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Elaine FUCHS, *et al.*
Beta-Catenin vs Alopecia

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http://www.sciencedaily.com/releases/1998/11/981126103434.htm
November 26, 1998

Substance Discovered That Induces Hair Follicle Formation In The Mature Skin Cells Of Mice

Everyone has bad hair days. For 30 million men in the United States, roughly 40 percent of those over 35, every day is a no hair day. The good news is that thanks to new research, baldness may be fading away.

Researchers from the Howard Hughes Institute at the University of Chicago have induced hair follicle formation in the mature skin cells of mice. Follicle formation is a once-in-a-lifetime event that ordinarily happens only during early embryogenesis.

Their findings, reported in the November 25 issue of Cell, indicate that a molecule called beta-catenin may be the long sought message that instructs embryonic cells to become hair follicles, suggesting possible treatments for premature baldness.

"Beta-catenin can cause adult epithelial cells to revert to an embryonic-like state where they have the ability to choose to become a hair follicle," says Elaine Fuchs, Amgen Professor in the Departments of Molecular Genetics and Cell Biology, Howard Hughes Investigator, and lead author of the paper. “This is exciting because current treatments for baldness only work if there are living follicles left, or if the patient undergoes hair transplant surgery. Our research shows that new follicles can be created from adult skin cells if certain molecular players are induced to act.

Beta-catenin performs two very different functions. In adult epithelial cells, it participates in binding neighboring cells together to facilitate cell-cell communication. But during embryogenesis, beta-catenin appears to have another role: it reacts with a molecule called LEF-1, which is expressed only in cells that will eventually become hair follicles. Together, beta-catenin and LEF-1 form a transcription factor that binds to the cell’s DNA and activates the genes that instruct the cell to become a hair follicle.

Uri Gat, a postdoctoral fellow in Fuchs’s lab, created mice that constantly produced a stabilized form of beta-catenin in their skin. (Normally any excess beta-catenin that is not needed for cell-cell adhesion is quickly degraded.) "The process caused some epidermal cells to make the partner molecule, LEF-1," explains Fuchs. “Wherever both stabilized beta-catenin and LEF-1 were present in an epithelial cell, a new hair follicle formed. If we can induce those two partners at the right time, new hair could be formed in places where it has been lost.

The genetically engineered mice were exceptionally hairy. In some mice, most skin cells became hair follicles.

Unlike in embryogenesis, however, the genetically engineered skin of the transgenic mice made an endless supply of beta-catenin and benign follicle tumors formed.

"This is a case of too much of a good thing leading to a bad thing," says Fuchs. She cautions that the use of these exciting findings will still need more work before hair growth can be induced without danger of unwanted side-effects. "If we can find a way to transiently express beta-catenin in these skin cells, just until new follicles are established, and then turn it off, we may be able to prevent tumor formation and still allow hair follicles to form," says Fuchs. The flip side of this, she says, is that it is also a potential tool to stop unwanted hair growth by inhibiting the pathway.

It could also have applications in the agricultural industry, for example to engineer sheep with denser wool or thicker skin, Fuchs explains.

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http://www.sciencedaily.com/releases/2014/04/140401102709.htm
April 1, 2014

Lose the paunch, halt the hair loss?

A new discovery showing how hair growth activated fat tissue growth in the skin below the hair follicle could lead to the development of a cream to dissolve fat. In particular, the protein that activated hair follicle growth was shown to also inhibit fat production.

The world first research confirmed that changes in the hair growth cycle led to fluctuations in the thickness of the underlying fat layer of the skin -- essentially meaning that the skin can regulate fat production.

The research was led by Professor Fiona Watt at King's College London in collaboration with Professor of Dermatology Rodney Sinclair from the University of Melbourne and Epworth Hospital.

Professor Sinclair said these findings could potentially be used both as a means to replace fat lost in scar tissue or as a localized treatment for obesity.

"The specific chemicals identified in this study could be produced synthetically and used in creams for topical application to the skin to modulate growth of fat beneath the skin."

"A cream could trim fat specifically where it was applied by 'pausing' the production of factors that contribute to fat cell growth." he said.

The effect of changes in the fat tissue on the synchronized patterns of hair follicle growth has long been established.

"This is the first demonstration that the opposite also holds true in that the skin below the hair

follicle can regulate the development of fat," Professor Sinclair said.

This discovery could also affect future treatment of obesity, male and female pattern baldness and alopecia -- male and female baldness -- an autoimmune condition that affects one to two per cent of the general population at some stage in their life.

Related R&D by Dr Fuchs, et al.:

Method for Modulating Hair Growth
US2009203574

Inventors: FUCHS ELAINE / RHEE HORACE

The present invention relates to the use of Lhx2 as a target for modulating the hair growth. Screening assays for identifying agents which increase or decrease the expression or activity of Lhx2 are provided.

INTRODUCTION

[0001] This invention was made in the course of research sponsored by the National Institutes of Health (Grant No. R01-AR050452). The U.S. government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

[0002] Hair follicle morphogenesis involves a temporal series of reciprocal interactions between the ectoderm and its underlying mesenchyme (Hardy (1992) Trends Genet. 8:55; Millar (2002) J. Invest. Dermatol. 118:216; Schmidt-Ullrich and Paus (2005) Bioessays 27:247). During embryogenesis, the skin epidermis develops from a single uniform layer of multipotent cells, separated from the mesenchymally-derived dermis by a basement membrane of extracellular matrix. Cells of this proliferative basal layer can be committed into one of two major lineages. To serve its function as a protective barrier, cells directed towards the epidermal lineage begin a program of terminal differentiation by detaching from the basement membrane, moving outward toward the skin surface, and undergoing metabolic changes to create a keratinized, stratified squamous cell layer. Alternatively, cells of the basal layer can give rise to hair follicles. In response to inductive signals, embryonic hair morphogenesis begins with a localized thickening of epidermal cells and a subsequent bud-like down-growth into the dermis. Known as a hair placode or hair germ, these cells send a reciprocal signal back to the underlying mesenchymal cells to organize into a dermal condensate, the precursor of the dermal papilla. As the hair follicle continues to develop by growing further down into the dermis, a group of rapidly proliferating follicular cells called the matrix surrounds the dermal papilla, forming the hair bulb. Cells losing contact with the hair bulb become the outer root sheath, contiguous with the interfollicular epidermis. The close association between the matrix and dermal papilla within the hair bulb likely results in another set of epithelial-mesenchymal exchange of signals to begin terminal hair differentiation. Specific hair lineages are adopted by the matrix cells as they move upward in concentric rings of cells to form the different layers of the hair follicle, including the inner root sheath and hair shaft. At some point during this morphogenetic process, stem cells

residing in the bulge are specified and set aside for the postnatal hair cycle and epidermal repair.

[0003] Some of the molecular events involved in hair follicle morphogenesis have been elucidated. In response to an inductive Wnt and an inhibitory Bmp signal (Noggin), small hair placodes bud from the epithelium, giving rise to larger hair germs (DasGupta and Fuchs (1999) Development 126:4557; Huelsken, et al. (2001) Cell 105:533; Botchkarev, et al. (1999) Nature Cell Biol. 1:158; Jamora, et al. (2003) Nature 422:317). In the presence of the mitogen Shh, these hair germs develop further and grow downwards to form a mature follicle that actively produces hair (Chiang, et al. (1999) Dev. Biol. 205:1; Oro and Higgins (2003) Dev. Biol. 255:238; St-Jacques, et al. (1998) Curr. Biol. 8:1058). Although the molecular details of bud formation are still being defined, the general features of this process are repeated at the start of each postnatal hair cycle when multipotent stem cells in the hair follicle bulge become activated to initiate a new round of hair growth. In addition, the early epithelial remodeling to form the hair germ shares many features with the development of other epithelial tissues and organs, including feathers, teeth, and mammary glands (Hogan (1999) Cell 96:225; Pispa and Thesleff (2003) Dev. Biol. 262:195; Yue, et al. (2005) Nature 438:1026). Understanding how tissues form buds which then progress along different lineages is predicated on elucidating the molecular mechanisms that funnel these early signaling pathways into a transcriptional program that drives morphogenesis.

SUMMARY OF THE INVENTION

[0004] The present invention is a method for modulating hair growth by regulating the expression or activity of Lhx2. The present invention also relates to the use of Lhx2 in a screening assay to identify an agent which modulates hair growth. The screening assay involves contacting a test cell expressing a reporter operably linked to an Lhx2 promoter with an agent and detecting expression of the reporter in the test cell, wherein a decrease in reporter expression is indicative of an agent which stimulates hair growth and an increase in reporter expression is indicative of an agent which inhibits hair growth.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] FIG. 1 shows that Lhx2 maintains follicle stem cells in a quiescent, inactive state. FIG. 1A shows CD34 quantification by flow cytometry in telogen and anagen follicles during the first postnatal cycle. FIG. 1B shows loss of BrdU label retention in KO follicles. Following a 3-day BrdU pulse on d26-28 at the onset of anagen in both wild-type and KO skin grafts, and a 4-week chase when follicles had entered telogen, label retaining cells (LRCs) concentrated in the infrequently dividing bulge stem cells of wild-type follicles, but LRCs were diminished in Lhx2 KO skin. FIG. 1C shows increased BrdU incorporation by KO follicle stem cells. Following a 4-hour BrdU pulse at d40, when wild-type and KO follicles were in mid-anagen of their first postnatal hair cycle, cells were isolated and [alpha]6-integrin expressing S-phase cells were quantified by flow cytometry.

[0006] FIG. 2 shows the normal program of hair development.

DETAILED DESCRIPTION OF THE INVENTION

[0007] It has now been found that Lhx2 is a transcription factor positioned downstream of signals necessary to specify hair follicle stem cells, but upstream from signals required to

drive activated stem cells to terminally differentiate. Using gain and loss of function studies, Lhx2 was found to maintain the growth and undifferentiated properties of hair follicle progenitors. Accordingly, the present invention relates to the use of Lhx2 as a target for modulating hair growth. For example, by increasing the expression or activity of Lhx2, hair follicles can be maintained in a resting or quiescent state thereby preventing or reducing unwanted hair growth, whereas decreasing the expression or activity of Lhx2 can be employed in the stimulation or activation of follicle stem cell proliferation and therefore stimulation of hair growth. Thus, the present invention also embraces screening assays for identifying agents which modulate the expression or activity of Lhx2. Such agents can be identified in in vitro or in vivo screening assays which monitor the activity or expression of Lhx2 (e.g., via reporter protein expression). Agents which can be screened in accordance with the instant assays include the Lhx2 protein or fragments thereof, as well as agonistic or antagonistic anti-Lhx2 antibodies. Ribozymes, siRNA, antisense oligonucleotides and the like can be screened for inhibiting the expression of Lhx2 and small organic molecules can be identified which inhibit or stimulate the expression or activity of Lhx2.

[0008] Embryonic hair progenitors were isolated using mice doubly transgenic for a Keratin 14-GFP gene expressed in skin keratinocytes and the Wnt reporter gene TOPGAL, transcribed in hair placodes and germs where [beta]-catenin/Lef1 complexes are active (DasGupta and Fuchs (1999) supra; Vaezi, et al. (2002) Dev. Cell 3:367). In these early hair progenitors, E-cadherin is down-regulated and P-cadherin is upregulated (Jamora, et al. (2003) supra). By embryonic day 17 (E17), dispase was used to separate the epidermis, including hair placodes and germs, from the underlying dermis, which harbored more mature hair pegs and follicles. Using fluorescence-activated cell sorting (FACS) on the epidermal fraction, the early "PCAD+" hair progenitors (K14-GFP+, [alpha]6-integrin+, P-cadherin+) were then separated from the "PCAD-" interfollicular epidermis (K14-GFP+, [alpha]6-integrin+, P-cadherin-) based on their differential surface P-cadherin expression. Characterization of these two cell populations confirmed their similarities in K5 and [beta]4-integrin expression, but their distinct activities of TOPGAL and expression of known hair placode markers.

[0009] The gene expression profiles of purified PCAD+ hair progenitors and PCAD- interfollicular basal keratinocytes were further analyzed using oligonucleotide microarrays. Utilizing fold differences of known hair placode markers as a sensitivity gauge, a 2-fold cut-off was assigned as a genuine difference between the two populations. A total of 1394 probes (660 in PCAD+; 734 in PCAD-) were preferentially expressed greater than 2-fold in one population over the other (Table 1). The Mean Log2 Ratio of Table 1 was calculated for PCAD+ with respect to PCAD- signal values.

[0010] A short list of differentially expressed genes relevant to the present study is provided in Table 2. Genes designated with "#" were upregulated and genes designated with "*" were downregulated within the bulge stem cells of postnatal hair follicles compared against the total skin epithelial cell population (Blanpain, et al. (2004) Cell 118:6). A number of these genes have documented roles in either hair morphogenesis (PCAD+) or epidermal differentiation (PCAD-). The interfollicular epidermal population was typified by adhesive and cytoskeletal components, Notch signaling factors, C-myc, Kruppel-like factors, as well as Bmp-responsive transcription factors (Grainyhead-like, Ovol) previously implicated in epidermal differentiation (Fuchs and Raghavan (2002) Nature Rev. Genet. 3:199; Tao, et al. (2005) Development 132:1021; Ting, et al. (2005) Science 308:411; Arnold and Watt (2001) Curr. Biol. 11:558). In contrast, the hair germ signature featured Wnts, Shh, Bmps,

Tgf[beta]s, and tyrosine kinase receptor signaling morphogens, as well as a number of different transcription factors. Although some of these transcription factors have not been previously implicated in the specification of skin progenitor fates, others have previously been associated with postnatal genetic hair disorders, including Cutl1, Gli1, Hoxc13, Sox9, Trps1, and Vdr (Millar (2002) supra; Schmidt-Ullrich, et al. (2005) supra).

[0011] Unexpectedly, several of the uncharacterized transcription factors on this list were also found to be differentially expressed in the postnatal hair follicle bulge (Blanpain, et al. (2004) supra; Morris, et al. (2004) Nature Biotechnol. 22:411) (Table 2), indicating that the embryonic hair germ may exhibit functional properties similar to adult stem cells. Although the hair germ is committed to a follicular cell fate, it remains undifferentiated like bulge stem cells, yet capable of differentiating into all the lineages of the hair follicle, including the sebaceous gland (Ito, et al. (2005) Nature Med. 11:1351; Levy, et al. Dev. Cell 9:855).

[0012] To determine whether the early hair germs may reflect hair follicle stem cells and regulate key steps in progenitor cell differentiation, focus was placed on the transcription factors emanating from the screen that were known to govern developmental cell fate specification in other tissues and organs. Lim-homeodomain transcription factor Lhx2 was of interest since Lhx2 null mutant animals display defects in patterning and cell fate determination during brain development (Porter, et al. (1997) Development 124:2935; Bulchand, et al. (2001) Mech. Dev. 100:165; Hirota and Mombaerts (2004) Proc. Natl. Acad. Sci. USA 101:8751). In addition, they lack definitive erythropoeisis and conversely, hematopoetic progenitor cells can be maintained in vitro by forced expression of Lhx2 (Pinto do, et al. (2002) Blood 99:3939). Lhx2 null animals die between E15.5-E16.5, and a possible role for Lhx2 in skin has not been examined.

[0013] Lhx2 was upregulated 18-fold in the PCAD+ population relative to PCAD- population by microarray. Semi-quantitative RT-PCR and in situ hybridization confirmed this marked differential expression. By immunofluorescence, Lhx2 first appeared in early hair placodes, and as morphogenesis progressed, became prominent at the leading front of invaginating hair germs and pegs. As down-growth neared completion and hair differentiation began, Lhx2 concentrated in the upper outer root sheath (ORS) at a presumptive site (bulge) of the developing postnatal follicle stem cell compartment. Concomitantly, expression diminished at the base of the follicle, where highly proliferative matrix cells give rise to the differentiating inner root sheath and hair shaft. In adult follicles, Lhx2 concentrated in the bulge, and as the new hair cycle initiated, Lhx2 extended to the emerging secondary hair germs. Based upon these patterns, Lhx2 appeared to function in specifying the embryonic hair follicle progenitor cells that then persist as bulge stem cells in adult follicles.

[0014] To more precisely define Lhx2's role in hair follicle stem cell specification and/or maintenance, its status was examined in various genetic mutant embryos defective in different aspects of hair morphogenesis. In the complete absence of hair follicle induction or bulge maintenance, as reflected in [beta]-catenin conditionally null (cKO) skin, Lhx2 was not expressed. In Shh knockout embryos, where hair germs are specified but unable to progress, Lhx2 expression was dramatically reduced. This positioned Lhx2 downstream of Wnt and Shh, where it could play a role in establishing or expanding the early progenitors necessary for hair follicle morphogenesis.

[0015] Bmp signaling is not required for hair follicle induction, even though Bmp ligands and receptors are expressed in embryonic hair germs and in postnatal follicle stem cells.

Correspondingly, in BmpR1a cKO skin, Lhx2 was expressed in both embryonic hair germs and the presumptive bulge of developing follicles. Conversely, Bmp signaling is required for differentiation, and in the absence of BmpR1a, proliferating undifferentiated hair progenitor cells accumulate at the follicle base (Andl, et al. (2004) Development 131:2257; Kobielak, et al. (2003) J. Cell Biol. 163:609). Lhx2 was noticeably enhanced in these follicles, with strong staining throughout the ORS and matrix. These cells were also positive for Shh and Lef1. Thus, in the absence of terminal hair differentiation, cells accumulating in postnatal BmpR1a null follicles resembled early embryonic hair follicle progenitors.

[0016] If Lhx2 governs the gene expression program of undifferentiated follicle stem cells or their early progenitors, then misexpression of Lhx2 in interfollicular epidermis might result in an induction of hair follicle progenitor genes. Accordingly, K14-Lhx2 transgenic mice were generated to examine this possibility. Although more hair follicles were not induced, Lhx2 markedly suppressed morphological and biochemical signs of epidermal differentiation and failed to produce a functional lipid barrier. Most notable was the induction of Tcf3 and Sox9, two key transcription factors of adult hair follicle stem cells (Merrill, et al. (2001) Genes Dev. 15:1688; Vidal, et al. (2005) Curr. Biol. 15:1340). Lhx2 also suppressed differentiation in tongue epithelium. These findings indicate that Lhx2 can maintain cells in an undifferentiated state, further enforcing the link between Lhx2 and stemness.

[0017] If Lhx2 is required for follicle stem cell maintenance, then its absence could alter the ability of hair follicles to form. In support of this notion, E16 Lhx2 null embryos displayed an ~40% reduction in overall density of P-cadherin positive hair follicles, with no noticeable defect in the epidermis or embryo size. Marked reduction in follicle density is a feature of other mouse mutants in key hair follicle morphogenetic genes. Although Lhx2 KO follicle density was reduced, Shh, Wnt10b, Bmp2, Bmp4 and Lef1 expression appeared unaffected in those hair placodes and germs that developed. In Lhx2 null skin engraftments, follicles appeared morphologically and biochemically indistinguishable from their wild-type counterparts. Taken together, the gain and loss of function studies indicate that Lhx2, reflecting its expression pattern, functions to specify and maintain hair follicle stem cells, but does not function in their differentiation.

[0018] If Lhx2 maintains the undifferentiated state of embryonic and adult follicle stem cells, then Lhx2 null follicles might exhibit alterations in the transition of stem cells from the resting (telogen) to the growing (anagen) phase of the postnatal hair cycle. Using skin grafts, the hair cycles of wild-type and Lhx2 KO follicles were compared. The initial morphogenetic and first postnatal Lhx2 KO hair cycles progressed similarly to wild-type and by 8 weeks, KO follicles had returned to telogen. By contrast, at 11 weeks when most wild-type follicles were still in this extended telogen, KO follicles had precociously entered the next hair cycle. Moreover, upon shaving at 8 weeks, most wild-type hairs remained in telogen while KO hairs consistently and uniformly grew back within 3 weeks, confirming their shortened resting phase.

[0019] Immunofluorescence and FACS analyses revealed that KO follicles exhibited diminished CD34, a surface marker of bulge stem cells (FIG. 1A) (Blanpain, et al. (2004) supra). This reduction in CD34 was observed irrespective of hair cycle number or stage. Other stem cell markers examined (i.e., Tenascin C and S100A6) were comparably expressed in wild-type and KO bulges.

[0020] Although CD34 marks adult stem cells, it is not found in embryonic skin progenitors,

suggesting that its reduction could be an indication of enhanced proliferative activity within KO follicle stem cells. This was supported by bromodeoxyuridine (BrdU) pulse-chase experiments conducted prior to marked deviations in hair cycling (FIG. 1B). Only the wild-type follicle bulge compartment retained appreciable BrdU label administered at the onset of anagen and chased for 4 weeks (Blanpain, et al. (2004) supra; Taylor, et al. (2000) Cell 102:451). By contrast, KO hair follicles displayed very few label retaining cells (LRCs), confirmed and quantified by flow cytometry.

[0021] The reduction in label retention was accompanied by enhanced proliferation within the KO bulge. After a 4-hour BrdU pulse during full anagen, the percentage of S-phase labeled bulge cells was 2* higher than normal (FIG. 1C). By contrast, the number of S-phase cells in the interfollicular/ORS of wild-type and KO skins was comparable, underscoring the specificity of this hyperproliferation. The elevated proliferative activity of the KO bulge did not appear to alter the overall size of the stem cell niche. It was concluded that without Lhx2, follicle stem cells are more readily activated to proliferate and differentiate along the hair lineage. On the other hand, Lhx2 is not sufficient to induce quiescence as transgenic expression did not suppress proliferation or induce CD34 in the skin epithelium.

[0022] By isolating and transcriptionally profiling embryonic hair placodes and interfollicular epidermis, genes implicated in hair development have been identified (Table 1) and novel differences have been uncovered that could be important in orchestrating lineage specification of multipotent skin progenitors. By way of illustration, Lhx2 studies revealed that it functions as a molecular brake in regulating the switch between hair follicle stem cell maintenance and activation. Although follicles can be specified embryonically without Lhx2, their overall numbers are reduced, and Lhx2 null follicles that do form are not proficient in maintaining the resting state and precociously activate. Once committed, cells no longer require or express Lhx2 and progress along a normal program of terminal differentiation.

[0023] Finally, Lhx2 is the first identified marker expressed specifically by both embryonic hair placodes and postnatal follicle stem cells of the bulge. Lhx2 now provides a segue to dissect the transcriptional mechanisms that underlie stem cell maintenance within the hair follicle and also serves as a target for modulating hair growth. Further, one or more of the genes identified as being involved in embryonic hair placodes and interfollicular epidermis (Table 1) can be used as a signature of the early hair germ that makes a follicle. Moreover, as with Lhx2, it is contemplated that one of more of the genes listed in Table 1 can be used as targets for modulating hair growth.

[0024] The invention is described in greater detail by the following non-limiting examples.

EXAMPLE 1
Mice

EXAMPLE 2
Engraftment and BrdU Experiments

EXAMPLE 3
Histology, Immunofluorescence, and in situ Hybridizations

EXAMPLE 4
Isolation of Hair Progenitors and Flow Cytometry

EXAMPLE 5
RNA Isolation and Microarray Analyses

EXAMPLE 6
Semi-Quantitative RT-PCR

Extract of Hovenia Dulcis (Asian Raisin Tree) triggers b-catenin production, which triggers the formation of new hair follicles. The substance that is responsible for triggering the beta-catenin production is apparently methyl-Vanillate.

http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0085546
DOI: 10.1371/journal.pone.0085546
January 22, 2014

Hovenia dulcis Thunb Extract and Its Ingredient Methyl Vanillate Activate Wnt/ß-Catenin Pathway and Increase Bone Mass in Growing or Ovariectomized Mice

Pu-Hyeon Cha, et al.

Abstract

The Wnt/ß-catenin pathway is a potential target for development of anabolic agents to treat osteoporosis because of its role in osteoblast differentiation and bone formation. However, there is no clinically available anti-osteoporosis drug that targets this Wnt/ß-catenin pathway. In this study, we screened a library of aqueous extracts of 350 plants and identified Hovenia dulcis Thunb (HDT) extract as a Wnt/ß-catenin pathway activator. HDT extract induced osteogenic differentiation of calvarial osteoblasts without cytotoxicity. In addition, HDT extract increased femoral bone mass without inducing significant weight changes in normal mice. In addition, thickness and area of femoral cortical bone were also significantly increased by the HDT extract. Methyl vanillate (MV), one of the ingredients in HDT, also activated the Wnt/ß-catenin pathway and induced osteoblast differentiation in vitro. MV rescued trabecular or cortical femoral bone loss in the ovariectomized mice without inducing any significant weight changes or abnormality in liver tissue when administrated orally. Thus, natural HDT extract and its ingredient MV are potential anabolic agents for treating osteoporosis.

Introduction

...Natural products such as plant extracts and their individual ingredients have been used traditionally to treat various diseases including obesity and inflammation in eastern Asia and western Africa [20], [21]. These natural products are regarded as relatively safe for drug development and are increasingly being used in medicines. The global market of medicinal plants was estimated at approximately 83 billion US dollars in 2008, and the World Health Organization reports that over 80% of the world's population use natural products for medicinal treatment of primary health care needs [22], [23]. This resource, however, has not been fully exploited and many more natural products remain to be identified.

In this study, we searched plant extracts that activate Wnt/ß-catenin signaling pathway and

induce osteoblast differentiation. Through the screening of plant extracts library, we identified an extract from Hovenia Dulcis Thunb (HDT) as an activator of the Wnt/ß-catenin pathway, and characterized its ability to modulate osteoblast differentiation and bone mass in vitro and in vivo, respectively. We determined that methyl vanillate (MV), an ingredient of HDT, activates the Wnt/ß-catenin pathway and is involved in osteoblast differentiation. The non-toxic concentration of MV rescued femoral bone loss in ovariectomized mice after oral administration, and the effect was equivalent to that of intraperitoneal PTH (1–34) injection. HDT extract and the small molecule MV have potential for development as anabolic agents to treat osteoporosis.

Figure 1. Identification of Hovenia dulcis Thunb (HDT) extract as an activator of Wnt/ß-catenin signaling pathway.

(A) Each of the 350 plant extracts (1 μg/ml each) was added to HEK293 reporter cells for 24 h, and TOPflash activity was measured. (n = 3). (B) Fourteen plant extracts, which showed increased TOPflash activity compared with control, were subjected to calvaria ex vivo assay. Of 14 plant extracts, six plant extracts, which increased the thickness of the ex-vivo cultured calvaria, were marked by blue bars (n = 2). (C–E) HDT extract was added to HEK293 reporter cells (C) or calvarial osteoblasts (D, and E) for 24 h. (C) Luciferase activity of HEK293 reporter cells (left) and calvarial osteoblasts transfected with TOPflash or FOPflash (right) was measured, respectively (n = 3). (D–E) ß-catenin proteins were detected by immunoblotting (D) and immunofluorescence staining (E, left), respectively (white arrows indicate nuclear localized ß-catenin). Scale bars, 50 μm. Intensities of ß-catenin were measured from the immunofluorescence staining images (E, right) (n>3). (C, and E) *p<0.05, ***p<0.001 versus control.

Hovenia Dulcis & Related Patents

NATURAL COMPOSITION FOR PREVENTING LOSS OF HAIR AND RECOVERING OF SILVERHAIR, AND ITS MANUFACTURING METHOD KR20130075207

PURPOSE: A natural composition for preventing alopecia and gray hair is provided to thicken hair, to prevent alopecia, to promote hair growth, to blacken hair, and to enhance cleansing ability using Brownian motion of a colloidal cleansing composition. CONSTITUTION: A natural composition for preventing alopecia and gray hair contains: 100-300 parts by weight of Curcuma longa L. which is produced by a sulfur spray method; 300-700 parts by weight of a sulfur extract which is prepared with one or more kinds of first medicinal materials selected among Eclipta prostrate, pine nut coat, Ligustrum japonicum, blueberry, complex vitamin, an antler, Atractylodes japonica Koidz. ex Kitam, and yew leaves; and 30-700 parts by weight of one or more kinds of second medicinal herb material extracts which are selected among a mix of amino acids, Cynachi wilfordii radix, Rubi fructus, eastern prickly pear, prickly pear, grape liquid tea, purple sweet potato, liquid collagen, pumpkin, Lycium chinense miller, Rehmanniae radix preparata, Ganoderma lucidum, Hovenia dulcis Thunb., Eucommia ulmoides OLIV., Acanthopanax sessiliflorum seeman, Solanum nigrum, Ulmus pumila, and salicornia herbacea. [Reference numerals] (AA) Before test; (BB) 5 days after finishing the test

Oral Chinese patent medicinal capsules for treating middle and old-aged white hair
CN102018943

The invention relates to Chinese patent medicinal capsules for treating middle and old-aged white hair, which is characterized by comprising the following raw materials in part by weight: 40 parts of black sesame, 20 parts of black bean peel, 40 parts of black nonglutinous rice, 60 parts of tuber fleeceflower root, 30 parts of flos chrysanthemi, 40 parts of rehmanniae vaporata, 40 parts of rehmanniae praeparatum, 25 parts of dried ginger, 30 parts of gardenia fruit, 40 parts of medlar, 40 parts of cornus fruit, 30 parts of safflower, 30 parts of cinnamomvine, 40 parts of astragalus, 30 parts of curcuma aromatica, 30 parts of peach seed, 30 parts of hovenia dulcis, 30 parts of tangerine peel, 30 parts of hawthorn fruit, 40 parts of szechwon tangshen root, 40 parts of spina date seed, 40 parts of senna leaf, 30 parts of thinleaf milkwort root-bark, 30 parts of epimedium,; 30 parts of root of red-rooted salvia, 30 parts of bupleurum, 30 parts of dwarf lilyturf tuber, 30 parts of cortex moutan, 20 parts of liquorice root and 40 parts of cassia seed. The Chinese patent medicinal capsules belong to pure Chinese medicinal preparations, are safe and reliable, and have the characteristics of short treatment course, high curative effect, low cost and relapse prevention in the treatment of white hair, wherein the effective rate is 100 percent, and the cure rate is 98 percent. The ideal Chinese patent medicinal capsules for treating middle and old-aged white hair have the excellent effects on hypertension, cardiovascular and cerebrovascular diseases and endocrinopathy, and prolong life and improve immunity.

MANUFACTURE METHOD SHAMPOO FOR STOPPING THE HAIR FROM FALLING OUT AND SHAMPOO FOR STOPPING THE HAIR FROM FALLING OUT
KR100806125

A manufacturing method of shampoo for inhibiting the hair loss and the shampoo for inhibiting the hair loss manufactured therewith are provided to inhibit growth of bacteria in the scalp by the function of gold and silver microparticles, and induce skin protection and hair loss inhibition by the function of traditional Oriental medicine materials. The manufacturing method of shampoo for inhibiting the hair loss comprises the steps of: applying the electric source to a cathode and an anode which are made of an alloy containing 8-16 wt.% of gold and 84-92 wt.% of silver to prepare gold and silver solution; adding 4-7 wt.% of red ginseng, 8-14 wt.% of Mori radicis cortex, 4-7 wt.% of Angelica gigas Nakai, 6-11 wt.% of ox bezoar, 4-7 wt.% of Glycyrrhiza uralensis FISCH., 4-7 wt.% of Platycodon grandiflorum, 5-10 wt.% of wild chrysanthemum, 4-7 wt.% of Acorus calamus, 4-7 wt.% of black bean, 5-10 wt.% of Hovenia dulcis Thunb. ex Murray, 6-11 wt.% of Dictamnus dasycarpus TURCZ., 4-7 wt.% of Codonopsis lanceolata, 1-5 wt.% of fruit of the trifoliate orange, 8-14 wt.% of Cervi parvum Cornu and 1-5 wt.% of black sesame into the gold and silver solution, and slightly boiling the mixture for 65-80 hours to prepare the extracts of medicinal herbs; fermenting and maturing the medicinal herb extracts at room temperature for 2 months or more and purifying the matured products with fine threads; and mixing 5-15 wt.% of matured extracts of medicinal herbs, 2-5 wt.% of humectants, 20-26 wt.% of surfactant, 0.2-0.8 wt.% of antioxidant, 0.05-0.4 wt.% of preservative and 52-70 wt.% of purified water, and adding a flavoring agent into the mixture.

KR20130134887
Stimulation of hair growth with extract of Spirodela polyrhiza

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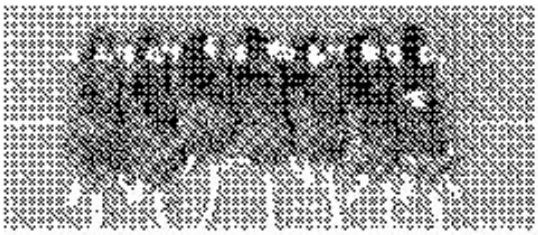

< 시협악 1 >

< 시협악 2 >