Expression patterns of the Siah genes in postnatal mouse skin

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Introduction:

Hypoxia-inducible HIF signaling pathway plays a key role in a wide spectrum of physiological processes in the skin in homeostasis (keratinocyte proliferation and differentiation), and in stress-related responses (e.g., skin regeneration after injury). HIF pathway is under tight control from numerous regulatory factors (including protein degradation machinery), which modulate hypoxia response in a tissue/cell-specific manner. Recently, the involvement of HIF pathway in control of hair growth was confirmed (Rathman-Josserand et al., 2013). This study has showed that in normoxia (21%O₂) stabilization of HIF1 with ER 3721 (precursor diethyl pyridine-2,4-dicarboxylate) promotes hair growth. The balance of HIF1 protein stabilization and degradation is controlled by the activity of prolyl hydroxylases (PHD) which, in turn, are controlled by the Siah family of E3-ubiquitin ligases, mammalian homologs of the drosophila Sina protein, which target the degradation of diverse protein substrates (Jianfei Qi et al., 2013). Under hypoxia, Siah1 and Siah2 ubiquitinate proteins, which take part in HIF1a degradation under normoxia (PHDs, Hsp2 and others).

Given all of the above, we decided to assess the patterns of Siah1/2 expression in mouse skin during the first month after birth. This period was chosen because it covers most dramatic phases of HF remodeling – the morphogenesis and the first HF cycle.

Materials and methods:

Expressional patterns of Siah-family genes have been assessed using immunohistochemistry and qPCR methods. Akin samples have been harvested from the back of C57Bl mice during the normal development from the 1st to 30th day after birth. RNA was extracted with ExtractRNA reagent (Evrogen). For Immunohistochemistry skin samples were frozen in OST, sectioned and incubated with antibodies to Siah1 (Santa Cruz, sc-5507) and to Siah2 (Santa Cruz, sc-5505).

Results 1:

Siah 1 expression is primarily associated with epithelial skin compartment (epidermis and HF) and is hair cycle-dependent

Immunohistochemistry shows that Siah1 positivity localizes to epithelial parts of the skin (epidermis and hair follicles). After birth during the normal development protein changes its expression pattern. These changes can be associated with epithelial development and hair follicle cycle (Fig.1).

In the epidermis, Siah1 protein localizes to all basal and suprabasal layers from the 1st day after birth to 5th day. Then, Siah1 is detected in suprabasal layers, but at 12th day of postnatal development the protein localizes exclusively to granular layer, where it stays until 17-19th days after birth. From this time point and up to the 30th day, the Siah1 is detected in suprabasal epidermal layers.

In hair follicle, Siah1 is detected in inner root sheath (IRS) during first month of mice life. Protein also localizes in outer root sheath (ORS) in late stages of hair follicle morphogenesis. During stages 7 and 8 of HF morphogenesis Siah1 is seen in lower portion of both root sheaths. In early catagen, Siah1 is expressed in IRS, but in late catagen and during telogen there is no signal in hair follicle. Siah1 positivity appears again in anagen. These results show that Siah1 is linked to keratinocyte differentiation in hair follicle during normal development and hair cycle. In epidermis Siah1, probably, takes part in keratinocyte differentiation and in cornification.

Figure 1: Siah1 localization in mouse skin during first month after birth. Immunohistochemistry with antibodies to Siah1 (sc-5507) - Siah1 signal is red, Dapi is blue.

Stages 4 – 8 are stages of hair follicle morphogenesis. **Stage 4:** Siah1 is expressed in all layers of epidermis and IRS (inner root sheath) cone; **Stage 6:** Siah1 is expressed in all layers of epidermis, IRS hair channel and ORS (outer root sheath); **Stage 7:** Siah1 is expressed in suprabasal layers of epidermis, IRS and ORS around hair bulb; **Stage 8:** Siah1 is expressed in granular layer of epidermis, IRS, ORS nearly hair bulb; **Catagen I:** Siah1 is expressed in granular layer of epidermis, IRS, keratinogenous zone; **Catagen VII and Telogen:** Siah1 is expressed in suprabasal layers of epidermis; **Anagen IV:** Siah1 is expressed in all layers of epidermis and IRS.

Other Abbreviations: DP- dermal papilla, SG-sebaceous gland, ES- epithelial strand, CTS- connective tissue sheath
In schemes* Siah1 localization is shown in Red.

Schemes are adopted from articles R. Paus et al., 1999 and S. Müller-Röver et al., 2001

Results 2:

Siah2 expression is detected in mesenchymal skin compartment (dermis, dermal papilla and dermal sheath) and is hair cycle-dependent

Siah2 has primarily dermal localization. During the first month of mice life protein are seen in dermis. It also is detected in dermal sheath and in dermal papilla. Interestingly, in dermal papilla protein is expressed during stages of HF active growth and degradation (morphogenesis, anagen and catagen) (Fig.2).

Siah2 is also detected in epidermis during days 7-12 of postnatal development. The signal is seen exclusively in suprabasal epidermal layers.

These results suggest that Siah2 takes part in the processes associated with remodeling of the dermis.

Figure 2: Siah2 localization in mouse skin during first month after birth. Immunohistochemistry with antibodies to Siah2 (sc-5505) - Siah2 is in Red, Dapi is blue.

Siah2 expression during hair follicle morphogenesis. **Stage 4:** Siah2 is expressed in the dermis and in DP (dermal papilla); **Stage 6:** Siah2 is expressed in the dermis and DP; **Stage 7:** Siah2 is expressed in suprabasal layers of the epidermis, in the dermis and in dermal sheath; **Stage 8:** Siah2 is expressed in granular layer of the epidermis, in the dermis and dermal sheath; **Catagen I:** Siah2 is expressed in the dermis, dermal sheath and in DP; **Catagen VIII and Telogen:** Siah2 is expressed in single cells in the dermis; **Anagen IV:** Siah2 is detected in dermis, dermal sheath and DP. **Other Abbreviations:** DP- dermal papilla, SG-sebaceous gland, ES- epithelial strand, CTS- connective tissue sheath, IRS- inner root sheath, ORS- outer root sheath
In schemes* Siah2 localization is shown in Red.

Schemes are adopted from articles R. Paus et al., 1999 and S. Müller-Röver et al., 2001

Results 3:

Expression of Siah family genes in mice skin during first month after birth

Expression of Siah-family genes (qPCR) in the full-skin samples during first 30 days of postnatal mouse development.

A: two peaks of Siah1 expression are seen at days 12 and 15 after birth. This time-interval corresponds to the end of morphogenesis and beginning of the first catagen.

B: Siah2 mRNA level in whole-skin samples (qPCR). The high level of expression is seen at days 5, 12 and 30 after birth. The 5th and 30th days are periods of active hair grow while the 12th day demarcates transition from morphogenesis and catagen

Conclusions:

Siah1 positivity is primarily associated with epithelial skin compartment (epidermis and HF) and shows dynamic patterns during the hair cycle. Expression of Siah1 appears to be linked to keratinocyte differentiation.

Siah2 is detected in mesenchymal skin compartment (dermis, dermal papilla and dermal sheath). Nevertheless, the intensity of its expression is linked to hair follicle cycle. Siah2 positivity is associated with dynamic changes in dermis during hair growth and degradation. Siah2 also seems to play a role in dermal papilla structural transformations during hair follicle cycle. So, its activity may be associated with papilla functions in hair growth.

Therefore, both Siah genes show dynamic patterns of expression during postnatal skin development. These patterns imply their potential role in modulation of hair follicle-specific regulatory pathways (including HIF) in a context-specific manner.

Reference:

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