

ECM VIII: Bone Tissue Engineering: “Expression of mutant RhoA in osteoblasts in transgenic mice results in altered bone formation *in vitro*”

G. Bluteau^{1,2}, A.E. Coudert¹, A. Danikas¹, A.E. Grigoriadis¹

¹*Dept of Craniofacial Development, Kings College London, Guy's Hospital, London, UK,*

²*Current address: Institut für Orale Biologie, ZZMK, Universität Zürich, Switzerland*

INTRODUCTION: Small GTPases of the Rho family are molecular switches controlling several aspects of cell behaviour, such as cytoskeletal organization, cell growth, motility and differentiation. We have recently reported that inhibition of Rho and its downstream effector, Rho Kinase (ROCK), enhance differentiation of primary mouse osteoblasts and bone formation *in vitro* [1] although the precise role of Rho/ROCK on the *in vitro* differentiation of osteoblasts and mesenchymal stem cells in rodents and humans is not clear [2-4]. Moreover, the role of altered Rho levels on osteoblast differentiation *in vivo* is not known. To begin to understand the possible role of Rho in osteoblast differentiation *in vivo*, we have generated transgenic mice expressing mutant RhoA proteins specifically in osteoblasts.

METHODS: Transgenic mice overexpressing either V14RhoA (constitutively active) or N19RhoA (dominant negative) from the 2.3kb type I collagen promoter were generated which ensured high expression of the two transgenes to osteoblasts. Mice were analysed by x-ray, histology and skeletal preparation. *Ex vivo* adult bone marrow stromal and early post-natal primary calvarial cell cultures were prepared from each transgenic line to analyse the *in vitro* osteoblast differentiation potential in the presence of osteogenic media (ascorbic acid and β -glycerophosphate). The expression of the transgenes and osteoblast marker genes was carried out by RT-PCR and Western blot analyses.

RESULTS: Two V14RhoA founders and five N19RhoA founders were obtained and specific transgene expression was confirmed by PCR. Both transgenic mice were viable and fertile. To date, there are no obvious macroscopic abnormalities between transgenic mice and their wild-type littermates as assessed by x-ray analysis. *In vitro* bone marrow stromal cell cultures, however, revealed that osteoblastic differentiation was markedly increased in cells derived from V14RhoA mice, and severely impaired in N19RhoA cultures as demonstrated

by alkaline phosphatase von Kossa staining (Fig.1).

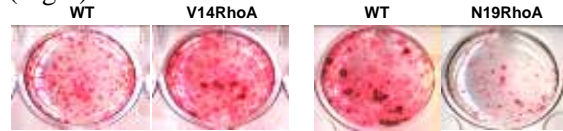


Fig. 1: Bone marrow cell cultures under osteogenic conditions: WT & V14RhoA; WT & N19RhoA. Cultures were stained for alkaline phosphatase activity and von Kossa for mineralized nodules.

RT-PCR analysis of osteoblast markers confirmed the changes in bone nodule formation. Preliminary analysis of proliferation of bone marrow-derived osteoblasts has indicated an increase in cell number in the presence of the V14RhoA transgene, and a decrease in cell number in the presence of N19RhoA.

DISCUSSION & CONCLUSIONS: To date, mice expressing RhoA mutant proteins specifically in osteoblasts exhibit no obvious macroscopic skeletal abnormalities. MicroCT and detailed histological analyses are currently underway to investigate whether transgenic bones display any alterations *in vivo*. The mechanisms underlying the clear *in vitro* phenotypes observed are not yet known, and we are currently investigating the role of the downstream Rho effector, ROCK in these cultures, which we have previously demonstrated to be important for osteoblast differentiation [1]. Finally, we are also analysing osteoclast parameters both *in vivo* and *in vitro* to investigate whether altered RhoA expression by osteoblasts affects the balance between formation and bone resorption.

REFERENCES: ¹Harmey et al. *JBMR* (2004) 19:661. ²Ohnaka et al. *BBRC* (2001) 287:33. ³Meyers et al., *JBMR* (2005) 20:1858. ⁴McBeath et al., *Dev Cell* (2004) 6:483.

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