ORIGINAL ARTICLES

Repigmentation of Gray Hair After Thyroid Hormone Treatment

P Redondo,[a] M Guzmán,[b] M Marquina,[a] M Pretel,[a] L Aguado P Lloret,[a] and A Gorrochategui[c]

[a]Departamento de Dermatología and [b]Área de Terapia Celular, Clínica Universitaria de Navarra, Pamplona, Spain
[c]Clínica Dermatológica, Bilbao, Spain

Abstract. *Introduction and objectives.* Darkening of gray and white hairs occurred in 2 patients with increased exogenous triiodothyronine (T3) due to treatment of myxedema coma in one case and iatrogenic hyperthyroidism in the other. We hypothesized that thyroid hormone may affect the homeostasis of hair follicles. To test our hypothesis and investigate the influence of thyroid hormone on the hair cycle, we used an in vivo murine model and an in vitro model based on culture of follicular units. *Methods.* We used the standard C57BL/6 murine model of the hair cycle. T3 (0.5 #mg) dissolved in ethanol was applied topically once daily for 10 days to a depilated area in the telogen phase on the backs of the mice. Follicular units, obtained from hair transplant interventions, were cultured in vitro with different concentrations of T3. *Results.* On day 5, all T3-treated mice entered the anagen phase, whereas the anagen phase started spontaneously in control mice on day 9, and not until day 15 had all controls entered this phase. In the in vitro experiment, follicular units treated with 100 nmol/L T3 grew significantly larger compared to the control group. *Conclusions.* These data suggest that follicles in the telogen phase can be induced to enter the anagen phase by the topical application of T3. In the in vitro experiments, T3 stimulated hair shaft growth. Follicular melanocytes may be the target cell for these actions.

Key words: thyroid hormone, hair follicle, melanocytes, mice.

REPIGMENTACIÓN DEL PELO CANOSO TRAS TRATAMIENTO CON HORMONA TIROIDEA

Resumen. *Introducción y objetivos.* Basándonos en dos pacientes con oscurecimiento de sus canas que sufrieron una elevación exógena de T3, debida a un coma mixedematoso y a una descompensación de su enfermedad, sugerimos que la hormona tiroidea puede tener un efecto en la homeostasis del folículo piloso. Para ello se utilizan un modelo animal de ratón y un modelo de cultivo *in vitro* de unidades foliculares, con el objeto de valorar la influencia de la hormona tiroidea en el ciclo capilar.
Métodos. Sobre un modelo de ratón estandarizado para el estudio del ciclo capilar (C57BL/6), aplicamos tópicamente T3 (0,5 μg) disuelta en etanol una vez al día durante 10 días en el dorso de ratones depilados en fase de telogen. Cultivamos *in vitro* unidades foliculares, obtenidas de cirugía capilar, con diferentes concentraciones de T3.
Resultados. In vivo, al quinto día del tratamiento el 100 % de los ratones estudiados entraron en anagen, mientras que en el grupo control se apreció un inicio del anagen el día 9, que no se completó en todos los ratones hasta el día 15. *In vitro*, las unidades foliculares tratadas con 100 nM de T3 crecieron de forma significativa respecto al grupo control.
Conclusiones. Estos datos sugieren que los folículos en telogen pueden estimularse para entrar en anagen tras la aplicación tópica de T3. Esta hormona puede revertir el encanecimiento del pelo terminal. En cultivo, la administración de T3 estimula el crecimiento del tallo piloso. Los melanocitos foliculares podrían ser la célula diana para estas acciones.

Palabras clave: hormona tiroidea, folículo piloso, melanocitos, ratones.

Correspondence:
Pedro Redondo.
Departamento de Dermatología.
Clínica Universitaria de Navarra.
31008 Pamplona. Spain.
predondo@unav.es

Introduction

Graying of hair correlates with chronological aging and occurs in all individuals regardless of sex and race. At least half the hair of half of all white individuals is thought to be gray by the time they are 50 years old.[1] Although premature grayness usually appears with no underlying

disease, the graying process has been associated with pernicious anemia, hypothyroidism, osteopenia,[2] and a range of uncommon syndromes. There have been isolated reports of reversal of graying after epinephrine injections[3] and sympathectomy[4] and by vasoconstriction or continuous exposure to low temperatures.

In human skin, at least 2 different populations of melanocyte are present: those in the epidermis and those in hair follicles.[5,6] Melanogenesis in hair follicles is related to the stage in the hair growth cycle, whereas epidermal melanogenesis is continuous[7] and can be affected by UV radiation. Hair follicle melanocytes can be divided into 3 different subpopulations. The first, found in the hair bulb, only expresses tyrosinase-related protein (TRP) 2, does not proliferate, and presumably represents the melanocyte stem cells of the hair follicles. The second, found in the outer root sheath, expresses TRP2 and to a lesser extent TRP1, shows proliferative activity during the early and intermediate anagen phase, and represents differentiated melanocytes. The third, localized to the hair matrix over the dermal papilla, expresses TRP1, TRP2, and tyrosinase, proliferates only during the intermediate anagen phase, produces melanin during the intermediate and late anagen phase, and progressively disappears in the catagen phase.[8] The last 2 subpopulations express c-kit and depend on stem cell factor (SCF)/c-kit signaling during the anagen phase.[9]

The best model for studying the hair growth cycle is a highly standardized C57BL/6 mouse model, in which manual depilation induces the synchronous growth of terminal hair.[10] From the skin color of the mice after depilation, we can readily distinguish the telogen phase (pink color) from the anagen phase (gray to black color). This model is widely used and the duration of each phase of the hair growth cycle and the correlation with clinical and pathological variables are perfectly well defined.[10] Between the telogen and anagen phase, the resting melanocytes proliferate, differentiate, and migrate within the hair follicle in synchronous fashion to regenerate and form a new hair bulb.

Thyroid hormone is essential for homeostasis in cells derived from the neural crest—indeed, congenital hypothyroidism causes severe neurological damage. In the skin, it is known that thyroid hormone plays a role in epidermal differentiation, in enhancing local response to growth factors, in the physiology of sebaceous, eccrine, and apocrine glands, and in hair growth.

In this study, we discuss the possible role of thyroid hormone in the darkening of gray hair with initial reference to clinical findings from 2 patients. Furthermore, we investigated the effect of triiodothyronine (T3) on hair follicles in a mouse model with a view to defining the effect of this hormone on melanocyte function. Finally, we corroborated that T3 stimulates hair shaft growth in an in vitro experiment with human follicular units. On the basis of these results and a review of the literature, we formulate several hypotheses on the role of melanocytes not only in repigmentation but in the whole process of follicular homeostasis.

Materials and Methods

Case Reports

Patient 1

A 63-year-old man, who and been progressively graying over the years and who had a 1-year history of fungoid mycosis (stage IA) treated with psoralen-UV-A (PUVA) (3 sessions/wk), went into a myxedema coma of unknown cause. When he attended the clinic for a PUVA session accompanied by a family member, he showed a low level of consciousness, irregular breathing, inflammation, reddening of the face and both hands, and periorbital edema. His vital signs were as follows: systolic blood pressure, 110 mm Hg; diastolic blood pressure, 60 mm Hg; pulse rate, 56 beats/min; and body temperature, 35ºC. Of note in the laboratory tests were free T3 levels of 0.4 pg/mL (normal range, 1.5-4.3 pg/mL), free thyroxine (T4) levels of 1.8 pmol/mL (normal range, 10.5-25.0 pmol/mL), and thyroid stimulating hormone (TSH) levels of 102 mU/L (normal range, 0.45-7.0 mU/L). The patient was admitted to the intensive care unit (ICU) for 10 days, where he received support measures and intravenous therapy with 0.5 mg of L-thyroxine. He responded satisfactorily and continued oral therapy with 0.1 mg of L-thyroxine. He was able to return home after a month. On discharge from the ICU, complete repigmentation of all gray hairs of his scalp was observed and these have retained their coloration during 2 years of follow-up. His underlying disease did not show significant changes and he still needed specific treatment. The patient did not receive any other medication while he was in hospital.

Patient 2

The second patient was a 51-year-old man with Cowden syndrome and a history of thyroid carcinoma treated by surgery and thyroid hormone replacement therapy. His hair had gone progressively gray over time. Two years later, he developed non-Hodgkin lymphoma of the tonsils and cervical lymph nodes, which was treated with chemotherapy and radiotherapy (total dose, 36 Gy). At the time, 60% of his hair was white. After completing 4 cycles of chemotherapy (cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin) he presented in the clinic suffering from nervousness, anxiety, and insomnia. He had lost 15 kg. He was diagnosed with iatrogenic hyperthyroidism caused by

hormone replacement therapy (0.2 mg/d of L-thyroxine). The laboratory tests performed revealed free T3 levels of 1.62 pg/mL, free T4 levels of 34.4 pmol/L, and undetectable TSH (<0.1 mU/L). During this time, complete repigmentation of his hair occurred. The levels of free T4 were normal 3 months after daily treatment with 0.15 mg of L-thyroxine. The pigmentation of his hair has persisted during the 3 years of follow-up.

Both patients received high doses of thyroid hormone, one due to myxedema coma and the other due to overcompensation during thyroid hormone replacement therapy. Although other causes for the reversal of the graying processes cannot be ruled out, such as for example progression of cutaneous lymphoma or PUVA therapy in the first patient,[11] or chemotherapy in the second one,[12] hair pigmentation due to thyroid hormone seems the most plausible and reasonable explanation. In the first patient, repigmentation cannot be attributed to psoralens or UV-A, as this treatment was being performed for months before any affect on hair color was noted.

Mice

The animals were cared for and handled in accordance with the standard protocols. Six-week-old female C57BL/6 mice were used (Jackson Labs, Bar Harbor, USA) with an approximate weight of 20 g. Once acclimatized and after a week in observation, the animals were divided into 2 groups of 5 and the dorsum was depilated with cold wax.[10] The experiment was approved by the corresponding animal ethics committee.

T3 was purchased from Sigma (Barcelona, Spain). We used C57BL/6 experimental mice, standardized for studies of the hair growth cycle.[10] Specifically, cold wax was applied to the dorsum of the 7-week-old mice. After application and depilation, a pink skin color was noted, indicative that all follicles were in the telogen phase. With a micropipette, 0.5 µg of T3 dissolved in 20 µL of ethanol was applied to the dorsum of all the mice in one of the groups, while 20 µL of ethanol was applied to the other group as control. Application was daily, and the study lasted 10 days.

Culture of Follicular Units

After obtaining the appropriate informed consent, follicular units in the anagen phase were obtained by dissection under a microscope of surplus samples from the occipital region of 2 women (aged 30 and 38 years) undergoing hair transplantation. The follicular units were cultured in Williams E medium (Gibco, Madrid, Spain) with glutamine, fetal bovine serum (1%), insulin (10 ng/mL), transferrin (10 µg/mL), hydrocortisone (10 ng/mL), and sodium

selenite (10 ng/mL). To avoid superinfection, the culture medium was treated with penicillin/streptomycin (50 U/mL-50 µg/mL) and amphotericin B (Fungizone, 2.5 µg/mL).[13] The follicular units were divided into 2 groups: a control group and a group with the same culture medium but with 100 nmol/L of T3 added. The follicular units were kept half immersed in 24-well plates in an incubator set to a uniform temperature of 37ºC under an atmosphere of 95% air and 5% carbon dioxide. The medium was changed every 72 hours. Photographs were taken with a digital camera using an inverted binocular microscope at the beginning of culture (baseline), and at 72 and 144 hours. All photographs were taken using the same settings.

Results

Mice

At the age of 7 weeks, the skin of all C57BL/6 mice visible after depilation had the characteristic pink color of the telogen phase (Figure 1). When the mice entered the anagen phase, their hair started to grow again, as reflected by a progressive gray to black pigmentation of the dorsum. After 5 days of treatment with 0.5 µg/d of T3, all study animals had entered the anagen phase (Figure 2). In the control

Figure 1. Seven-week-old female C57BL/6 mice that have been depilated with cold wax after application of surface anesthesia with ketamine at a normal dose. An even pink coloration of the bare skin can be seen, characteristic of a synchronous telogen phase.

Figure 2. After 5 days of treatment, a change in the skin coloration of the mouse treated with topical T3 (right) was observed compared to the control mouse (left), suggesting synchronous entry into the anagen phase.

group, increased pigmentation—suggestive of the start of the anagen phase—was observed from day 9, but it was not observed in all mice until day 15.

After 8 days of treatment, a histological study was undertaken to confirm induction of the anagen phase. The skin, stretched and adhered to photographic paper to prevent creasing, was fixed in formalin, washed with ethanol, and embedded in paraffin according to standard techniques. Hematoxylin-eosin and Masson trichromic staining was performed (Figure 3). All experiments were repeated twice.

Cultures

Eight follicular units per group were used from each donor. The results were very similar in both cases. In one of the groups, after 72 hours, significant growth was seen in 6 of the 8 follicular units treated with T3 whereas growth only occurred in 3 of the 8 follicular units in the control group (Figure 4). During this time, the follicular architecture was completely conserved. The measurements were made using hardcopies of uniformly enlarged photographs. The mean (SD) growth for the group treated with 100 nM of T3 was 1.2 (0.05) mm at 72 hours compared to 0.65 (0.05) mm for the control group. At the 200 nmol/L dose of T3, the differences were not increased (data not shown).
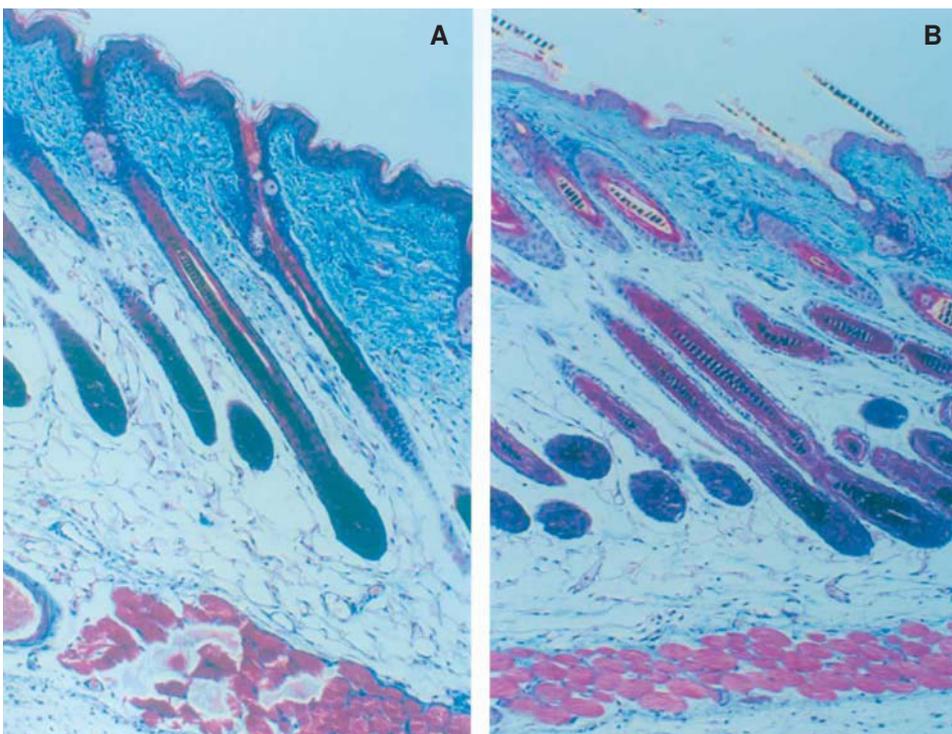

Figure 3. Masson trichromic staining of the biopsy performed at day 10. (A) Control group: follicles in the anagen phase (IV) in which the tip of the new hair shaft still had not reached the epidermis (×100). (B) Topical T3-treated group: a higher follicular density can be seen in a more advanced anagen phase (VI), with hair protruding through the epidermis (×100).
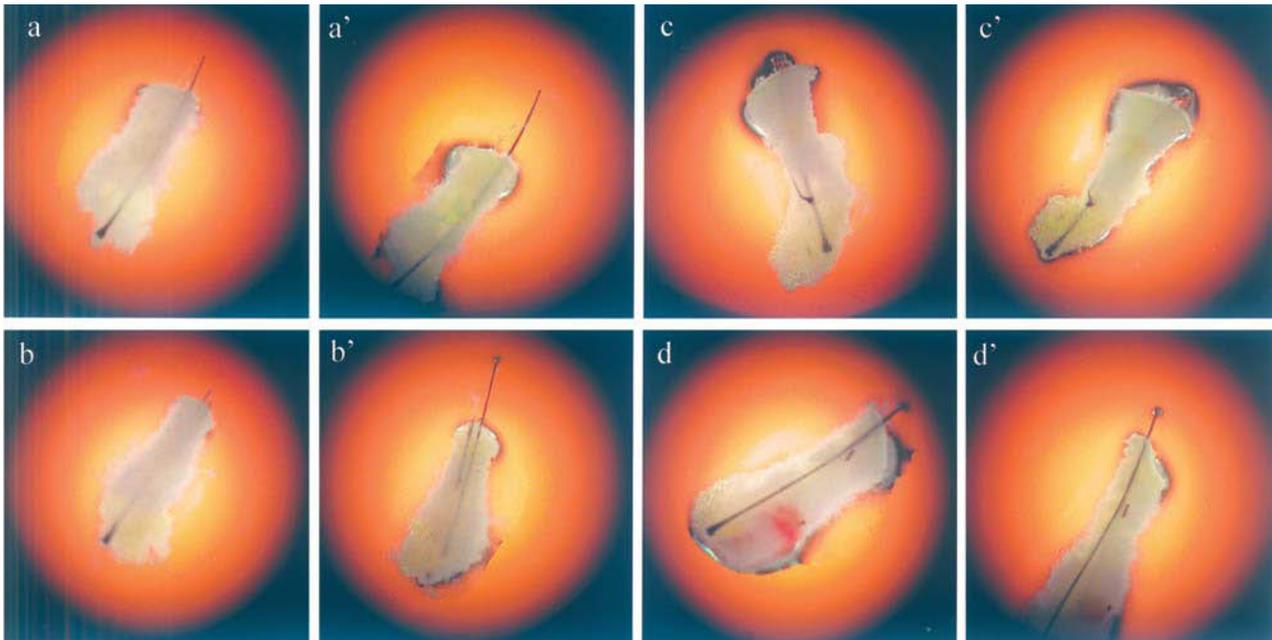
Figure 4. Follicular units at baseline (a-d) and after 72 hours (a'-d') of in vitro culture. Significant growth of the hair shaft can be seen in samples treated with 100 nmol/L T3 (a, b) compared to control samples (c, d). The images correspond to photographs representative of all experiments.

Discussion

Hair grays due to changes (slowing or failure) in the repopulation of the new hair bulb in anagen phase with "fresh" melanocytes derived from the outer root sheath of the hair.[14] Expression of tyrosinase and melanogenic activity can only be detected in the subpopulation of follicular melanocytes close to the papillary dermis. This structure, the papillary dermis, secretes growth factors such as SCF and hepatocyte growth factor, which are probably involved in the conversion of hair follicles to fully differentiated melanin producers.[9]

Several studies have shown that hair graying is not an irreversible process. There have been isolated reports of repigmentation of hair after radiotherapy for cancer or after inflammatory processes such as erythrodermic eczema, erosive candidiasis of the scalp,[15] carbuncle,[16] and furunculosis.[17] There have also been reports of temporary hair darkening after administration of high doses of para-aminobenzoic acid[18] and after using drugs such as verapamil,[19] tamoxifen,[20] cyclosporine,[21] etretinate,[22] levodopa,[23] latanoprost,[24] and arsenic.[25] Likewise, anecdotal cases have been published of repigmentation of gray hair after exposure to x-rays and following use of PUVA in a patient with Sézary syndrome,[11] in 2 patients with celiac disease,[26] and in 2 patients with porphyria cutanea tarda.[27]

The skin is a well-known target of thyroid hormones. Low hair density on the scalp, thinning of the outer third of the eyebrows, and decreased body hair have often been associated with hypothyroidism and are due to an increased proportion of follicles in the telogen phase compared to those in the anagen phase.[28] Given that these situations can be corrected after the administration of L-thyroxin, thyroid hormone is implicated in the growth of human hair.[29] The thyroid hormone receptors TR-α and TR-β have been detected in hair follicles, with TR-β being the predominant form.[30,31] These receptors have been detected in cells of the outer root sheath, cells of the papillary dermis, and fibroblasts surrounding the outer lining of the follicle. In addition, in vitro studies with cells of the outer root sheath and the papillary dermis have shown that thyroid hormone may have a direct effect on the growth of hair follicles through its specific receptors.[32] Our study showed how topical application of T3 accelerates conversion to the anagen phase in a standard murine model. In an in vivo study with SKH-1 mice (hairless mice due to a genetic mutation) and rats, topical administration of T3 in liposomes was seen to significantly increase the number and size of hairs compared to the control group and another group treated with low doses, although this study did not assess the influence of the intervention on any specific phase of the hair growth cycle.[33] More interesting is a recent study that shows a variable influence of thyroid hormone on the skin according to route of administration (topical or intraperitoneal).[34] Specifically, the mice that received T3 intraperitoneally had an epidermis 10% thinner and a lower hair density (48% lower); in contrast, mice that were administered T3 topically had a 78% thicker epidermis and a higher hair density

(160% greater). These findings suggest that although T3 stimulates keratinocyte proliferation, it also favors release of proliferation inhibitors by dermal fibroblasts. In addition, administration of T3 to hypothyroid dogs increases conversion of hair to the anagen phase compared to euthyroid dogs or untreated hypothyroid dogs.[35]

In vitro studies of follicular units have shown how transforming growth factor (TGF)-β, phorbol esters (tetradecanoyl phorbol acetate [TPA]), bFGF, and fetal bovine serum reduce the growth of hair shafts and play an important role in inducing the catagen phase of the hair growth cycle.[13,36] This effect, of unknown origin, could be explained by the fact that both TGF-β[37] and bFGF[38] stimulate expression and production of c-kit. Melanocytes can regulate the hair growth cycle, pigmentation, and conversion to and from the anagen and catagen phases through expression of c-kit.[39] If expression of c-kit is inhibited with anti-TGF-β antibodies or with selective TGF-β inhibitors, for example, pigmentation of the hair shaft is lost and the anagen phase is extended.[40] All these substances (TGF-β, TPA, bFGF, etc) have in common that they favor proliferation and differentiation of melanocytes in pure cultures. Thyroid hormone is also necessary for the culture of melanocytes, as is the case with other cells derived from the neural crest. We can therefore infer that the melanocytes could be one of the cellular targets of all these substances (including thyroid hormone) when they act on hair follicles. Along these lines, we can speculate on the role played by thyroid hormone in melanocyte homeostasis, both with regard to nevus cells (nevus growth)[41] and epidermal melanocytes in a process as common as vitiligo, in which thyroid dysfunction has been documented in as many as 30% of newly appearing cases.[42] Specifically, as discussed earlier, thyroid hormones participate in the maturation of cells derived from the neural crest through regulation of β$_2$-adrenergic receptors. Thus, for example, the maturation and differentiation of astrocytes occurring after administration of thyroid hormone is mediated by β$_2$-adrenergic receptors.[43] With regard to the culture of melanocytes, some adrenergic drugs enhance melanogenesis and cell proliferation by stimulating these β$_2$ receptors, thereby increasing levels of cyclic adenosine monophosphate.[44] In contrast, the use of β-blockers may trigger the onset of vitiligo in predisposed patients.[45] Finally, some of the preparations that have proved effective in the treatment of certain cases of vitiligo have contained thyroid hormone.[46]

On a much more theoretical level, and without getting into the interaction of multiple neuroendocrine factors, we can infer that decreases in thyroid hormone levels affect melanocytes, as occurs with other cells derived from the neural crest. At times, sudden hair whitening may be preceded by a stressful experience or neuropsychiatric disorders, often related with decreased thyroid hormone or increased demand. It is popularly accepted that premature graying over a short period of time is caused by worries and being in a state of tension. Thus, events such as a fright, a stressful episode, and sustained anxiety (situations in which the appearance of alopecia areata or sudden graying of hair is widely documented both historically and culturally) may lead to a sudden increase in demand for T3. In situations in which it is impossible to produce more hormone, the first cells affected may be the follicular melanocytes. Epidermal melanogenesis is constant and may have lower hormonal demands, whereas follicular melanogenesis is cyclic, momentary, and may have a higher hormonal demand.

Melanocytes of the hair follicles reach their peak proliferative capacity during the early and intermediate anagen phase, which is associated with expression of c-kit on the surface of the melanocytes. Loss of pigmentation in the hair shafts in gray hairs is not due to complete loss of all follicular melanocytes; in fact, melanocytes negative for dopa and many other markers have been found in the outer root sheath of senile white hairs.[47] This finding leaves the door open to the possibility of stimulating the migration and differentiation of melanocytes to favor repigmentation of gray hair follicles.

Obviously, thyroid hormone may influence other cell types that are not studied or discussed here but that are present in normal skin, such as keratinocytes and fibroblasts. In addition, an essential point to be verified in the above hypothesis, and something that we have not been able to do because it was impossible for us to obtain depigmented follicular units, is whether in vitro repigmentation by selective culture with T3 is possible. Although gray hairs are not usually present in the occipital region (the region from which donated follicles are taken during hair transplantation), we do hope to have such follicular units available in the near future to confirm the described findings.

In summary:

1. Thyroid hormone stimulates in vitro growth of hair shafts in a culture of follicular units.
2. After topical application of T3 to C57BL/6 mice, follicles in telogen phase entered the anagen phase earlier.
3. In humans, thyroid hormone may reverse graying by repigmentation of terminal hair. Follicular melanocytes may be the target cell for these actions.

This work stemmed from clinical findings concerning the role of thyroid hormone in follicular repigmentation; subsequently, using standard models, we have shown other actions, more or less known, of T3 in the regulation of the hair growth cycle. At this point, we can postulate a possible role for melanocytes not only in repigmentation but in the whole process of follicular homeostasis. It therefore seems logical that these cells with neuronal morphology, derived from the neural crest, may play an essential role in the

regulation of the hair growth cycle and are not just limited to providing hair color. In everyday life, we see how gray hair of the beard and scalp is thicker and more resistant to thinning than pigmented hair. Pharmacological intervention, using melanocytes as new targets, might open up new lines of research in hair growth besides the preferred current ones aimed at blocking miniaturization due to sex hormones.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Tobin DJ, Paus R. Graying: gerontobiology of the hair follicle pigmentary unit. Exp Gerontol. 2001;36:29-54.
2. Rosen CJ, Holick MF, Millard PS. Premature graying of hair is a risk marker for osteopenia. Clin Endocrinol Metab. 1994; 79:854-7.
3. Lerner AB. Gray hair and sympathectomy. Report of a case. Arch Dermatol. 1966;93:235-6.
4. Ortonne JP, Thivolet J, Guillet R. Graying of hair with age and sympathectomy. Arch Dermatol. 1982;118:876-7.
5. Tobin DJ, Bystryn JC. Different populations of melanocytes are present in hair follicles and epidermis. Pigment Cell Res. 1996;9:304-10.
6. Botchkareva NV, Botchkarev VA, Gilchrest BA. Fate of melanocytes during development of the hair follicle pigmentary unit. JID Symp Proc. 2003;8:76-9.
7. Paus R, Cotsarelis G. The biology of hair follicles. N Engl J Med. 1999;341:491-7.
8. Tobin DJ, Hagen E, Botchkarev VA, Paus R. Do hair bulb melanocytes undergo apoptosis during hair follicle regression (catagen)? J Invest Dermatol. 1998;111:941-7.
9. Botchkareva NV, Khlgatian M, Longley BJ, Botchkarev VA, Gilchrest BA. SCF/c-kit signaling is required for cyclic regeneration of the hair pigmentation unit. FASEB J. 2001;15: 645-58.
10. Müller-Röver S, Handjiski B, van der Veen C, Eichmüller S, Foitzik K, McKay IA, et al. A comprehensive guide for the accurate classification of murine hair follicles in distinct hair cycle stages. J Invest Dermatol. 2001; 117:3-15.
11. Staughton RCD. Neoplasms of the scalp. In: Staughton RCD, editor. The color atlas of hair and scalp disorders. Ipswich: Cowell Ltd; 1988:59.
12. Robinson A, Jones W. Changes in scalp hair after cancer chemotherapy. Eur J Cancer Clin Oncol. 1989;25:155-6.
13. Philpott MP, Green MR, Kealey T. Human hair growth in vitro. J Cell Sci. 1990;97:463-71.
14. Takada K, Sugiyama K, Yamamoto I, Oba K, Takeuchi T. Presence of amelanotic melanocytes within the outer root sheath in senile white hair. J Invest Dermatol. 1992;99: 629-33.
15. Verbov J. Erosive candidiasis of the scalp, followed by the reappearance of black hair after 40 years. Br J Dermatol. 1981;105:595-8.
16. Bean WB. A note on post inflammatory hair darkening. Arch Dermatol. 1959;79:681-3.
17. Pinkus H. Postinflammatory hair darkening. Arch Dermatol. 1960;82:155-6.
18. Sieve BF. Darkening of grey hair following para-amino benzoic acid. Science. 1941;94:257-302.
19. Read GM, Verapamil and hair colour change. Lancet. 1991; 338:1520.
20. Hampson JP, Donnelly A, Lewis-Jones MS, Pye JK. Tamoxifen-induced hair colour change. Br J Dermatol. 1995;132: 483-4.
21. Rebora A, Delmonte S, Parodi A. Cyclosporin A-induced hair darkening. Int J Dermatol. 1999;38:229-30.
22. Vesper JL, Fenske NA. Hair darkening and new growth associated with etretinate therapy. J Am Acad Dermatol. 1996; 34:860.
23. Reynolds NJ, Crossley J, Ferguson I, Peachey RDG. Darkening of white hair in Parkinson's disease. Clin Exp Dermatol. 1989;14:317-8.
24. Wand M. Latanoprost and hyperpigmentation of eyelashes. Arch Ophtalmol. 1997;115:1206-8.
25. Juhlin L, Ortonne JP. Red scalp hair turning dark brown at 50 years of age. Acta Derm Venereol (Stockh) 1986;66:71-3.
26. Hill LS. Reversal of premature hair greying in adult coeliac disease. Br Med J. 1980;281:115.
27. Shaffrali FCG, McDonagh AJG, Messenger AG. Hair darkening in porphyria cutanea tarda. Br J Dermatol. 2002;146: 325-9.
28. Freinkel RK, Freinkel N. Hair growth and alopecia in hypothyroidism. Arch Dermatol. 1972;106:349-52.
29. Messenger AG. Thyroid hormone and hair growth. Br J Dermatol. 2000;142:631-5.
30. Billoni N, Buan B, Gautier B, Gaillard O, Mahé Y, Bernard BA. Thyroid hormone receptor (beta) 1 is expressed in the human hair follicle. Br J Dermatol. 2000;142:645-52.
31. Thompson CC, Bottcher MC. The product of a thyroid hormone responsive gene interacts with thyroid hormone receptors. Proc Natl Acad Sci USA. 1997;94:8527-32.
32. Ahsan MK, Urano Y, Kato S, Oura H, Arase S. Immunohistochemical localization of thyroid hormone nuclear receptors in human hair follicles and in vitro effect of L-triiodothyronine on cultured cells of hair follicles and skin. J Med Invest. 1998;44:179-84.
33. Safer JD, Fraser LM, Ray S, Holick MF. Topical triiodothyronine stimulates epidermal proliferation, dermal thickening, and hair growth in mice and rats. Thyroid. 2001;11:717-24.
34. Safer JD, Crawford TM, Fraser LM, Hoa M, Ray S, Chen TC, et al. Thyroid hormone action on skin: diverging effects of topical versus intraperitoneal administration. Thyroid. 2003;13:159-65.
35. Credille KM, Slater MR, Moriello KA, Nachreiner RF, Tucker KA, Dunstan RW. The effects of thyroid hormones on the skin of beagle dogs. J Vet Inter Med. 2001;15:539-46.
36. Soma T, Tsuji Y, Hibino T. Involvement of transforming growth factor-beta2 in catagen induction during the human hair cycle. J Invest Dermatol. 2002;118:993-7.
37. Kawakami T, Soma Y, Kawa Y, Ito M, Yamasaki E, Watabe H, et al. Transforming growth factor beta 1 regulates melanocyte proliferation and differentiation in mouse neural crest cells via stem cell factor/KIT signaling. J Invest Dermatol. 2002;118:471-8.
38. Burger P, Lukey PT, Coetzee S, Wilson EL. Basic fibroblast growth factor modulates the expression of glycophorin A and

c-kit and inhibits erythroid differentiation in K562 cells. J Cell Physiol. 2002;190:83-91.

39. Peters EMJ, Tobin DJ, Botchkareva N, Maurer M, Paus R. Migration of melanoblasts into the developing murine hair follicle is accompanied by transient c-kit expression. J Histochem Cytochem. 2002;50:751-66.

40. Tsuji Y, Denda S, Soma T, Raftery L, Momoi T, Hibino T. A potential suppressor of TGF-beta delays catagen progression in hair follicles. JID Symp Proc.2003;8:65-8.

41. Niepomniszcze H, Amad RH. Skin disorders and thyroid diseases. J Endocrinol Invest 2001;24:628-38.

42. Hegedus L, Heidenheim M, Gervil M, Hjalgrim H, Hoier-Madsen M. High frequency of thyroid dysfunction in patients with vitiligo. Acta Derm Venereol 1994; 74:120-3.

43. Gharami K, Das S. Thyroid hormone-induced morphological differentiation and maturation of astrocytes are mediated through the beta-adrenergic receptor. J Neurochem. 2000;75: 1962-9.

44. Hu DN, Woodward DF, McCormick SA. Influence of autonomic neurotransmitters on human uveal melanocytes in vitro. Exp Eye Res. 2000;71:217-24.

45. Schallreuter KU. Beta-adrenergic blocking drugs may exacerbate vitiligo. Br J Dermatol. 1995;132:168-9.

46. Ichimiya M. Immunohistochemical study of ACTH and alpha-MSH in vitiligo patients successfully treated with a sex steroid-thyroid hormone mixture. J Dermatol. 1999;26: 502-6.

47. Horikawa T, Norris DA, Johnson TW, Zekman T, Dunscomb N, Bennion SD, et al. DOPA-negative melanocytes in the outer root sheath of human hair follicles express premelanosomal antigens but not a melanosomal antigen or the melanosome-associated glycoproteins tyrosinase, TRP-1, and TRP-2. J Invest Dermatol. 1996;106: 28-35.