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Expression of COUP-TF-interacting protein 2 (CTIP2) in mouse skin during development and in adulthood

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Abstract

COUP-TF-interacting protein 2 (CTIP2), also known as Bcl11b, is a transcriptional regulatory protein that is highly expressed in and plays a critical role(s) during development of T lymphocytes and the central nervous system. We demonstrate herein that CTIP2 is also highly expressed in mouse skin during embryogenesis and in adulthood as revealed by immunohistochemical analyses. CTIP2 expression in the ectoderm was first detected at embryonic day 10.5 (E10.5), and became increasingly restricted to proliferating cells of the basal cell layer of the developing epidermis in later stages of fetal development and in adult skin. In addition, CTIP2 expression was also detected in some cells of the suprabasal layer of the developing epidermis, as well as in developing and mature hair follicles. Relatively fewer cells of the developing dermal component of skin were found to express CTIP2, and the adult dermis was devoid of CTIP2 expression. Some, but not all, of the cells present within hair follicle bulge were found to co-express CTIP2, keratin K15, but not CD34, indicating that a subset of $K15^{+}$ $CD34^{-}$ skin stem cells may express CTIP2. Considered together, these findings suggest that CTIP2 may play a role(s) in skin development and/or homeostasis.

Keywords: COUP-TF-interacting protein 2; Bcl11b; Nuclear receptor; Transcription; Expression; Zinc finger; Skin; Epidermis; Dermis; Ectoderm; Mesoderm; Keratinocyte; Stratification; Development; Stem cells; Proliferation; Differentiation; Morphogenesis; Basal cell; Suprabasal cell; Hair bulb; Hair follicle; Hair bulge; Immunohistochemistry; *In situ* hybridization; Marker; Mouse; Embryo; K10; K14; K15; Ki67; CD34

1. Results and discussion

CTIP2 (Chicken ovalbumin upstream promoter transcription factor (COUP-TF)-interacting protein 2), also known as Bcl11b, is a C_2H_2 zinc finger protein (Avram et al., 2000) that has been shown to repress transcription though interaction with COUP-TF nuclear receptor proteins as well as through direct, sequence-specific DNA binding (Avram et al., 2002). CTIP2 is required for normal T cell development and CTIP2-null mice exhibit arrested thymocyte development (Wakabayashi et al., 2003b). Additionally, deregulation of CTIP2 may be implicated in immune system malignant transformation (Wakabayashi et al., 2003a; Bezrookove et al., 2004; Kamimura et al., 2007). It was shown that CTIP2 is also expressed in layer V of cerebral cortex and plays a critical role in the establishment of connections of corticospinal motor neurons to the spinal cord (Arlotta et al., 2005).

Mouse epidermis develops from a single-layered embryonic ectoderm (Mack et al., 2005). Subsequent stratification events lead to the formation of the periderm (around E9–E12), which overlies the ectoderm (Byrne et al., 2003; Mack et al., 2005). Cells of this two-layered epidermis then undergo a series of proliferation and terminal differentiation events which results in the formation of new cell layers of the future epidermis. Formation of the mature epidermis

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is completed by E18, at which the epidermis consists of four layers: the basal, spinous, granular, and cornified layers (Mack et al., 2005).

Epidermis undergoes constant renewal, which is required to maintain normal tissue homeostasis. This is possible due to the presence of two populations of proliferating cells: transit amplifying cells with limited proliferative potential and keratinocyte stem cells, which are slow-cycling cells with high proliferative capacity (Lavker et al., 1993; Slack, 2000).

Previous RNA *in situ* hybridization using a CTIP2 RNA probe performed in our laboratory demonstrated that CTIP2 was highly expressed in developing and mature central nervous system and spinal cord as well as in the thymus (Leid et al., 2004). The epidermis was not specifically identified as a site of CTIP2 expression in the previous *in situ* hybridization studies, although CTIP2 mRNA is found in the skin (see Fig. 1G and I from Leid et al., 2004). Preliminary attempts to define CTIP2 expression pattern during mouse embryogenesis using a CTIP2-specific monoclonal antibody revealed high-level expression of CTIP2 in developing skin. To our knowledge this is the first evidence for expression of CTIP2 in skin, during development or in the adulthood, and it therefore provided a rationale to perform additional analyses.

1.1. Expression of CTIP2 during epidermal morphogenesis

To characterize the expression profile of CTIP2 during mouse skin ontogenesis, we performed immunohistochemistry at different stages of development using an anti-CTIP2 monoclonal antibody, which has been previously described (Senawong et al., 2003; Arlotta et al., 2005; Topark-Ngarm et al., 2006).

CTIP2 expression was detected in the ectoderm as early as embryonic day 10.5 (E10.5), where CTIP2-positive cells were found in the outermost layer of the cross-section of a developing fetus (Fig. 1Aa). Some of these cells were already expressing the basal cell marker keratin 14 (K14) (Fig. 1Ab), which signifies a commitment of these cells to give rise to stratified epithelia, (Byrne et al., 1994).

Expression of CTIP2 persisted at E12.5 (Fig. 1Ac). This stage of skin development is marked by formation and stratification of the embryonic basal layer and all cells of that layer were found to be positive for CTIP2 expression (Fig. 1A). CTIP2 expression precisely co-localized with basal cell marker K14 (Vassar et al., 1989) at E12.5 (Fig. 1Ad).

Strong expression of CTIP2 was detected in the rapidly dividing basal cell layer at E14.5, and expression of CTIP2 appeared to be co-localized with that of K14 (Fig. 1Ba and b). At this stage, the early differentiating layers started to form, and were identified using differentiation marker keratin 10 (K10) (Byrne et al., 1994). CTIP2 expression also extended to some cells in the differentiating suprabasal cell layer at E14.5, as seen by co-localization of CTIP2 and K10 staining (Fig. 1Bc). However, the level of CTIP2 expression in the suprabasal cell appeared to be lower than that of basal cells (Fig. 1B, compare b and c). In addition, some cells of the future dermis were also found to express CTIP2 at this developmental stage (Fig. 1Ba).

CTIP2 expression was further investigated at E16.5 and E18.5 (Fig. 1Bd and i). High levels of CTIP2 expression were consistently observed in the basal layer of the epidermis at these two stages (Fig. 1Bd, e, g, and h). Some CTIP2 positive cells were observed in the suprabasal layers that expressed high levels of K10 at E16.5 (Fig. 1Bf), whereas the suprabasal expression of CTIP2 at E18.5 was primarily restricted to a small number of K10-positive cells that were adjacent to the basal cells (Fig. 1B and I; see Supplemental Fig. 1).

Later stages of skin morphogenesis were marked by the formation of hair follicles, and anti-CTIP2 antibody robustly stained hair bulbs and follicles at E16.5 and E18.5, respectively (Fig. 1Bd and g). These stages were also characterized by expansion of the dermal compartment of the skin, and some of these dermal cells were also found to express CTIP2 (Fig. 1Bd and g, and data not shown).

1.2. CTIP2 expression in proliferating cells

To evaluate the proliferation status of CTIP2-expressing cells we performed co-labeling studies using anti-CTIP2 and an antibody against the proliferation marker Ki67 (Schluter et al., 1993) at different stages during epidermal morphogenesis (Fig. 2). At early stages of development (E10.5), almost all cells of the fetus were positive for Ki67 expression (Fig. 2b and data not shown). This was expected as early stages of fetal development are marked by rapid and ubiquitous proliferation. All cells of the ectodermal compartment, as well as those of the underlying mesenchyme were found to express Ki67 (Fig. 2b). Two days later (at E12.5) virtually all cells of the ectoderm were positive for Ki67 expression (Fig. 2e). Most, but not all, cells of mesenchymal origin were negative for Ki67 staining at this developmental stage (Fig. 2f).

The basal cell layer of epidermis is highly proliferative at E14.5 (Mack et al., 2005), which is reflected in the presence of multiple Ki67-positive cells (Fig. 2h and i). A smaller fraction of cells of the future dermis were positive for Ki67 staining at this stage (Fig. 2h and i), and all layers of the developing skin became less proliferative with developmental progression. For example, only a fraction of basal cells, as well as those cells of the dermal compartment, were positive for Ki67 staining at E16.5 (Fig. 2k and l). By E18.5 only a few cells were still proliferating in the basal cell layer, and the dermis was mostly non-proliferative. The developing hair follicle, which represents an epithelial invagination into the dermis, still harbored a considerable number of Ki67-positive cells at this developmental stage (Fig. 2n).

At early, highly proliferative stages of development (E10.5–E14.5) almost all of the CTIP2-positive epidermal cells were found to be dividing as indicated by co-localization of CTIP2 and Ki67 staining (Fig. 2a and i). The total