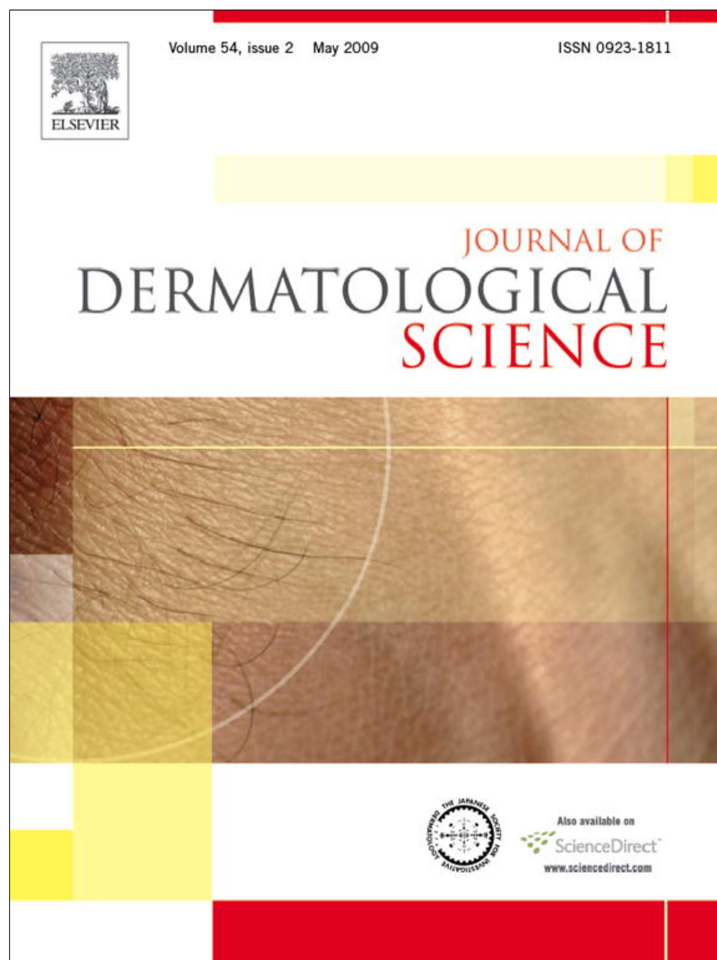
Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/copyright

Author's personal copy

Table 1
LCs in *émigrés* from normal skin and CA ($\bar{x} \pm s$).

Surface markers of LCs	Temperature	Normal skin ($n = 5$)	CA ($n = 5$)	p
CD1a+ (%)	37 °C	0.67 ± 0.26	1.22 ± 0.28	<0.05
	42 °C	1.17 ± 0.20	3.14 ± 0.16	<0.01
	45 °C	2.25 ± 0.36	4.71 ± 1.11	<0.01
p		<0.01	<0.01	
CD1a+/CD83+(%)	37 °C	0.59 ± 0.08	0.68 ± 0.25	0.471
	42 °C	1.14 ± 0.17	2.37 ± 1.17	<0.05
	45 °C	1.67 ± 0.54	4.44 ± 0.48	<0.01
p		<0.01	<0.01	

The significances of differences between the numbers of CD1a+ and CD1a+/CD83+ LCs in *émigrés* subjected to different temperatures were analyzed by repeated measures analysis of variance (ANOVA) and, the significances of differences between the numbers of CD1a+ and CD1a+/CD83+ LCs in *émigrés* from normal skin and CA at the same temperatures were analyzed by independent *t*-test.

Acknowledgments

This work was supported by grants from Program for Changjiang Scholars and Innovative Research Team in University (IRT0760), National Natural Science Foundation of China (No. 30740082), and Program for New Century Excellent Talents in University (NCEP-04-028).

References

[1] Ghaznawie M, Papadimitriou JM, Heenan PJ. The repopulation of murine Langerhans cells after depletion by mild heat injury. Br J Dermatol 1999; 141:206–10.
[2] Ostberg JR, Kabingu E, Repasky EA. Thermal regulation of dendritic cell activation and migration from skin explants. Int J Hyperthermia 2003;19: 520–33.
[3] El-Tonsy MH, Anbar TE, El-Domyati M, Barakat M. Density of viral particles in pre and post Nd: YAG laser hyperthermia therapy and cryotherapy in plantar warts. Int J Dermatol 1999;38:393–8.
[4] Hong K, Greer CE, Ketter N, Van Nest G, Paliard X. Isolation and characterization of human papillomavirus type 6-specific T cells infiltrating genital warts. J Virol 1997;71:6427–32.
[5] Prechtel AT, Steinkasserer A. CD83: an update on functions and prospects of the maturation marker of dendritic cells. Arch Dermatol Res 2007;299: 59–69.
[6] Saeki H, Moore AM, Brown MJ, Hwang ST. Cutting edge: secondary lymphoid-tissue chemokine (SLC) and CC chemokine receptor 7 (CCR7) participate in the emigration pathway of mature dendritic cells from the skin to regional lymph nodes. J Immunol 1999;162:2472–5.
[7] Annels NE, Da Costa CE, Prins FA, Willemze A, Hogendoorn PC, Egeler RM. Aberrant chemokine receptor expression and chemokine production by Langerhans cells underlies the pathogenesis of Langerhans cell histiocytosis. J Exp Med 2003;197:1385–90.
[8] Hildebrandt B, Wust P, Ahlers O, Dieing A, Sreenivasa G, Kerner T, et al. The cellular and molecular basis of hyperthermia. Crit Rev Oncol Hematol 2002;43:33–56.
[9] Fleming MD, Pinkus JL, Fournier MV, Alexander SW, Tam C, Loda M, et al. Coincident expression of the chemokine receptors CCR6 and CCR7 by pathologic Langerhans cells in Langerhans cell histiocytosis. Blood 2003;101: 2473–5.
[10] Danno K, Sugie N. Effects of near-infrared radiation on the epidermal proliferation and cutaneous immune function in mice. Photodermatol Photoimmunol Photomed 1996;12:233–6.

Xiaodong Li[a,b], Xing-Hua Gao[a,*], Li Jin[c],
Yakun Wang[a], Yuxiao Hong[a], Uwesu Omari Mchepange[a],
Xiaoqin Wang[a], Yi Jiang[a], Huachen Wei[ad], Hong-Duo Chen[a]

[a]*Department of Dermatology, No. 1 Hospital of China Medical University, 155N. Nanjing Street, Shenyang 110001, China*
[b]*Department of Dermatology, Fengtian Hospital Affiliated to Shenyang Medical College, China*
[c]*Department of Gynecology, No. 1 Hospital of China Medical University, Shenyang 110024, China*
[d]*Department of Dermatology, Dermatology Research Laboratories, Mount Sinai Medical Center, New York, NY 10029, United States*

*Corresponding author. Tel.: +86 24 8328 2326;
fax: +86 24 8328 2633
E-mail address: gaobarry@hotmail.com
(X-H. Gao)

8 August 2008

doi:10.1016/j.jdermsci.2008.12.004

Letter to the Editor

Silencing the androgen receptor: New skills for antiandrogen oligonucleotide skin and hair therapy

ARTICLE INFO

Keywords:
Antiandrogen oligonucleotides; Skin and hair; Cutaneous therapy

Hair growth and follicular cycle are regulated predominantly through androgens under complex genetic and hormonal control. Human hair growth occurs in cycles of three phases, anagen (continuous growth), catagen (cessation of growth) and telogen (resting phase). In genetically susceptible subjects, hair follicles in vertex and frontal regions of the scalp respond to androgens by reducing length of the anagen phase and regression of the follicles producing weaker and thinner hairs. More than 50% of men by the age of 50 years and women over 60 years suffer from androgenetic alopecia. The mesenchymal-derived dermal papilla cells (DPC) exert a control on the hair growth cycle, express androgen receptor, are responsive to androgen hormones and are implicated in triggering baldness in humans. Today, androgens are considered inhibiting hair follicle activity by early inducing catagen. Although the pathogenic mechanisms underlying androgenetic alopecia are not fully understood it is now accepted that androgens (mainly testosterone and dihydrotestosterone) inhibit hair follicle activity, probably by triggering the expression or repression of some genes (not yet identified) involving modification of DPC and epithelial cells relationship, thereby leading to the induction of programmed cell death. Consequently, the anagen length period shortens, favoring catagen and finally miniaturizing the follicle and thinning the hair [1,2]. Moreover, other skin disorders such as acne vulgaris, hirsutism and seborrhea are dependent or sensitive to the androgens action [3,4]. With this in view, the developing of new topical therapeutics

Author's personal copy

aiming at down regulating or silencing the expression of the AR seems the right choice as current therapeutic strategies are not completely satisfactory in terms of clinical efficacy and potential secondary effects [5].

In this sense, oligonucleotides deriving from human genome knowledge and molecular genetics constitute a very appealing new class of therapeutics as they act selectively at the level of gene expression, allowing the design of more effective and less toxic therapeutics.

There are two main kinds of therapeutic oligonucleotides, antisense oligonucleotides and small interfering RNA (siRNA) that clearly differ in mode of action, stability, mRNA interaction and outcome in silencing gene. In a therapeutic point of view the use of one or the other would depend on doses, persistence and toxicity and the type of condition to be treated and the possibility of an optimal delivery formulation.

We assessed the use of both molecules antisense and siRNA oligonucleotides to achieve the downregulation of the AR gene expression in diverse human cellular models to evaluate their potential for skin and hair treatments.

The best way to be confident on an antiandrogen oligonucleotide action is through measuring the reduction of both target AR mRNA and AR protein levels. In accordance, our experiments showed reductions of AR protein and function, indicating antiandrogen activity. Actually, the stimulation of an androgen-responsive gene by DHT was cut down in DPC by treatment with different molecules of antisense antiandrogen oligonucleotides (ASAO). An example of antiandrogen activity inhibition by the 18.1 ASAO in DPC is shown in Fig. 1a. Moreover, ASAO efficacy varies depending on which accessibility region, as disclosed previously [6] is matching the ASAO. In fact, we also demonstrate that the unraveling of those access sites is essential to the efficacy in the siRNA design targeting to the AR. It is noteworthy in these results the pharmacological evidence that the potency of inhibition of AR expression by siRNA correlates with the pattern corresponding to the tested ASAO. Thus, siRNA 71 was mild whereas siRNA 18.1 as well as siRNA 24 (Fig. 1b) were, in our cellular models, potent inhibitors of the androgen-dependent gene expression comparable to usual antiandrogens such as flutamide. These results as a whole open new insights in antiandrogen therapy.

Therapeutic oligonucleotide technology seems a good choice for dermatology treatments because of the suitability of the target organ. On the other hand, both active molecules are today seen as promisingly therapeutic drugs in number of clinical trials on several pathologies [7–10]. Antiandrogen therapeutic oligonucleotides targeting the downregulation of the AR expression is advantageous because both will be possible to eliminate the only way for androgens to act and simultaneously this strategy allows the medication to be topically administrated. In fact, this could be very useful in a long-term treatment of, for instance, androgenetic alopecia, being an alternative way to systemic administration of 5 alpha reductase inhibitors such as finasteride. Moreover it could be used as a specific antiandrogenic contribution in the treatment of acne if prescribed as a choice of topical administration instead of the oral use of drugs such as the retinoid isotretinoin, or others. From the therapeutic point of view the use of one or the other antiandrogen oligonucleotide would depend on features such as doses, persistence, toxicity, and also the possibility of an optimal delivery formulation.

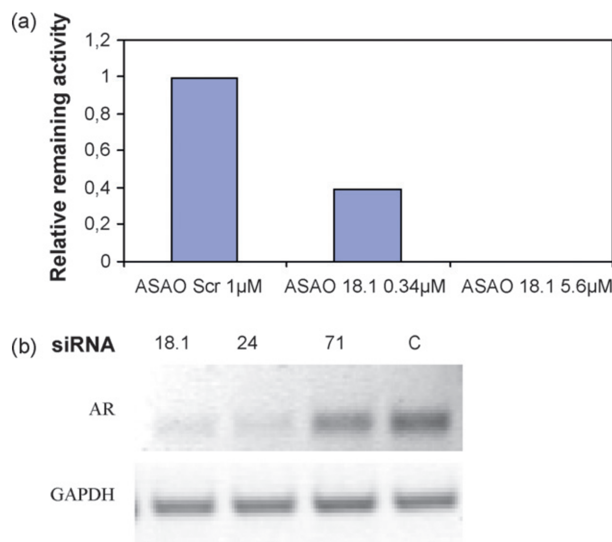


Fig. 1. Effect of antiandrogen oligonucleotides on AR activity and expression. (a) Androgen-responsive gene expression inhibition by the ASAO 18.1 in dermal papilla cells. Dermal papilla cells were transfected by electroporation with pSVAR, pCMVSportBgal and $p(Pre)_2$TATALuc (20 μg each). 24 h later cells were transfected with liposomes containing ASAO 18.1 or Scr AS and stimulated with DHT 10 nM. Oligonucleotide transfection was repeated after 3 h. After 5 h from the first oligonucleotide transfection luciferase activity was assessed and normalized by beta galactosidase activity. Each condition was repeated five times. Results are expressed as % inhibition. (b) Modulation of AR mRNA expression in human skin fibroblasts and dermal papilla cell cultures. Agarose gel electrophoresis of the RT-PCR fragments. Results are shown for skin fibroblasts as they were identical for dermal papilla cells. Cells were transfected with 1 μM siRNA by electroporation. RNA was extracted after 48 h, cDNA was synthesized and AR mRNA was analyzed by semiquantitative RT-PCR. GAPDH was analyzed as control.

To be functional, therapeutic oligonucleotides must properly enter the cell and remain relatively stable. Encapsulation in or complexation with liposomes could be suitable carrier for cell internalization as well as bonding oligonucleotides to lipophilic compounds such as neutral or cationic lipids. However, different formulations of liposomes could do different works, each delivery formulation with liposomes should be tested for any given oligonucleotide molecule because of the variability in cellular uptake or oligonucleotide protection.

In our hands liposomes comprising the lipid phosphatidylcholine worked efficiently in delivering the strong ASAO ODN 18.1 into dermal papilla cells in primary cultures as almost 100% transfection efficiency (Fig. 2a) and 100% AR function inhibition (Fig. 1a) were achieved. Moreover, cutaneous administration allows reaching almost exclusively the hair follicle structure upon one application on the animal skin probably via the follicular infundibulum (Fig. 2b).

The relatively low concentrations needed to the pharmacological action, the efficacy of these antiandrogen therapeutic oligonucleotides in modulating the AR expression and the high level of cell transfection with adequate liposomes open the way to new safer and more efficient therapies of dermatological androgen-dependent disorders. The multiplicity of antiandrogen oligonucleotide types enlarges the scope of formulations to be applied satisfying the clinical needs.

Author's personal copy

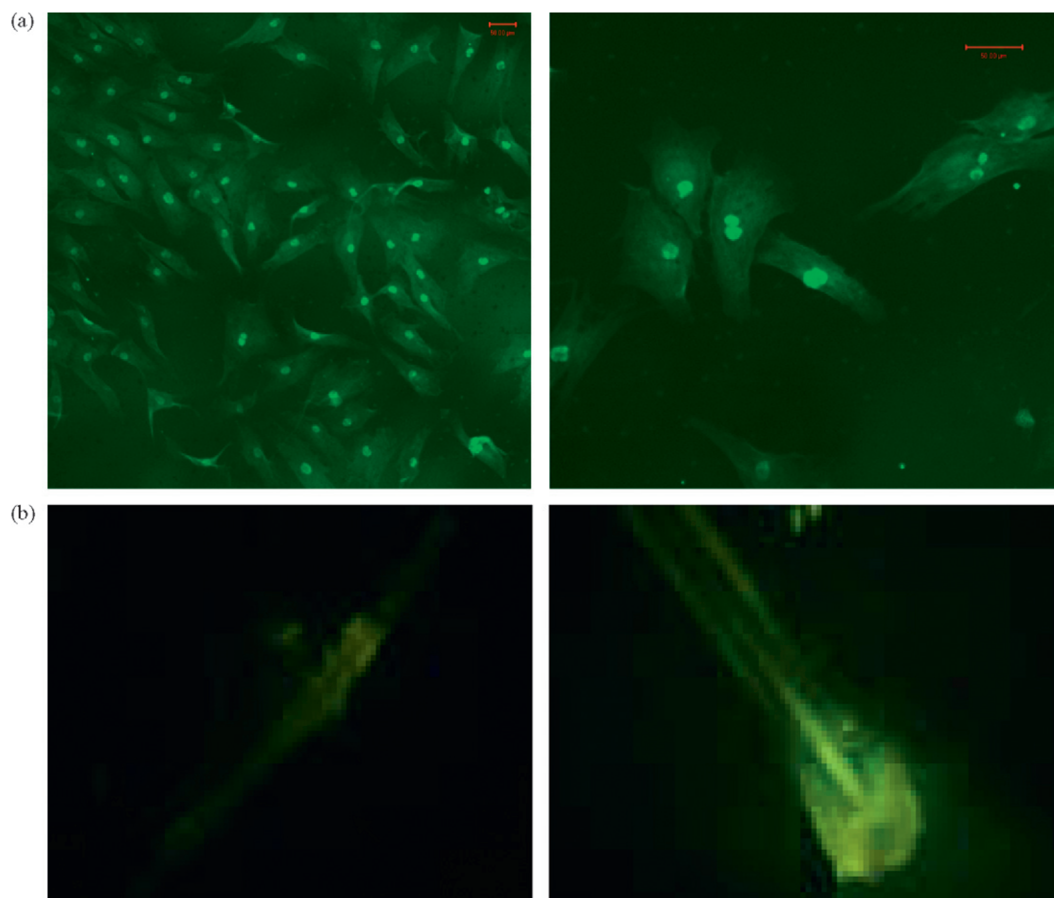


Fig. 2. Efficient vectorization of fluoresceinated ASAO 18.1 by specially designed liposomes. (a) *Confocal laser scanning microscopy*. Dermal papilla cells were transfected with liposomes containing a fluoresceinated antisense oligonucleotide against AR (ASAO 18.1, 8 μM). Left panel shows an almost 100% transfection. A more detailed view is in right panel. (b) *Fluorescent microscopy*. Intact pig skin was treated with liposomes containing the fluoresceinated antisense oligonucleotide ASAO 18.1. Left panel shows a control untreated skin section; right panel shows a treated skin section where the hair follicle silhouette is easily visualized by fluorescence.

Acknowledgements

We are indebted to Dr. Arturo Gonzalez Marlia and Dr. Juan Cebrian for discussions and support. We are also grateful to Dr. Fernando Stengel for permanent suggestions, discussions and encouraging support.

References

[1] Inui S, Fukuzato Y, Nakajima T, Yoshikawa K, Itami S. Androgen inducible TGF-Beta 1 from balding dermal papilla cells inhibits epithelial cell growth: a clue to understand paradoxical effects of androgen on human hair. FASEB J 2002;16:1967–9.
[2] Brodland D, Muller S. Androgenetic alopecia (common baldness). Cutis 1991;47:173–6.
[3] Sinclair R. Male pattern androgenetic alopecia. BMJ 1998;317:865–9.
[4] Shaw JC. Acne: effect of hormones on pathogenesis and management. Am J Clin Dermatol 2002;20:3001–15.
[5] Sawaya M, Shapiro J. Androgenetic alopecia, new approved and approved treatments. Dermatol Clin 2000;18:47–61.
[6] Balaña ME, Alvarez Roger C, Dugour AV, Kerner NA. Antiandrogen oligonucleotides: active principles in hair- and skin-derived culture cells. J Drugs Dermatol 2004;3:287–94.
[7] Maksymowych WP, Blackburn Jr WD, Tami JA, Shanahan Jr WR. A randomized, placebo controlled trial of an antisense oligodeoxynucleotide to intercellular adhesion molecule-1 in the treatment of severe rheumatoid arthritis. J Rheumatol 2002;29:447–53.
[8] van Assche G, Rutgeerts P. Antiadhesion molecule therapy in inflammatory bowel disease. Inflamm Bowel Dis 2002;8:291–300.
[9] Morris MJ, Tong WP, Cordon-Cardo C, Drobnjak M, Kelly WK, Slovin SF, Terry KL, et al. Phase I trial of BCL-2 antisense oligonucleotide (G3139) administered by continuous intravenous infusion in patients with advanced cancer. Clin Cancer Res 2002;8:679–83.
[10] Vitravene Study Group. A randomized controlled clinical trial of intravitreous fomivirsen for treatment of newly diagnosed peripheral cytomegalovirus retinitis in patients with AIDS. Am J Ophthalmol 2002;133:467–74.

Andrea Dugour[1], Karin Hagelin[1], Cintia Smus,
María Eugenia Balañá, Néstor Kerner*

Fundación Pablo Cassará, Centro de Ciencia y Tecnología Dr. Cesar Milstein, Saladillo 2468 C1440FFX, Ciudad de Buenos Aires, Argentina

*Corresponding author. Fax: +54 11 4687 4065
E-mail address: nkerner@fundacioncassara.org.ar

[1]Both authors contributed equally.

27 June 2008

doi:10.1016/j.jdermsci.2008.12.008