Phenotyping of a mouse model for Paget's disease of bone

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Aims. Paget's disease of bone (PDB) is a common, late onset (usually after the age of 50) bone disease, characterised by a focal increase in bone turnover. The main problem in PDB is overactive bone resorption by osteoclasts. The osteoblasts then try to compensate for this by rapidly producing more bone, but this leads to disorganized and structurally weak bone (1). The consequences of this are bone deformation, fracture, and bone pain, and, in cases where the skull is affected, deafness. In some patients only a single bone is affected, whereas in others multiple bones are affected. Although the underlying cause of PDB is not yet clear, in many cases PDB is an inherited disease, and mutations in SQSTM1 gene are observed in a large proportion of patients with PDB (2-4). However, it is at present not clear whether these mutations are sufficient to cause PDB. To study this we have developed a transgenic mouse that carries the most common SQSTM1 mutation, P392L.

Method. Mice were analysed for the presence of PDB-like bone lesions by μCT at 12 months of age. The animals were killed and the hind limbs fixed for 24h in buffered formalin. Micro-CT analysis was performed using a Skyscan 1172, at 50 kV, 0.5mm Al filter and 17µm resolution to analyse whole hind limbs. A 5µm resolution was used for detailed analysis of regions of interest in the bones. Lesions were scored as mild (score=1), moderate (2) or severe (3), and all scores for each animal added to give a total severity score per animal.

Results. Originally, we tried to look for bone lesions in the transgenic mice using a traditional X-Ray machine (Faxitron). However, the images were hard to analyse, and we only observed a lesion in one animal. This turned out to be an uncharacteristic very large lesion. Using μCT analysis we observed a wide range of focal abnormalities in hind limbs of the transgenic mice. The most common lesions observed were small lytic areas close to the growth plate in the distal femur and proximal tibia (Fig. 1A). In addition, large lytic lesions penetrating the cortex were observed in the metaphysis of the tibia (Fig. 1D-F). Intracortical lesions accompanied by disorganised bone were observed in the midshaft of the femur, and at the base of the trochanter (Fig. 2). No lesions were observed in age-matched wild type control animals. The distribution and severity of the lesions differed strongly between the affected animals, and in most cases the lesions were a-symmetric. Penetration was almost complete in the homozygotes (19 out of 20 animals were affected), whereas only 14 out of 18 heterozygotes were affected. The homozygote animals were also more severely affected than the heterozygote animals (lesion score HOM 3.5±2 versus HET 1.4±1; p<0.01). Since the bone lesions were clearly visible at a resolution of 17µm in the 1172 desktop scanner, we next tested whether they could be visualized using the 1076 in vivo scanner. When scanned at a resolution of 18µm the PDB-like lesions were easily visualized (Fig. 3), and we are now using this technique to study when the lesions first appear, and how they develop over time.
Fig. 1: PDB-like lesions in the tibia. A: knee scan of a WT animal; B: knee scan of a P392L animal; C: unaffected left tibia of P392L HOM; D: Right tibia of same mouse. Note large lesion in cortex. E, F: lesion in tibia penetrating the cortex in P392L HOM.

Fig. 2: PDB-like lesions in the femur. Note the abnormal “foamy” cortical bone indicated by the arrows in the P392L animal. The position of the cross-sectional image for the mid shaft was taken at the green arrow, for the base of the trochanter at the red arrow.
Some of the bones that showed big lesions by µCT were embedded in MMA and sections prepared and stained for microscopy. We used the 3D reconstructions from the µCT to guide us to the lesions in the bones during sectioning. The sections from mutant mice clearly showed increased numbers of osteoclasts (Fig. 4A). Normally, mouse osteoclasts only have up to 3-4 nuclei in sections like this. The osteoclasts from the mutant mice though were also abnormally large and had many more nuclei (up to 20) than normal osteoclasts (Fig 4B).

The main growth factor for osteoclasts is called RANKL (5), and one of the current theories is that PDB is caused by hypersensitivity of osteoclast precursors to RANKL. When we generated osteoclasts from bone marrow cells in culture by stimulating the cells with RANKL, we observed indeed an increase in sensitivity to RANKL in the mutant cells (Fig. 4C). However, in contrast to the µCT analysis of the bones where the phenotype was more severe in homozygotes than in heterozygote animals, the increase in sensitivity to RANKL was the same for both genotypes in the osteoclast cultures.

**Fig. 3: Development of a lytic lesion in the tibia studied using a 1076 in vivo system.**

**Conclusion.** In conclusion, mice carrying the P392L mutation of the SQSTM1 gene show skeletal abnormalities that closely resemble PDB in humans. Our results therefore suggest that mutations of SQSTM1 are sufficient to cause PDB. The fact that disease was more severe in homozygotes, while the increase in RANKL sensitivity was the same in homozygote and heterozygote animals, suggests that increased sensitivity to RANKL is not the only cause of PDB. This mouse is the first animal model for PDB, and will allow us to investigate the causes of this disease, and to test new therapeutic approaches.
Fig. 4: Increased osteoclast number and size in P392L mutant mice. A and B: sections trough an affected tibia shown in Fig 1A. Note the large numbers of osteoclasts (red, indicated by arrows), and the large number of nuclei in the high power view of an osteoclast in B. C: Bone marrow cultures were stimulated with different concentration of RANKL for 6 days, and osteoclasts stained and counted. Both P392L HET and HOM cells are more responsive to RANKL, and there is no difference between HET and HOM cells in this assay.

Reference List