < 0.001; **P-value < 0.01; *P-value < 0.05.*
(C) IGV-sashimi plot showing the reads distribution of genes in the up panel and the transcripts of each gene are shown below.

cis-regulates KRT82, KRT84, and KRT85, and RP11-686F15.4 cis-regulates HOXC13 (Figure 4).

Discussion.

Alopecia areata is an autoimmune disease with unknown etiology and mechanism [22], which has received increasing attention in recent years [23] due to the demonstrated involvement of lncRNA in the onset and progression of autoimmune diseases [24]. So far, lncRNA and mRNA have been poorly reported in alopecia areata. To this end, we studied the expression profiles of lncRNA and mRNA in the scalp of patients with alopecia areata.

To the best of our knowledge, reports on the expression profiles of lncRNA and mRNA in the scalp of patients with alopecia areata analyzed by high-throughput sequencing are relatively rare. By analysing the sequencing data, 344 differentially expressed genes were identified, of which 300 genes were down-regulated, and most of the down-regulated genes were keratin family genes.

Keratin (KRT) is a part of the epithelial cell skeleton and belongs to the intermediate filament protein [25], which is of great significance for the mechanical stability and integrity of epithelial cells and tissues [26]. It is the main structural protein in human hair fibers [27]. Research has shown that abnormal KRT genes can lead to various hair diseases [28]. In male androgenic hair loss, KRT82 and KRT39 may play a crucial role [29].

Moreover, both the alopecia areata mouse model and AA patients showed morphological damage to hair and differential expression of hair related genes, including keratin associated proteins (KRTAPs) [30]. Therefore, its significant downregulation may be the core link of hair loss in patients with alopecia areata. And the GO analysis results showed that downregulated genes were mainly enriched in biological processes such as intermediate silk tissue, keratinization, and epithelial cell differentiation, which is consistent with Previous research.

At the same time, we also detected 116 differentially expressed lncRNAs. Among the numerous differentially expressed lncRNAs, the overall expression level of upregulated differentially expressed lncRNAs is low. Therefore, we focus on downregulated differentially expressed lncRNAs, which can be mainly divided into two categories: newly predicted and known [31].The vast majority are members of the RP11 lncRNA family, which have similar regulatory effects, such as lncRNA RP11-33A14.1, RP11-423H2.3, RP11-838N2.4, RP11-436H11.5, and RP11-708H21.4, which are associated with malignant tumors [32], including glioblastoma, renal cell carcinoma, and colon cancer. At the same time, research has reported [33] that various RP11 lncRNAs, including RP11-11G251.23 and RP5-11E231, may play a key role in the pathogenesis of alopecia areata by regulating the cytokine receptor interaction pathway, and can serve as therapeutic targets for alopecia areata in clinical interventions.

To better understand the potential interactions and related functions of the co-expressed lncRNAs and mRNAs. We conducted correlation analysis on the expression levels of all differentially expressed lncRNAs and DEGs genes in this project, and identified KRT31, KRT85, and KRTAP11-1 involved in hair follicle development [34]. KRT85 was found in this study, and multiple studies also found that KRT85 can inhibit the proliferation and differentiation of hair follicle cells, thereby affecting the progression of alopecia areata [35-36], which is consistent with our results. The KRTAP gene also plays an important role in the pathogenesis of alopecia areata [37]. In addition, KRT31 is associated with hair growth [38]. Meanwhile, most of these differential genes are enriched to be related to biological processes such as intermediate filament organization, keratinization, and epithelial cell differentiation. However, the role of these genes in alopecia areata remains to be further investigated. Meanwhile, GO functional enrichment analysis of these co-expressed DEGs was performed, and the top three enriched pathways were consistent with the DEGs enriched pathways.

The transcriptional regulation of lncRNAs is divided into trans- and cis-target [39], in which lncRNAs regulating the expression of genes on the chromosome where they are transcribed are cis-target; and lncRNAs regulating the expression of genes on other chromosomes are trans-regulate [40]. On the basis of the co-expression of differential lncRNAs and DEGs obtained in this project, we further analyzed the overlap of 100 kb of DEGs upstream and downstream of the lncRNAs, and finally found that 11 lncRNAs cis-target 15 target mRNAs. Of these, RP11-845M18.6 cis-targets KRT82, KRT84, and KRT85, and RP11-686F15.4 cis-targets HOXC13.KRT82 is a type II keratin that is expressed in the keratin layer of the hair shaft, and along with other hair keratin proteins, plays a role in the formation of intermediate filaments necessary for the structural integrity of the hair shaft [41].

Studies have shown that KRT82 expression is reduced in the skin and hair follicles of patients with alopecia areata, and that its aberrant expression and its effect on hair shaft integrity may be relevant to the pathophysiology of AA. KRT84, a type II hair keratin, heterodimerises with type I keratin to form hair and nails [42]. Gene expression analyses showed a significant downregulation of KRT84 expression in alopecia areata. KRT85 is expressed in matrix cells, cortical cells, and throughout the hair cuticle. Mutations in KRT85 result in the inability of functional type II hair keratin to pair with the corresponding type I hair, leading to an abnormal hair phenotype [43]. KRT85 is significantly suppressed in AA lesion skin and is an early marker of baldness. KRT85 was undetectable in Hirosaki hairless rat, suggesting that it is associated with hair follicle development. HOXC13 regulates the expression of hair, metaceratin, keratin-associated proteins, bridging calreticulin, and plays an important role in hair follicle morphogenesis and the hair cycle [44]. Knockdown or overexpression of HOXC13 resulted in severe hair growth and defects in the mouse. In addition, HOXC13 has been shown to be a key transcriptional regulator of a variety of KRT and KRTAP genes. any imbalance in HOXC13 expression (low or high) may result in severe keratin-related abnormalities, including alopecia and brittle hair phenotypes [45]. Taken together, these genes may be closely related to human hair composition, to follicle regeneration and skin development, and their significant changes may be central to hair loss in AA patients.

There are some limitations to this study. For example, our sample size was relatively small (Only 5 samples). Therefore, we need to expand the sample size in order to be more convincing with our results. The present study is our preliminary work so far. Although a large number of differential genes were obtained by transcriptome sequencing in this study, future studies need to experimentally verify the mechanism of action of these differential genes as well as the related signalling pathways.

Conclusion.

The pathogenesis of alopecia areata is closely related to multiple genes and multiple pathways, in which keratin family genes may play a key role. This study provides new and promising biomarkers for the diagnosis of alopecia areata.

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Date sharing statement.

The data in this study is available from the corresponding author on reasonable request.

Ethics statement.

This study is conducted in accordance with the principles of the Declaration of Helsinki and approved by the Ethical Committee for Lanzhou University Second Hospital.

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