Genes & Diseases (2023) 10, 51–54

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.keaipublishing.com/en/journals/genes-diseases

RAPID COMMUNICATION

Androgen receptor inhibits the hair follicle induction potential of dermal papilla cells by binding with Tcf4 at the A574 binding site

Androgenic alopecia (AGA) is the most common type of clinical alopecia. Androgen receptor (*AR*) is the most logical candidate gene for regulating the occurrence of AGA. Dermal papilla cells (DPCs) are a special kind of mesenchymal cells, located in the hair bulb of hair follicles. DPCs play a role in maintaining and inducing the periodic cycling of hair follicles, and are considered as a key cell target of androgen in hair follicles. Tcf4 is a positive regulator of the maintenance of DPC biological features. Previously, we reported that Twist1 can enhance the inductive effect of Tcf4.[1] As a transcription factor, AR can bind with Tcf4 to regulate the proliferation in prostate growth and tumorigenesis.[2,3] However, whether and how AR interacts with Tcf4 in DPCs remains unknown.

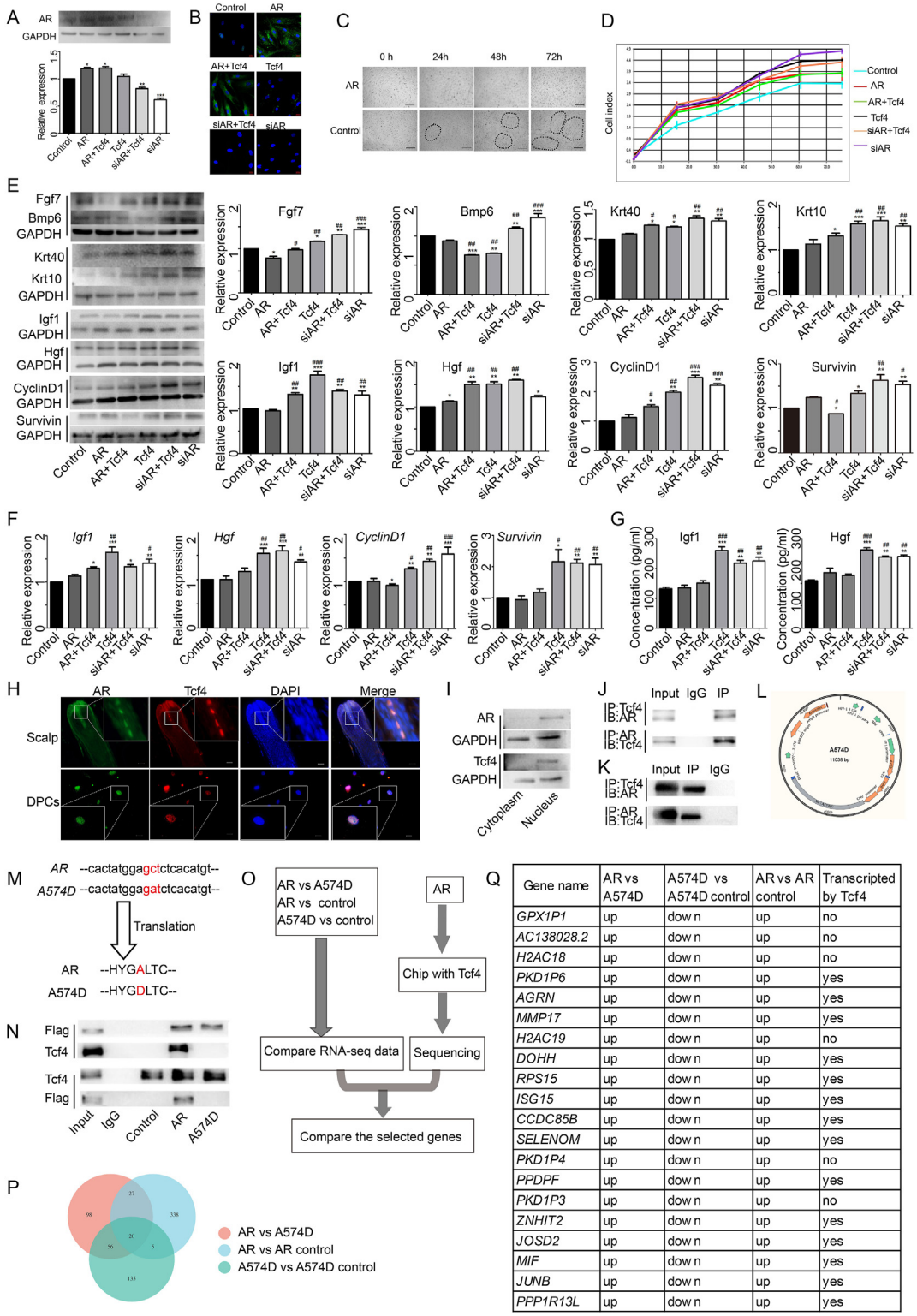
In this study, we employed lentivirus or adenovirus vectors to overexpress or knockdown *AR* or *Tcf4* in primary cultured DPCs (Supplementary Methods). First, adenovirus for overexpression of *AR* (Ad-AR) or knockdown of *AR* (Ad-siAR) was added to the culture medium of DPCs, and *AR* was successfully overexpressed or knocked down (Fig. 1A, B). After *AR* overexpression, the agglutinative behavior of DPCs disappeared (Fig. 1C). Based on our previous studies that Tcf4 can promote the proliferation of DPCs *in vitro*, we further investigated whether AR plays a role in Tcf4-induced proliferation. We used the label-free proliferation technique to determine the cell index of DPCs. Compared with that of the control group, the proliferation of the *Tcf4*-overexpression (Ad-Tcf4)-treated DPCs was significantly increased. As expected, the proliferation rate of the DPCs treated with both Ad-Tcf4 and Ad-AR was significantly lower than that of the DPCs treated with Ad-Tcf4, indicating that *AR* overexpression can inhibit the proliferation of DPCs induced by Tcf4. In accordance with this finding, the DPCs treated with Ad-siAR proliferated faster than the control cells or even Ad-Tcf4-treated cells (Fig. 1D), indicating that *AR* knockdown can further enhance the proliferation of DPCs. These results suggest that AR inhibits the proliferation of DPCs, and may interact with Tcf4 in regulating DPC proliferation.

We then tested the expression of DPC markers in the Ad-AR-, Ad-siAR-, Ad-Tcf4-, Ad-AR- and Ad-Tcf4-, Ad-siAR- and Ad-Tcf4-treated DPCs. The increased expression of Fgf7 induced by Tcf4 was inhibited by AR, whereas the decreased expression of Bmp6 was not inhibited by AR (Fig. 1E). HaCaT cells are from the basal layer of the skin and can be induced to differentiate into cells resembling various types of skin cells. To test the inductive ability of DPCs, we treated HaCaT cells with conditioned medium from adenovirus treated DPCs. The conditioned medium from the Ad-siAR and Ad-Tcf4 treated DPCs increased the expression levels of Krt40 and Krt10, compared to the conditioned medium from the Ad-Tcf4-treated DPCs (Fig. 1F). Then, we detected the expression and release of hair growth-related growth factors (Hgf and Igf-1) in these adenoviruses-treated DPCs or culture supernatants. The increased protein expression levels of Igf1 induced by Tcf4 were inhibited by Ad-AR (Fig. 1E). The mRNA expression levels of *Igf1* and *Hgf* induced by Tcf4 were both inhibited by Ad-AR (Fig. 1F). The secretion of these factors was detected by ELISA. The expression trend of these growth factors in the supernatant was inhibited by Ad-AR as well (Fig. 1G). These results demonstrated that AR was negatively correlated with the production and secretion of growth factors regulated by Tcf4 and partly related to the biological features of DPCs.

CyclinD1 and *Survivin* are known target genes transcribed by Tcf4. We also found the increased expression levels of these genes in the *Tcf4* overexpressing DPCs. The increased expression levels of both CyclinD1 and Survivin

Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2022.04.015



Q

Gene name	AR vs A574D	A574D vs A574D control	AR vs AR control	Transcripted by Tcf4
GPX1P1	up	down	up	no
AC138028.2	up	down	up	no
H2AC18	up	down	up	no
PKD1P6	up	down	up	yes
AGRN	up	down	up	yes
MMP17	up	down	up	yes
H2AC19	up	down	up	no
DOHH	up	down	up	yes
RPS15	up	down	up	yes
ISG15	up	down	up	yes
CCDC85B	up	down	up	yes
SELENOM	up	down	up	yes
PKD1P4	up	down	up	no
PPDPF	up	down	up	yes
PKD1P3	up	down	up	no
ZNHIT2	up	down	up	yes
JOSD2	up	down	up	yes
MIF	up	down	up	yes
JUNB	up	down	up	yes
PPP1R13L	up	down	up	yes

Figure 1 The roles and mechanism of AR in the function of dermal papilla cells (DPCs). **(A)** The efficiency of AR overexpression and knockdown. The expression of AR was detected by Western blot and standardized to the expression of GAPDH. **(B)** The expression of AR was detected by immunofluorescence. Scale bar = 20 μm. **(C)** Morphological changes in AR-overexpressing DPCs. The agglutinative growth behavior is designated with dashed line. **(D)** Growth curve of DPCs detected by an unlabeled real-time cell analyzer. $n = 3$, $^{*}P < 0.05$ compared with the control group. $^{***}P < 0.0001$ compared with the control group. **(E–G)** The effect of AR on Tcf4 induced DPC biological characteristics. $n = 3$. *Compared with the control, $^{*}P < 0.05$, $^{**}P < 0.01$. $^{***}P < 0.001$. $^{\#}$Compared with AR group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$. (E) DPCs were treated with Ad-GFP, Ad-AR, Ad-siAR, Ad-Tcf4, Ad-Tcf4 and Ad-AR, Ad-Tcf4 and Ad-siAR. The DPC markers FGF7 and BMP6, the hair inductive factors Igf1 and Hgf, and the Tcf4 target genes CyclinD1

were inhibited by the addition of Ad-AR and enhanced by Ad-siAR (Fig. 1E). The mRNA expression levels showed the same trend (Fig. 1F). These results indicate that the genes directly transcribed by Tcf4 may be inhibited by AR.

To determine how AR inhibits the Tcf4 target genes, we analyzed the expression patterns of AR and Tcf4 in DPCs both *in vitro* and *in vivo* through immunofluorescence. AR and Tcf4 were colocalized in some DPCs in scalp hair follicles. They were also partly colocalized in the nucleus of cultured DPCs (Fig. 1H). Western blot results also demonstrated that AR and Tcf4 were mostly expressed in the nucleus of cultured DPCs (Fig. 1I). Coimmunoprecipitation experiments were performed to further verify the coexpression pattern. AR was detected in Tcf4 precipitated proteins, and Tcf4 was also detected in AR precipitated proteins (Fig. 1J). Dihydrotestosterone (DHT) was reported to be a key factor for AGA. When DHT was added to the DPCs, the binding of Tcf4 and AR was not impacted (Fig. 1K). These results demonstrated that AR could physically interact with Tcf4 in DPCs.

We then explored how AR interacts with Tcf4 in DPCs. A574 was reported to be a DNA-binding site for AR, and mutation of A574—D574 resulted in the loss of AR transactivation.[4] To determine the binding site for AR and Tcf4 in DPCs, we constructed a lentivirus overexpressing mutated AR named Lenti-A574D (Fig. 1L, M). Ad-AR-Flag, Lenti-A574D-Flag or control treated DPCs were tested by coimmunoprecipitation. When the proteins were precipitated with anti-Flag, Tcf4 was only detected in the Ad-AR-Flag treated group. When the proteins were precipitated with anti-Tcf4, Flag was only detected in the Ad-AR-Flag treated group (Fig. 1N). These results demonstrated that A574 is a key binding site for AR and Tcf4 in DPCs. When this site is mutated, the molecules cannot bind with each other. To further validate the results, we sequenced and compared the transcriptomes of the Ad-AR- and Lenti-A574D-treated DPCs. The Ad-AR-treated DPCs were also immunoprecipitated with anti-Tcf4, and the precipitated DNA was sequenced. The differentially expressed genes in the transcriptome sequence were compared with the ChIP-sequence data (Fig. 1O). In total, 20 genes were selected by Venn analysis, and 14 of them were found in anti-Tcf4 ChIPed-DNA (Fig. 1P, Q). The sequence data indicate that most of the differentially expressed genes between the AR-treated and A574D-treated DPCs were the direct target genes of Tcf4 in DPCs, whereas some differentially expressed genes were indirectly impacted by the AR-Tcf4 interaction. This finding is in accordance with the result that some Tcf4-induced DPC markers are not impacted by *AR* overexpression (Fig. 1E).

In summary, we reported that AR inhibits the agglutination growth of DPCs, the proliferation of DPCs induced by Tcf4, the expression of Tcf4 target genes and the secretion of growth factors regulating hair follicle regeneration. We also reported that the reported DNA-binding site A574 is a key binding site for AR to bind with the Tcf4 protein. However, binding with Tcf4 is not the only pathway for AR signals to regulate the features of DPCs. In conclusion, we report new data to clarify whether and how AR regulates the biological features of DPCs *in vitro*. Targeting the AR/Tcf4 complex is a potential treatment strategy for AGA.

Author contributions

NY and TH performed most of the experiments. HY, LZ, XZ and FX performed part of the experiments. XY and YL designed the experiments. YL wrote the manuscript.

Conflict of interests

The authors declare no conflict of interests.

Funding

This work was supported by The National Natural Science Foundation of China (No. 82073460, 82173446, 82103761).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.04.015.

and Survivin were detected by Western blot with normalization to the expression of GAPDH. HaCaT cells were cultured in conditioned medium from adenovirus vector-treated DPCs. The differentiation markers Krt10 and Krt40 were detected by Western blot with normalization to the expression of GAPDH. (F) The mRNA expression of *Igf1*, *Hgf*, *CyclinD1* and *Survivin* was detected by qPCR and standardlized to that of β-*actin*. (G) The concentration of the secreted factors was detected by ELISA. **(H)** The coexpression of AR and Tcf4 was detected by immunofluorescence in human scalp slices (scale bar = 20 μm) and cultured DPCs (scale bar = 5 μm). The inserted photos are the enlarged picture of the framed area in the same photos. **(I)** The expression levels of AR and Tcf4 in the cytoplasm and nucleus of DPCs were separately detected by Western blot, and GAPDH was used as an internal control. **(J, K)** The expression of Tcf4 and AR in DPCs (J) or DHT-treated DPCs (K) were detected by coimmunoprecipitation. The upper panel shows the results of immunoprecipitation with anti-Tcf4 and immunoblotting with anti-AR. The lower panel shows the results of immunoprecipitation with anti-AR and immunoblotting with anti-Tcf4. **(L)** The plasmid vector map of A574D. It is coexpressed with GFP and can be detected by anti-Flag. **(M)** The mutation site of A574D at the gene and peptide levels. **(N)** DPCs were treated with Ad-AR-Flag or Lenti-A574D-Flag. The DPCs were immunoprecipitated with anti-Flag and immunoblotted with anti-Tcf4 (upper panel). The DPCs were immunoprecipitated with anti-Tcf4 and immunoblotted with anti-Flag (lower panel). **(O)** The strategies used in the analysis of the sequencing data. First, the RNA-sequenced genes between the AR and A574D, AR and AR control, and A574D and A574D control were compared separately, and the overlapping differentially expressed genes were selected. Then, ChIP-sequencing was performed in the AR-overexpressing DPCs. Finally, the selected differentially expressed genes were compared with the ChIP-sequence data. **(P)** Venn diagram of the differentially expressed genes in the RNA-seq data. **(Q)** The expression trends of the 20 selected differentially expressed genes in the RNA-seq data and ChIP-seq data.

References

1. Yu N, Hu T, Yang H, et al. Twist1 contributes to the maintenance of some biological properties of dermal papilla cells in vitro by forming a complex with Tcf4 and β-catenin. *Front Cell Dev Biol*. 2020;8:824.
2. Antony L, van der Schoor F, Dalrymple SL, et al. Androgen receptor (AR) suppresses normal human prostate epithelial cell proliferation via AR/β-catenin/TCF-4 complex inhibition of c-MYC transcription. *Prostate*. 2014;74(11):1118—1131.
3. Chesire DR, Ewing CM, Gage WR, et al. *In vitro* evidence for complex modes of nuclear beta-catenin signaling during prostate growth and tumorigenesis. *Oncogene*. 2002;21(17):2679—2694.
4. Scaramuzzino C, Casci I, Parodi S, et al. Protein arginine methyltransferase 6 enhances polyglutamine-expanded androgen receptor function and toxicity in spinal and bulbar muscular atrophy. *Neuron*. 2015;85(1):88—100.

Nanlan Yu [a,1], Tianxing Hu [a,b,1], Haichao Yang [a], Lian Zhang [a], Lin Zhu [a], Xiaofang Zhou [a], Fei Xiang [c], Xichuan Yang [a,**], Yuhong Li [b,*]

[a] *Department of Dermatology, Southwest Hospital, Army Medical University, Chongqing 400038, China*
[b] *Department of Cell Biology, Army Medical University, Chongqing 400038, China*
[c] *Institute of Burn Research, State Key Laboratory of Trauma, Burns and Combined Injury, Southwest Hospital, Army Medical University, Chongqing 400038, China*

*Corresponding author.
**Corresponding author.
E-mail addresses: doctoryxc@msn.com (X. Yang), liyuhongtmmu@hotmail.com (Y. Li)

25 February 2022
Available online 7 May 2022

[1] These authors contributed equally to the paper.