Neuroscience Letters xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

# Spatial and temporal expression of RA70/Scap2 in the developing neural tube

Yuko Tanabe[a,1], Akira Shiota[b,1], Yoriko Kouroku-Murakami[a], Eriko Fujita-Jimbo[a,c], Koko Urase[d], Kana Takahashi[e], Yoshihiro Mezaki[e], Haruki Senoo[e], Takashi Momoi[a,*]

[a] Center for Medical Science, International University of Health and Welfare, Kitakanemaru, Ohtawara, Tochigi, Japan
[b] PhoenixBio, Ltd., Iwazo, Utsunomiya, Tochigi, Japan
[c] Department of Pediatrics, Jichi Medical University, Yakushiji, Shimotsukeshi, Tochigi, Japan
[d] Department of Biology, School of Medicine Tokyo Women's Medical University, Shinjuku-ku, Tokyo, Japan
[e] Department of Cell Biology and Morphology, Akita University Graduate School of Medicine, Hondo, Akita, Japan

## HIGHLIGHTS

- *Ra70/scap2* was temporarily expressed during the RA-induced neuronal differentiation.
- Homozygous *Ra70/scap2*-KO mice exhibited embryonic lethality.
- *Ra70/scap2* mRNA was expressed in the developing neural tube and hindbrain.
- RA70/Scap2 may be necessary for the RA-induced development of the neural tube.

## ARTICLE INFO

*Article history:*
Received 31 January 2014
Received in revised form 18 April 2014
Accepted 12 May 2014
Available online xxx

*Keywords:*
Retinoic acid
SKAP55
Scap
Neural tube
Integrin

## ABSTRACT

Src kinase-associated phosphoprotein 2 (*Ra70/scap2*), which was originally isolated as a retinoic acid (RA)-induced gene, associates with molecules that modulate integrin-survival signals. Although RA is essential for vertebrate organogenesis in the posterior region, little is known about the biological role of RA70/Scap2 during development. In the present study, we demonstrate that *Ra70/scap2* mRNA is temporally expressed during the RA-induced neuronal differentiation of P19 embryonic carcinoma cells. Homozygous knockout mice in which the *Ra70/scap2* gene was replaced with LacZ exhibited embryonic lethality, while heterozygous mice displayed preferential expression of LacZ in posterior neural tissues, including the neural tube and hindbrain during development (E7.5–11.5), but not the forebrain. *Ra70/scap2* was expressed in the ependymal layer and ventricular zone in the neural tube, where neuroepithelial cells and neuroblasts with proliferation capacity are localized, respectively. Thus, RA70/Scap2 may be necessary for RA-induced neuronal differentiation from the posterior neuroectoderm.



## 1. Introduction

*Ra70/scap2* (protein product also known as RA70, SKAP2, Scap2, SKAP-HOM, and SKAP55R) is a homolog of *Skap55/scap1* (encoding SKAP1, a 55-kDa Src kinase-associated phosphoprotein) [11]. Both RA70/Scap2 and SKAP55/Scap1 contain a pleckstrin homology (PH) domain and a carboxy-terminal src homology 3 (SH3) domain [7,9,10,12], but RA70/Scap2 harbors a unique N-terminal coiled-coil (CC) domain [9,12] and tyrosine phosphorylation sites [21].

RA70/Scap2 and SKAP55/Scap1 appear to associate with proteins that modulate the functions of integrin [1,27,29]. SKAP55/Scap1 associates with adhesion and degranulation promoting adaptor protein (ADAP) to regulate integrin adhesion [27]. RA70/Scap2 requires Signal regulatory protein-α (Sirpα) for its recruitment to engaged integrins, as well as for its coordination of downstream actin rearrangements [1]. RA70/Scap2 regulates actin assembly by interacting with Wiskott-Aldrich syndrome protein (WASP) family VErprolin-homologous protein 2 (WAVE2) and

*Abbreviations:* BAC, bacterial artificial chromosome; CC, coiled-coil; EC, embryonic carcinoma; ES, embryonic stem; IRES, internal ribosome entry sites; Neo, neomycin resistance; PH, pleckstrin homology; RA, retinoic acid; *Ra70/scap2*-KO mouse, *Ra70/scap2*-knockout mouse; Scap, Src kinase-associated phosphoprotein; SH3, src homology 3.

* Corresponding author. Tel.: +81 287 24 3000; fax: +81 287 24 3100.
*E-mail address:* momoi@iuhw.ac.jp (T. Momoi).

[1] Both authors equally contributed to this work.

http://dx.doi.org/10.1016/j.neulet.2014.05.013
0304-3940/

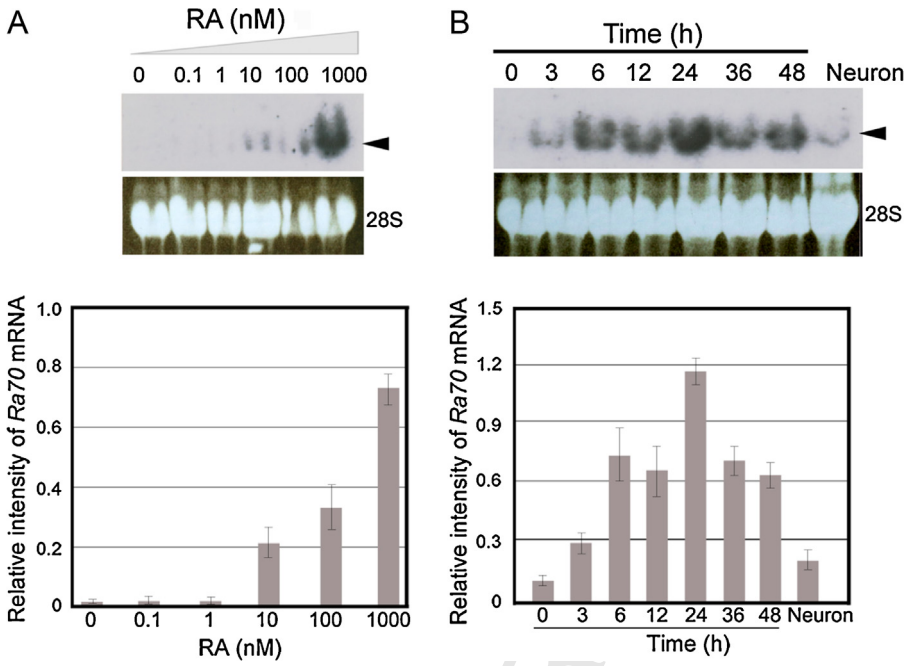


**Fig. 1.** *Ra70/scap2* expression during the RA-induced neuronal differentiation of P19 EC cells. (A) Effect of RA concentration on the expression of *Ra70/scap2* mRNA. P19 EC cells were incubated with various concentration of RA (0.1 nM–1 μM) for 48 h. (B) Time course of expression (for 3 to 48 h) of *Ra70/scap2* mRNA during RA (1 μM)-induced neuronal differentiation of P19 EC cells. Upper panel, northern blot analysis (*Ra70/scap2* mRNA and 28S ribosomal RNA); lower panel, quantitative analysis of the relative intensity of *Ra70/scap2* mRNA versus the intensity of 28S ribosomal RNA. Bars indicate the average value of the intensity scanned three times. Error bars indicate standard deviation (s.d.).

cortactin [18]. RA70/Scap2 also interacts with the SH2 domain of the non-catalytic region of tyrosine kinase adaptor protein 2 (NCK2) via its N-terminus [29] and forms a complex with NCK2 and F-actin that accumulates at the leading edge of the lamellipodium.

*Ra70/scap2* is a retinoic acid (RA)-induced gene isolated from P19 embryonic carcinoma (EC) cells [9]. In contrast with *Skap55/scap1*, which is expressed exclusively in the thymus and in T lymphocytes, *Ra70/scap2* is expressed ubiquitously in various adult tissues [4,9]. RA is an essential component of cell-cell signaling during vertebrate organogenesis [5]. For example, RA organizes the trunk by providing an instructive signal for posterior neuroectoderm and a permissive signal for trunk mesoderm differentiation. However, little is known about the role of RA70/Scap2 during organogenesis and the differentiation of various tissues during development, which require RA.

To examine the role of RA70/Scap2 during development, we generated *Ra70/scap2*-knockout (*Ra70/scap2*-KO) mice. The *Ra70/scap2* coding region has 13 exons that span approximately 173 kb of the genome: exons 1–3 encode the N-terminal CC domain, exons 5–8 encode the PH domain, and exons 11–12 encode the C-terminal SH3 domain. To make loss-of-function *Ra70/scap2* mutant mice, we added a nonsense mutation by inserting a stop codon in-frame into exon 2 and using the promoter-less internal ribosome entry sites (IRES)-LacZ-neomycin resistance (Neo) vector to prepare *Ra70/scap2*-KO mice. This strategy allowed LacZ to be expressed by the *Ra70/scap2* promoter via IRES in place of the PH and SH3 domains, thereby yielding *Ra70/scap2*-KO mice. In the present study, we demonstrate the spatial and temporal expression of *Ra70/scap2* in the developing neural tube.

## 2. Materials and methods

### 2.1. Cell culture

P19 EC cells were cultured in α-minimum essential medium (Sigma, St. Louis, MO, USA) supplemented with 10% fetal calf serum at 37 °C in a humidified atmosphere of 5% $CO_2$. For neuronal differentiation, P19 EC cells were cultured as originally described by McBurney et al. [13]. P19 EC cells were cultured in the aggregate form in the presence of 1 μM RA in bacterial dishes for 2 days and then cultured in the non-aggregate form in the absence of RA for 2 days in cell-culture dishes.

### 2.2. Northern blot analysis

Total RNA was prepared from untreated and RA-treated P19 EC cells with the RNeasy Mini Kit (Qiagen, Valencia, CA, USA). Northern blotting was performed as described previously [9]. Quantitative analysis of the relative intensity of *Ra70/scap2* mRNA versus the intensity of 28S ribosomal RNA was carried out with Image J software (National Institutes of Health).

### 2.3. Generation of Ra70/scap2-KO mice

The mouse *Ra70/scap2* bacterial artificial chromosome (BAC) clone RP22-477F17 was selected from RPCI-22 129S6/SvEvTac mouse BAC library (provided by the BACPAC Resources Center at Children's Hospital Oakland Research Institute) via hybridization of high-density arrayed nylon filters. A targeting vector was designed for embryonic stem (ES) cell selection as follows. The tandem 5′-arm, stop codon, IRES-LacZ-Neo cassette, and 3′-arm were subcloned into the *Spe*I and *Xho*I sites of the pBluescript-MCA/DTA plasmid. A stop codon was inserted into exon 2 of *Ra70/scap2*, and the IRES-LacZ-Neo cassette was substituted for exons 3 and 4 via the Red/ET Quick & Easy BAC Modification Kit (Gene Bridges, Heidelberg, Germany). The resulting *Ra70/scap2*-targeting plasmid was sequenced to confirm cassette insertion. As a result, LacZ expression was driven by the original *Ra70/scap2* promoter. Correctly targeted ES cells were microinjected into C57BL/6J blastocysts and the chimeras were set up for mating with the C57BL/6J mice, yielding germ-line transmission of the mouse *Ra70/scap2 LacZ* knock-in gene.



Please cite this article in press as: Y. Tanabe, et al., Spatial and temporal expression of RA70/Scap2 in the developing neural tube, Neurosci. Lett. (2014), http://dx.doi.org/10.1016/j.neulet.2014.05.013