Generation of Rac1 conditional mutant mice by Cre/loxP system

Dai Suzuki[1], Atsushi Yamada[1], Takanori Amano[2], Ayako Kimura[3], Rika Yasuhara[1], Mizuho Sakahara[4], Masaru Tamura[2], Noriyuki Tsumaki[5], Shu Takeda[3], Masanori Nakamura[6], Toshihiko Shiroishi[2], Atsu Aiba[4], Ryutaro Kamijo[1]

Departments of [1]Biochemistry and [6]Oral Anatomy and Developmental Biology, School of Dentistry, Showa University, 1-5-8 Hatanodai, Shinagawa, Tokyo 142-8555, Japan, [2]Mouse Genomics Resource Laboratory, National Institute of Genetics, 1111 Yata, Mishima, Shizuoka 411-0801, Japan, [3]Department of Orthopedic Surgery, Graduate School, Tokyo Medical and Dental University 1-5-45 Yushima, Bunkyo, Tokyo 113-8519, Japan, [4]Division of Molecular Biology, Department of Biochemistry and Molecular Biology, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan, [5]Department of Bone and Cartilage Biology, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

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 ($Rac1^{flox/flox}$) 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 ($Rac1^{flox/flox}$) were crossed with mice expressing

Table 1. The primer sequences used for PCR analysis

Primers	Direction	Sequence (5'-3')
Rac1	Sense primer	ATTTTCTAGATTCCACTTGTGAAC
	Antisense primer	ATCCCTACTTCCTTCCAACTC
Cre	Sense primer	GACGATGCAACGAGTGATGA
	Antisense primer	AGCATTGCTGTCACTTGGTC

The reaction conditions for all PCRs were 30 cycles of denaturation at 94℃ for 30 s, annealing at 58℃ for 30 s, and extension at 72℃ for 30 s.

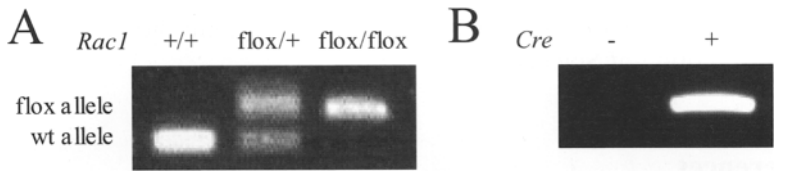

Fig. 1. Representative PCR genotyping reactions.
(A) The wild-type (wt) and floxed (flox) alleles of *Rac1* were detected by PCR using the primers indicated in Table 1. Genomic DNA was isolated from the tails of mice with genotypes $Rac1^{+/+}$, $Rac1^{flox/+}$, and $Rac1^{flox/flox}$. (B) PCR for the *Prx1-Cre* transgene (*Cre*) was performed using tail extracts of *Prx1-Cre* (-) and *Prx1-Cre* (+) mice.

Cre recombinase under the control of a *Prx1* limb enhancer (*Prx1-Cre* Tg) to obtain $Rac1^{flox/+}$/*Prx1-Cre* Tg mice. Then, $Rac1^{flox/+}$/*Prx1-Cre* Tg males were crossed with $Rac1^{flox/flox}$ females to obtain $Rac1^{flox/flox}$/*Prx1-Cre* Tg mice, to prevent the carryover of Cre recombinase in the cytoplasm of the oocyte (Ovchinnikov *et al.*, 2006). Genotypes of *Rac1* alleles ($Rac1^{+/+}$, $Rac1^{flox/+}$, $Rac1^{flox/flox}$) and the *Prx1-Cre* transgene were determined by PCR analysis (Fig. 1A, B).

Wang G. *et al.* demonstrated that the cartilage specific inactivation of Rac1 in vivo using mouse Collagen II promoter-driven Cre-expressing transgenic (*Col2-Cre* Tg) mice resulted in increased lethality, skeletal deformities, severe kyphosis, and dwarfism, which suggest that Rac1 is required for endochondral bone development (Wang *et al.*, 2007). However, the expression of Cre in *Col2-Cre* Tg is observed later in the limbs, after mesenchymal cells have committed to a chondrocyte lineage, while the perichondrium is not efficiently targeted (Terpstra *et al.*, 2003). Logan *et al*, showed that *Prx1-Cre* is active in the emerging forelimb bud mesenchyme at E9.5 and hindlimb mesenchyme at E10.5 (Logan *et al.*, 2002). Comparisons of the phenotype of these two mouse models suggest the spacious and temporal roles of Rac1 in embryonic endochondral bone formation during limb development.

In summary, the ablation of Rac1 in limb bud mesenchymal cells may provide new insights into limb development.

4 Acknowledgments

We are grateful to Dr. N Wada for critical discussion and Dr. T Sagai for technical support. This work was supported in part by the 'High-Tech Research Center' Project for Private Universities, a matching fund subsidy

from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and Grants-in-Aid for Scientific research from the Japan Society for the Promotion of Science.

5 References

Hall, A. (1994). Small GTP-binding proteins and the regulation of the actin cytoskeleton. Annu Rev Cell Biol 10, 31-54.

Hall, A. (1998). Rho GTPases and the actin cytoskeleton. Science 279, 509-14.

Kassai, H., Terashima, T., Fukaya, M., Nakao, K., Sakahara, M., Watanabe, M. and Aiba, A. (2008). Rac1 in cortical projection neurons is selectively required for midline crossing of commissural axonal formation. Eur J Neurosci 28, 257-67.

Logan, M., Martin, J. F., Nagy, A., Lobe, C., Olson, E. N. and Tabin, C. J. (2002). Expression of Cre Recombinase in the developing mouse limb bud driven by a Prxl enhancer. Genesis 33, 77-80.

Mizuno, T., Kaibuchi, K., Ando, S., Musha, T., Hiraoka, K., Takaishi, K., Asada, M., Nunoi, H., Matsuda, I. and Takai, Y. (1992). Regulation of the superoxide-generating NADPH oxidase by a small GTP-binding protein and its stimulatory and inhibitory GDP/GTP exchange proteins. J Biol Chem 267, 10215-8.

Olson, M. F., Ashworth, A. and Hall, A. (1995). An essential role for Rho, Rac, and Cdc42 GTPases in cell cycle progression through G1. Science 269, 1270-2.

Ovchinnikov, D. A., Selever, J., Wang, Y., Chen, Y. T., Mishina, Y., Martin, J. F. and Behringer, R. R. (2006). BMP receptor type IA in limb bud mesenchyme regulates distal outgrowth and patterning. Dev Biol 295, 103-15.

Ridley, A. J., Paterson, H. F., Johnston, C. L., Diekmann, D. and Hall, A. (1992). The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. Cell 70, 401-10.

Terpstra, L., Prud'homme, J., Arabian, A., Takeda, S., Karsenty, G., Dedhar, S. and St-Arnaud, R. (2003). Reduced chondrocyte proliferation and chondrodysplasia in mice lacking the integrin-linked kinase in chondrocytes. J Cell Biol 162, 139-48.

Wang, G., Woods, A., Agoston, H., Ulici, V., Glogauer, M. and Beier, F. (2007). Genetic ablation of Rac1 in cartilage results in chondrodysplasia. Dev Biol 306, 612-23.