Long Bone Phenotypic Analyzes Of A Rank Transgenic Mouse Line

Baaroun V.[1], . Coudert A.[1] , Marty C.[2], Castaneda B.[1], Berdal A.[1], Descroix V.[1]
[1] Laboratoire de Physiopathologie Orale Moléculaire – Equipe 5 – Centre de Recherche des Cordeliers – UMRS872 – Paris;
[2] Laboratoire Os et Articulations – Hôpital Lariboisière – UMRS 606 – Paris

INTRODUCTION

Osteoclast is a multinucleated cell which differentiation is controlled by a triad of molecules (figure 1): RANK, the receptor present at the surface of osteoclast precursors, binds to its ligand RANKL, allowing precursors differentiation to active osteoclasts. This RANK-RANKL binding can be inhibited by OPG, a decoy receptor for RANKL.

Many bone pathologies, as osteosarcoma [1] or odontogenic tumors [2], exhibit modified expression of these molecules, leading to a large and chronic osteolysis. In these cases, over-expression of RANK had been frequently observed. Hence, studying impact of RANK over-expression by osteoclasts would allow a better understanding of the biological processes underlying tumoral proliferation.

The aim of this study is to characterize long bone phenotype of transgenic mice over-expressing RANK in monocyte-macrophage cell lineage.

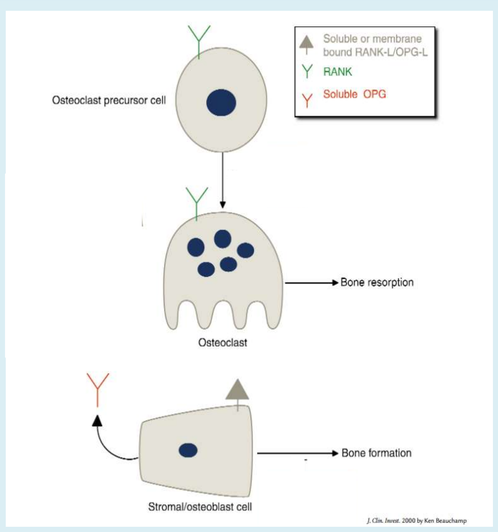


Figure 1: RANK-RANKL-OPG triad and osteoclastic differentiation

METHODS

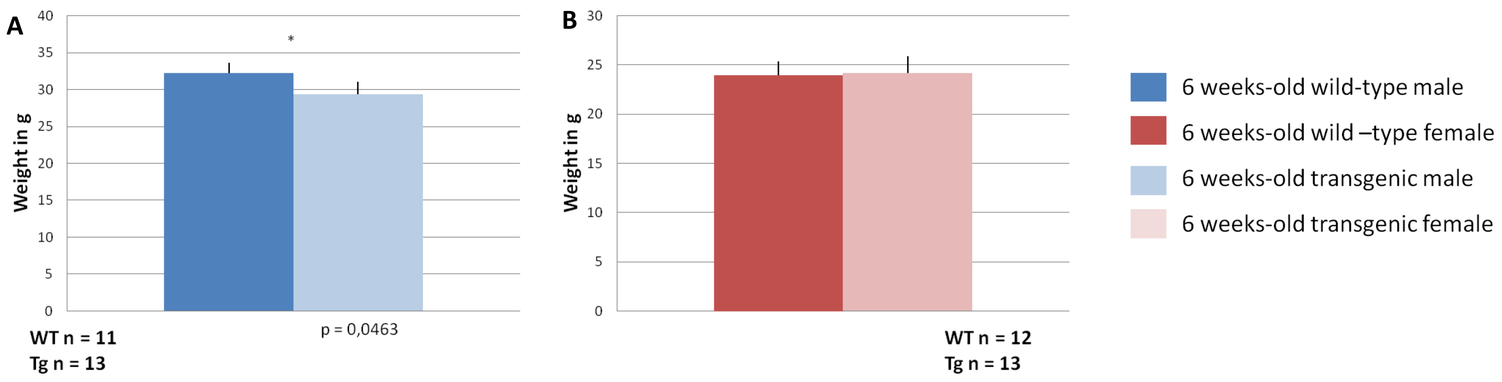
Long bone phenotype has been realized on 6-weeks-old mice, wild-type (WT) or transgenic (R-Tg). Transgenic mice over-express RANK in the monocyte-macrophage cell line, under the control of hMRP8 promoter (figure 2) [3][4].

Figure 2 : construction of transgenic model

Phenotypic analyzes were based on histomorphometric studies [5]. Femurs were used to analyze long bone phenotype. Before sacrifice, every animal was injected with tetracycline and calcein (respectively 72 and 24 hours before sacrifice for 6-weeks-old mice) to evaluate dynamic parameters, and was weighted.

RESULTS

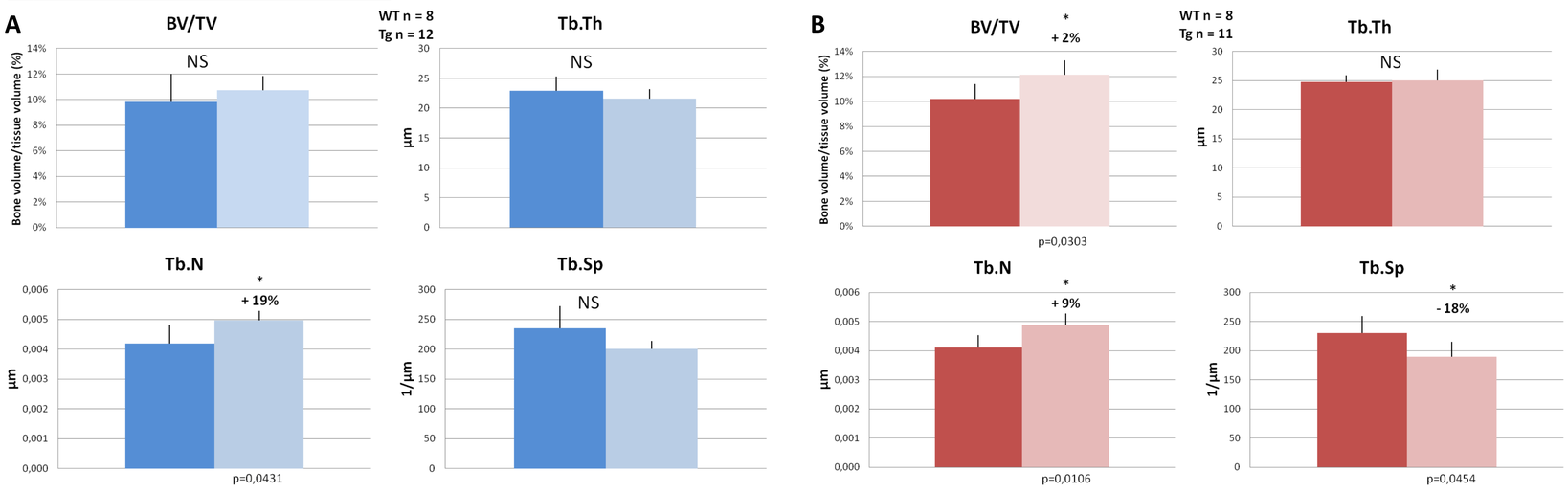
Figure 3 : Body weight



Every sacrificed animal was weighted. Here we compare weight of transgenic *versus* wild-type 6-weeks-old males (A) and females (B).

At 6 weeks, transgenic male had **a decreased weight** (-5,1 g; p = 0,0463).
No significant difference was found concerning 6-weeks-old females.

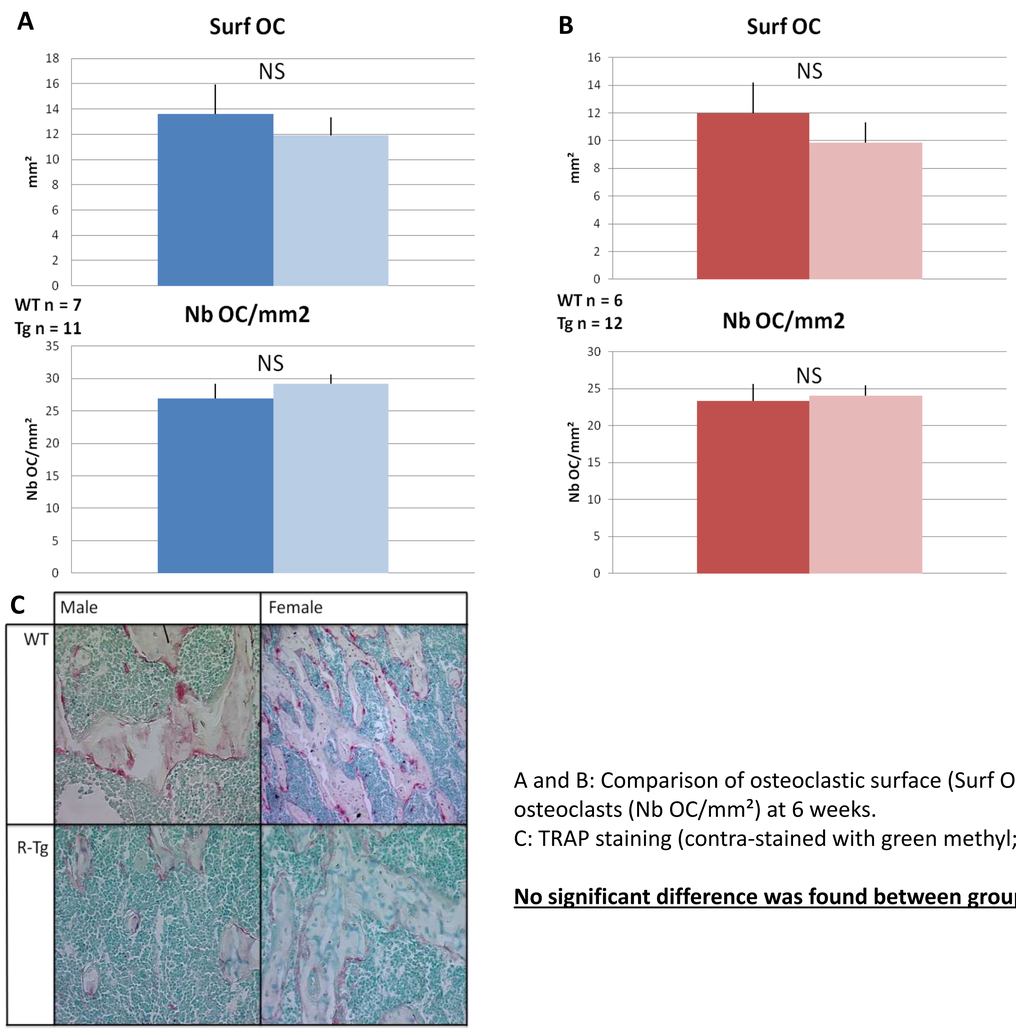
Figure 4 : Structural parameters



Measures of bone volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N) and trabecular separation (Tb.Sp) of 6-weeks-old males (A) and females (B).
C : blue aniline staining of 6-weeks-old WT (left) and R-Tg (right) males and females (objective x 5)

Transgenic males had an **increased trabecular number** (+19%; p = 0,0431).
Transgenic females had an **increased bone volume** (+2%; p = 0,0303), an **increased trabecular number** (+19%; p = 0,0106) and a **decreased trabecular separation** (-18%; p = 0,0454).

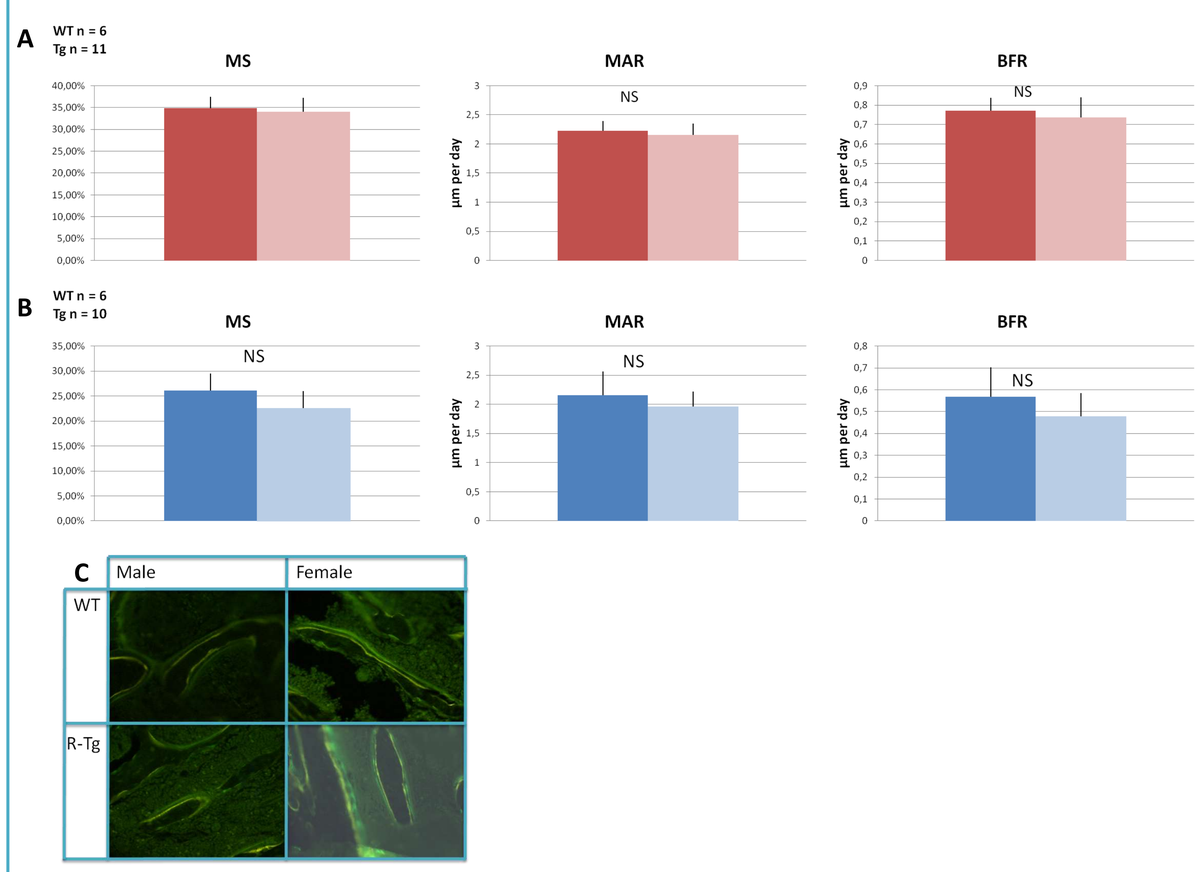
Figure 5 : Osteoclastic parameters



A and B: Comparison of osteoclastic surface (Surf OC) and number of osteoclasts (Nb OC/mm²) at 6 weeks.
C: TRAP staining (contra-stained with green methyl; objective x 25).

No significant difference was found between groups.

Figure 6 : Dynamic parameters



Measure of mineralizing surfaces (MS), mineral apposition rate (MAR) and bone formation rate (BFR) for 6-weeks-old males (A) and females (B).
C : fluorescence microscopy observation of tetracycline and calcein signal (objective x25).

No significant difference was found between different groups.

CONCLUSION

Those preliminary results of phenotypic analyses show some differences between male and female, WT and R-Tg. Transgenic males exhibit a decreased body weight and an increased Tb.N, whereas transgenic females have an increased BV/TV, Tb.N and a decreased Tb.Sp. No significant difference was found concerning osteoclastic and dynamic parameters.
RANK is a key regulator of osteoclastogenesis and osteoclastic differentiation. Our transgenic model over-express RANK in monocyte-macrophage cell line; initial hypothesis was that this over-expression will lead to increased osteoclastic surface and number, associated to a reduced BV/TV, *i.e.* an osteoporotic phenotype. But, at 6 weeks, transgenic mice have the same bone phenotype as wild-type concerning osteoclastic parameters; the only differences observed were for structural parameters. Furthermore, we found different phenotype between transgenic males and females. Indeed, transgenic males seem to exhibit almost no long bone phenotype, whereas females have a slightly more marked phenotype. Those differences can be explained by estrogenic osteoclast modulation [6].
This study will have to be completed by analyzes of jaw bone, in order to find if this RANK over-expression has different effects on different types of bone. We will also study femurs and jaws at different ages, specially 2-weeks-old, since this age corresponds to jaw growth stage.

REFERENCES

[1] Mori K et al. Osteosarcoma: current status of immunotherapy and future trends. Oncol Rep. 2006 Mar;15(3):693-700.
[2] Ruhin B et al. Facts and Hypothesis on Osteolytic Lesions Related to Normal and Tumoral Epithelial Dental Cell Differentiation. In: Bone Cancer: Progression and Therapeutic Approaches. Auteur: Heyman D, Editions Elsevier. 2009.
[3] Castaneda B et al. Bone resorption control of tooth eruption and root morphogenesis: Involvement of the receptor activator of NF-κB (RANK). J Cell Physiol. 2011 Jan;226(1):74-85.
[4] Duheron V et al. Receptor activator of NF-kappaB (RANK) stimulates the proliferation of epithelial cells of the epidermo-pilosebaceous unit. Proc Natl Acad Sci U S A. 2011 Mar 29;108(13):5342-7.
[5] Parfitt AM et al. Bone histomorphometry : standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res. 1987; 2(6):595-610.
[6] Robinson LJ et al. Estrogen inhibits RANKL-stimulated osteoclastic differentiation of human monocytes through estrogen and RANKL-regulated interaction of estrogen receptor-alpha with BCAR1 and Traf6. Exp Cell Res. 2009 Apr 15;315(7):1287-301.