Bone morphogenetic proteins are critical factors for hair follicle epithelial–mesenchymal interactions in androgenetic alopecia.

Julietta M. Ceruti, Valeria Y. Krum, Gabriela Kusinsky, Gustavo J. Leirós, María Eugenia Balaña.
Instituto de Ciencia y Tecnología Dr. César Milstein - Fundación Pablo Cassará, CONICET, Buenos Aires, Argentina
*mbalana@fundacioncassara.org.ar

Background

- During androgenetic alopecia (AGA) androgens cause hair follicle miniaturization and baldness through mechanisms which remain unclear.
- We previously reported that androgens impair dermal papilla-induced hair follicle stem cell (HFSC) differentiation inhibiting Wnt signalling suggesting that dermal papilla-secreted factors involved in normal HFSC differentiation are deregulated.
- DHT stimulation downregulated Wnt5a and Wnt10b mRNA while Dkk-1 was upregulated. WNT10b secreted by dermal papilla cells (DPC) overcame the HFSC differentiation inhibition involved in androgen-driven balding.
- Key differences between DPC cultured in vitro and intact dermal papilla have been reported, including molecular signature changes probably involved in loss of their hair inductive capacity.
- In DPC cultured as spheroids (DPC-Sph), Wnt agonists' basal expression (Wnt 10b and Wnt 5a) is enhanced; probably contributing to DPC inductivity.
- The HF bulb microenvironment is rich in bone morphogenetic proteins (BMPs) that act on DPC to maintain key signature features in vitro and hair-inducing activity in vivo.

Objectives

To evaluate the role of BMPs as mediators of androgen inhibition of DPC-induced HFSC differentiation and in DPC inductive properties
To compare BMPs expression in DPC monolayer (2D) or spheroids (3D) cultures.

Cell line derived from occipital dermal papilla that stably expresses the androgen receptor (AR)

AR translocates to the nucleus after stimulation with DHT.

In DPC spheroids dihydrotestosterone (DHT) significantly downregulated the expression of both BMP-2 and BMP-4 mRNA. However, in DPC cultured as ML, only BMP-2 was downregulated.

Fig. 1. Androgen effect on BMPs mRNA expression in human DPC. DPC were cultured as Monolayer (ML) or Spheroids (Sph) as indicated. mRNA expression analysis, by Real-time PCR, of BMP-2 (A) or BMP-4 (B) in both conditions in absence (C) or presence of 10^{-7} M DHT (DHT) for 72 h. Values are means of triplicates $\pm$ SD using HPRT mRNA as the internal control. Results are expressed relative to DPC Spheres Control. For DPC-spheroids cultures, 4×10^4 cells/well were seeded in 96-wells plate coated with 5% PVA (Polyvinyl alcohol). In case of monolayer cultures, 1.5×10^5 cells/well were seeded in 6-wells plate. The cultures were left undisturbed for 3 days. Then, cells were supplemented with 10^{-7} M DHT and cultured during 3 days for medium conditioning. C) Western blot of androgen effect on BMP-2 expression: DPC Sph were treated with 10^{-7} M DHT during 72 h (Sph DHT) or not treated (Sph Control).

Fig. 2. BMP-2 restores inductivity markers in androgen treated DPC spheroids. For each treatment, 60 DPC spheroids were prepared as described in Fig.1. Sph DPC were cultured during 3 days in presence of DHT (10^{-7} M) alone or DHT and hrBMP2 (300ng/ml).

A) Spheroids were collected, washed with PBS and sonicated in lysis buffer (NP40 0.4% in Cl2Mg 1mM). Alkaline phosphatase activity was measured in diethanolamide buffer with p-nitrophenyl phosphate as substrate (10mM). Color production was detected at 405 nm. Results represent Abs(405nm)/ μ g protein and are shown related to control sample (C) value. B) cDNA from spheroids treated or not with DHT or DHT plus hrBMP2 was used as template in quantitative real time PCR to measure Wnt5a and Wnt10b expression. HPRT gene was used as endogenous control.

BMP-2 restores inductivity markers in DPC spheroids treated with DHT

BMPs overcome the inhibitory effect of DHT on HFSC differentiation

Fig. 3. Androgen and BMPs effects on HFSC differentiation. A) Real-time PCR analysis or B) Western blot analysis of K6hf mRNA expression in untreated Tel-E6E7 (FL) and cultured during 3 days with: medium conditioned by DPC-Spheroids (Control), medium conditioned by DPC spheroids treated with 10^{-7} M DHT (DHT), medium conditioned by DPC treated with DHT 10^{-7} M and supplemented with 300 ng/ml BMP-2 (DHT+BMP2), BMP4 (DHT+BMP4) immediately before use or medium conditioned by DPC in presence of DHT and BMP-2 (DHT/BMP-2*)

BMP-2 and BMP-4 s are critical factors of the complex epithelial–mesenchymal cross-talk deregulated by androgens during AGA
Both BMPs overcome androgen inhibition in DPC-mediated HFSC differentiation
3D-tissue architecture impacts on BMPs expression in DPC cultures. This effect could explain in part why DPC cultured as monolayer lose their hair inductive ability through successive passages.