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The temporal and spatial expression of the novel Ca^{++}-binding proteins, Scarf and Scarf2, during development and epidermal differentiation

M. Hwang, A. Kalinin, M.I. Morasso*

Developmental Skin Biology Unit, NIAMS, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Building 50 Room 1525, Bethesda, MD 20892, USA

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Abstract

During the process of epidermal differentiation, intracellular and extracellular calcium (Ca^{++}) concentrations induce an array of signaling pathways [Berridge, M.J., Lipp, P., Bootman, M.D., 2000. The versatility and universality of calcium signaling. Nature Rev. Mol. Cell. Biol. 1, 11–21]. Keratinocytes follow a complex Ca^{++}-dependent program of differentiation moving from the basal proliferative layer, through the spinous and granular differentiated layers to ultimately culminate in the formation of the cornified layer of the epidermis. Members of the Ca^{++}-binding proteins play a central role in the transduction of Ca^{++} signals. Utilizing a suppressive subtractive hybridization screen comparing basal and differentiated keratinocytes, we identified the novel Ca^{++}-binding protein genes, Scarf (*s*kin *Ca*lmodulin-*r*elated *f*actor) and Scarf2, which have homology to calmodulin (CaM). In this study, we present a comprehensive analysis of the expression pattern for Scarf and Scarf2 transcripts and proteins in the developing mouse. To examine Scarf2 expression during embryogenesis, we performed in situ hybridization, and detected expression in the hair follicle, skin and nasal epithelium. These results showed substantial overlap with the previously reported Scarf gene expression [Hwang, M., Morasso, M.I., 2003. The novel murine Ca2+-binding protein, Scarf, is differentially expressed during epidermal differentiation. J. Biol. Chem. 278, 47827–47833]. Comparing the expression patterns of Scarf and Scarf2 proteins in neonatal and adult mouse skin with several structural epidermal proteins, i.e. keratin 14 (K14), keratin 1 (K1), loricrin (LOR) and filaggrin (FIL) showed that their expression overlaps K1, an early marker of keratinocyte differentiation. Interestingly, Scarf and Scarf2 were also detected in the tongue and oral epithelia, rib bone undergoing ossification and in the medullar region of thymus.

Keywords: Calcium-binding protein; Skin; Differentiation; Scarf; Scarf2; Epidermal development

1. Results and discussion

Ca^{++} is known as a secondary messenger in many cellular processes: proliferation, differentiation and apoptosis. Many aspects of these processes are mediated by Ca^{++}- binding proteins (Nelson and Chazin, 1998). CaM, the most characterized Ca^{++}-binding protein, is ubiquitously expressed and binds several dozen cellular proteins, from enzymatic to structural proteins, in response the Ca^{++} signals (Crivici and Ikura, 1995). CaM and CaM-like Ca^{++}-binding proteins are characterized by the presence of four EF-hand domains, the putative Ca^{++}-binding domains (Haeseleer et al., 2000; Haeseleer and Palczewski, 2002; Mehul et al., 2000; Rogers et al., 2001). It is through the binding of Ca^{++} that these proteins undergo conformational changes that allow for the association and regulation of their specific target proteins.

In contrast to CaM, some Ca^{++}-binding proteins such as the GCAPs show a cell-specific expression pattern (Dizhoor, 2000), suggesting that this family of genes plays a crucial function in the retina. Likewise, here we present evidence that Scarf and Scarf2 are CaM-like proteins that are expressed in a specific temporal and spatial pattern during epidermal development and are differentially expressed during the Ca^{++}-dependent differentiation process of the epidermis (Hennings et al., 1980; Yuspa et al., 1989).

1.1. Scarf2 expression during mouse embryo development

The initial characterization of the novel CaM-like protein Scarf was previously reported (Hwang and Morasso, 2003).

* Corresponding author. Tel.: +1 301 435 7842; fax: +1 301 435 7910.
E-mail address: morassom@mail.nih.gov (M.I. Morasso).

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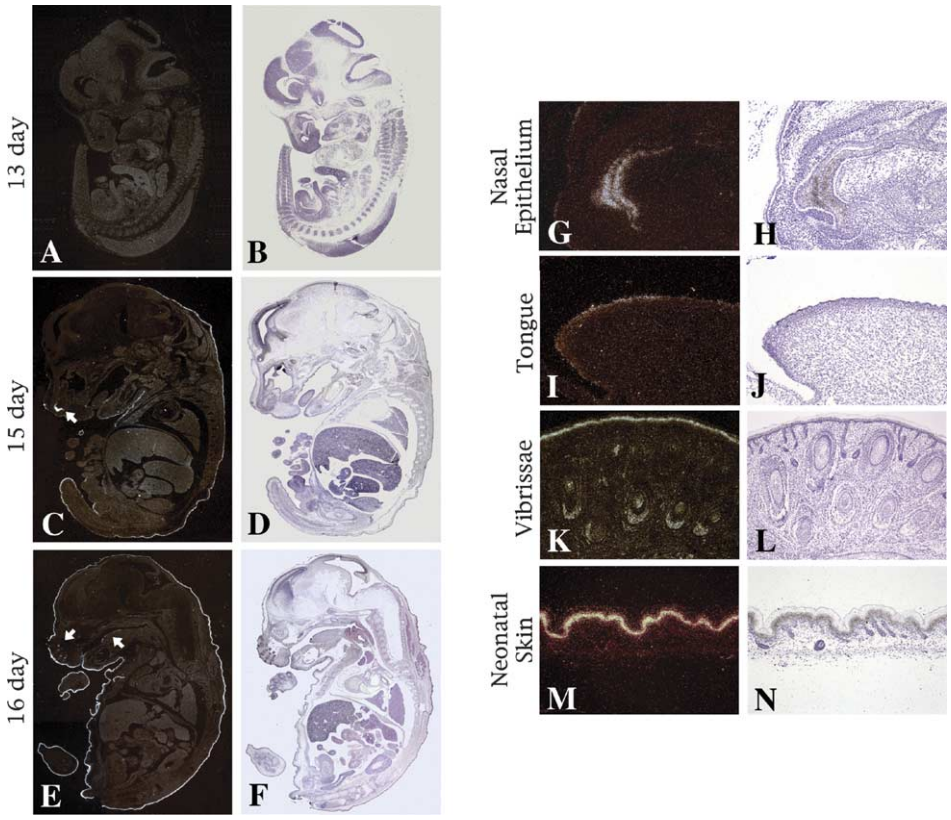

Fig. 1. Expression of Scarf2 during mouse development. In situ hybridization was performed with an antisense Scarf2 probe on sagittal sections of day 13 (A), day 15 (C) and day 16 (E) mouse embryos. Corresponding hematoxylin–eosin staining on panels B, D and F, respectively. Magnification 1.25×. Arrows point to sites of Scarf2 expression at 15-day, the nasal epithelium (C) and at day 16, in the vibrissae and dorsum of the tongue (E). (G–L) Magnified view of Scarf2 expression in the nasal epithelium (G) and tongue (I). Scarf2 expression in vibrissae (K) and in the differentiated layers of neonatal skin (M). Corresponding hematoxylin–eosin staining (H–N, respectively). Magnification 10× (G–L) and 20× (M–N).

The intronless Scarf gene was localized on chromosome 13, with its expression initiating at day 15 of mouse development and highly restricted to the differentiating epidermis. Scarf2 was reported as a gene with 83% nucleotide sequence homology to Scarf, that localized approximately 15 kb apart on chromosome 13. The high degree of homology extended to 1.6 kb upstream and 1.2 kb downstream sequence of the coding regions, indicating homology in the potential regulatory sequences including the putative proximal promoter region. To examine the level of similarity in the expression pattern between Scarf and Scarf2, the expression of Scarf2 was studied by radioactive in situ hybridization during mouse embryogenesis. Hybridization of sagittal sections of 13-day (Fig. 1A,B), 15-day (Fig. 1C,D) and 16-day (Fig. 1E,F) mouse embryos with an antisense Scarf2 probe, showed no expression at 13-day and strong epidermal expression at 15-day and 16-day developmental stages. Scarf2 expression was also detected in the nasal epithelial cells, the dorsum of the tongue and in the vibrissae (indicated by arrows in Fig. 1C,E, and presented in magnified view in Fig. 1G–J). In neonatal skin, the expression of Scarf2 was restricted to the suprabasal (differentiated) layers of the epidermis (Fig. 1M,N). The expression pattern of Scarf2 transcript was very similar to that of Scarf (Hwang and Morasso, 2003). No expression was detected with the use of Scarf2 sense probe (data not shown).

Using a commercial Northern blot of total embryo mRNA at different developmental stages, Scarf2 was detected at 15 days of embryogenesis and the level of expression increased by day 17, corroborating the results obtained by in situ hybridization (Fig. 2A). Scarf2 expression was not detected in heart, brain, spleen, lung, liver, skeletal muscle, kidney or testis (data not shown), whereas Scarf was weakly detected in skeletal muscle (Hwang and Morasso, 2003).

Percoll gradients are utilized to separate subpopulations of cells in the epidermis by density centrifugation (Fisher et al., 1982). Scarf2 expression was detected in the differentiated (suprabasal) epidermal cells obtained by Percoll gradient preparations from neonatal skins, corroborating the results obtained by in situ hybridization (Fig. 2B). During the gradient formation in the centrifugation, some suprabasal cells may remain and be detected as contaminant in the basal cell fraction (Fisher et al., 1982; Lichti and Yuspa, 1988). The differentiation-specific K1 marker was utilized to validate the Percoll separation method for basal (B) and suprabasal (SB) cells. Scarf, Scarf2 and K1 are suprabasal-specific transcripts primarily detected in