Generation of Rac1 conditional mutant mice by Cre/loxP system

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Summary. Rac1 is a small GTPase which belongs to the Rho family of proteins, and has multiple roles in cellular function, including actin cytoskeleton organization, transcriptional activation, microtubule formation, and endocytosis. In the present study, the mesenchyme of mouse limbs was made deficient in Rac1 in order to investigate its role in digit morphogenesis during limb development. We employed a Cre-loxP system for limb bud mesenchyme-specific inactivation of the Rac1 gene, as null mice show embryonic lethality.

Key words. Rac1, Cre-loxP system, limb bud mesenchyme

1 Introduction

The Rho family of small GTPases regulates the cytoskeleton and transcription by virtue of cycling between inactive GDP-bound and active GTP-bound forms (Hall, 1994). The Rac subfamily consists of Rac1, Rac2, and Rac3, and they participate in a wide range of cellular functions,
such as actin cytoskeletal reorganization (Ridley et al., 1992), cell adhesion (Hall, 1998), cell growth (Olson et al., 1995), and superoxide formation (Mizuno et al., 1992). However, the tissue-specific roles of Rac1 in mammalian growth and development in vivo remain largely unknown.

Herein, we describe the generation of limb mesenchymal cell-specific inactivation of the Rac1 gene in mice.

2 Materials and Methods

2.1 Generation of Rac1 conditional mutant mice

Rac1 alleles were used in this study. The first exon was flanked by loxP sites (floX) and deleted upon Cre-mediated recombination, causing the deletion of the exon1 allele, which is functionally equivalent to a null (Kassai et al., 2008). Rac1 conditional mutant mice were generated by mating Rac1 floX mice (Rac1floX/floX) with Prx1-Cre transgenic (Prx1-Cre Tg) mice (Logan et al., 2002).

2.2 Genotyping

Genotypes were assessed by PCR analysis using appropriate primer pairs (Table 1).

3 Results and Discussion

For the present study, we employed a Cre-loxP system for limb bud mesenchyme-specific inactivation of the Rac1 gene, as Rac1 null mice develop embryonic lethal. Mice with a conditional (floxed) mutation in both alleles of the Rac1 gene (Rac1floX/floX) were crossed with mice expressing

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<th>Table 1. The primer sequences used for PCR analysis</th>
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<td><strong>Primers</strong></td>
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<td>Rac1</td>
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The reaction conditions for all PCRs were 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s.