TARGETING OUTER ROOT SHEATH STEM CELLS AND DERMAL PAPILLA FIBROBLASTS TO REACTIVATE THE HAIR CYCLE.

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Objectives
Hair loss, a common affliction of humans, occurs in many pathophysiological conditions of the skin as well as in systemic disorders. The epithelial-mesenchymal interactions between keratinocyte stem cells, in particular Outer root sheath cells (ORSc) and dermal papilla fibroblasts cells (DPc) in the bulb region) are crucial for normal development of the hair follicle as well as during hair cycling. Hair cycle comprises three distinct phases anagen, catagen and telogen[1, 2]. During the cyclical regrowth of a new lower follicle, the multipotent hair follicle stem cells (ORSc) are stimulated to proliferate and differentiate through interactions with the underlying mesenchymal DPC[3]. In hair loss pathogenesis the hair cycle is perturbed[4]. Therefore, factors such as actives affecting the functions of ORSc and DPc are of great importance in hair loss therapeutic strategy. Literature data have described the benefit effect of Epigallocatechin Gallate (EGCG) on DPc proliferation and on hair follicle growth[5]. Based on this finding we decided to propose a new ingredient (DEGZ) composed in part by two polyphenolic components: EGCG-glucoside and Dihydroquercetin glucoside (DHQG), combined with glyicine and zinc. We have found that DHQG is able to activate these both cell types (DPc and ORSc). The cells activation under DHQG treatment encompassed the induction of cells proliferation, cells metabolism and specific genes expression involved in hair cycle and apoptosis. Based on this preliminary finding, we investigated the efficacy of this new ingredient on androgenic alopecia (AGA) hair growth. Efficacy studies were performed on ex vivo cultured AGA hair follicle explant and on AGA human volunteers. In parallel to these efficacy studies, fundamental studies were undergone in order to understand if DEGZ acts on the canonical wnt/beta catenin pathway and on the apoptosis mechanism.

Methodology
The effect of the compound DHQG was investigated on metabolism and proliferation of normal human follicle dermal papilla cells (DPc) and normal Outer root sheath cells (ORSc). The cellular metabolism was assessed by XTT reduction assay. BrdU incorporation assay was used to assess the cellular proliferation. Gene expressions of ORSc treated with DHQG were studied using RT-QPCR technology. The anti-apoptotic and beta catenin activation effect of DEGZ was also studied on ORSc obtained from AGA donors. Then, the efficacy of DEGZ (1%) on hair growth was assessed on hair follicle culture explant “Philpott model”[6]. The hair follicles (10 per condition) were obtained from 2 AGA donors. The study was performed in comparison to Minoxidil and to untreated follicles. The hair follicles were incubated 10 days and the culture medium containing DEGZ was changed everyday. The clinical effect of DEGZ (3%) on the hair growth cycle was assessed using photo-trichogram technic (PTG) on 30 human volunteers suffering of androgenic alopecia. Volunteers were divided in 2 groups: one receiving a placebo hair lotion, one receiving the same placebo lotion plus 3% of DEGZ. The subjects included in the study had hair loss evaluated at grade III to IV on the Hamilton scale amended by Norwood. They had at least a percentage of telogen hair ≥20% and a global density of hair ≥150 / cm² at the selection and inclusion visits. The volunteers used daily the hair lotion for 3 months in the whole head. The PTG analysis allowed the measurement of the proportion of hair in anagen and in telogen and the total density of the hair.

Results
First we have shown by in vitro experiments on normal human DPc and ORSc that DHQG is able to activate these cells. This activation encompassed the induction of cells proliferation for ORSc cells and stimulation of the metabolism for the DPc. Meanwhile we showed that the ORSc cells
maintain their stem cell phenotype (expression of the K15[3] and VdR markers[7]), and were expressing more beta-catenin mRNA. These cells once treated with DHQG expressed the BCL-2 anti-apoptotic gene. The beta catenin activation by DEGZ was confirmed at the protein level on AGA ORSc by western blot. DEGZ have demonstrated anti-apoptotic properties (detection studies of phosphatidylserine by Annexin V assay kit).

DHQG has been combined with EGCG-Glucoside (to prevent interleukin 8 release in scalp) and Zinc and Glycine (Zinc being a co-factor for cystine incorporation in hair[8], and glycine being one of the key amino acid of the hair shaft[9]). This mix has been called DEGZ. We confirmed on ex vivo cultured hair follicle explant obtained from males suffering of androgenic alopecia, the capability of the combination of these four molecules to induce hair growth in comparison to Minoxidil treatment, with up to 2 times better results than Minoxidil alone (Fig. 1). A double blind versus placebo clinical study on human volunteers suffering of androgenic alopecia confirmed the effect of DEGZ in AGA. 85% of the volunteers showed positive results, with +9% of hair in anagen phase, -17% of hair in telogen phase and an increased density up to +28,200 new hairs (+10,000 in average).

**Fig 1:** Androgenic alopecia hair follicles growth studies (microscopically measurements). DEGZ (DHQG+EGCG-glucoside+Glycine+Zinc ) at 1% induced a strong growth of AGA hair follicle.

**Fig 2:** Scalp’s pictures and phototrichogram pictures of some volunteers treated during 3 months with DEGZ at 3% (macrophotography)

**Conclusions**

We have demonstrated that a combination of DHQG with Epigallocatechin-gallate-Glucoside, Zinc and Glycine (DEGZ) is efficient to treat androgenic alopecia. Our studies ex vivo and in vivo have shown the hair growth and densification. The mechanism of action assumed is through the activation of ORSc and DPc (by the stimulation of their metabolism and their proliferation), more presumably by the activation of the WNT/beta-catenin pathway. The clinical study delivered visible results in 84 days on men suffering from grade III to IV androgenic alopecia (fig 2).