

Stem cells and bone loss

Dr Peter J Richards from the University of Zurich discusses his research into mesenchymal stem cells and their role in defining bone quality

Bone is constantly being remodelled by a process called bone turnover, which consists of the resorption of existing old bone and its subsequent replacement by new bone. In a healthy organism, this process enables regulation of bone stability as well as adaptation to changing environmental conditions, e.g. an increase in weight or sportive activities. Additionally, small tears or fractures can be directly repaired during bone remodelling. The disequilibrium of the remodelling parameters has the potential to cause deficits in the mechanical strength of bone resulting in an increased risk of fracture, as is the case in osteoporosis.

Osteoporosis

Osteoporosis is defined as a systemic disease characterised by reduced bone mass and low bone mineral density, with a subsequent increase in bone fragility and vulnerability to fractures. Postmenopausal osteoporosis is further characterised as high turnover remodelling in comparison to the low turnover remodelling observed in age-related osteoporosis. The total number of fractures, and hence the cost to society, will increase dramatically over the next 40 years as a result of demographic changes in the number of elderly people. It has been estimated that by 2050, the incidence of hip fractures worldwide will be approximately 6.3 million.

As many as one in two women and one in three men will sustain an osteoporotic fracture in later life, and there is a greater than 30% risk that patients will die within the first year of sustaining a hip fracture. In addition, almost one third will require placement in a nursing home following hospital discharge. Statistics for Switzerland alone estimated the direct medical costs related to hospitalisation of osteoporotic patients in 2000 as being more than CHF350m (~€284m). As such, osteoporosis is now regarded as being a global healthcare problem and represents a significant economic burden.

Current treatment of osteoporosis

The steady turnover of bone is directed through the contrasting actions of bone-forming cells (osteoblasts) and bone-resorbing cells (osteoclasts), and alterations in this homeostatic balance can lead to detrimental effects on bone quality, as seen in osteoporotic patients. The availability of effective treatments to combat bone loss and subsequent fractures is still an unsatisfactorily solved problem today, despite the numerous pharmacological and non-pharmacological treatments currently in use. The most widely used



Dr Peter J Richards

approach to enhancing bone strength in osteoporosis is through the prevention of bone loss using various anti-resorptive agents including bisphosphonates, oestrogen, calcitonin and selective estrogen-receptor modulators (SERMs). However, there is now a growing body of experimental evidence to suggest that improvements not only in bone mass, but also in bone quality, maybe brought about through the use of anabolic agents. This has led to a shift in the treatment regimes previously employed to combat bone loss, with emphasis now being placed on targeting osteoblast-dependent bone formation as opposed to osteoclast-dependent resorption. To date, the only approved anabolic compounds currently available are two forms of parathyroid hormone (PTH), a hormone secreted by the parathyroid gland. Both forms, PTH₁₋₃₄ and PTH₁₋₈₄, have protective roles on skeletal integrity through stimulation of bone formation.

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However, the use of PTH over the current anti-resorptive therapies has not been substantiated by the efficacy data and therefore suggests that, along with the high expense and extended treatment period associated with PTH, alternative bone-forming therapies may be required. For this reason, new treatment strategies are now being developed to target the cell source from which the bone-forming osteoblasts arise, namely the bone marrow stromal cells (BMSCs).

The role of BMSCs

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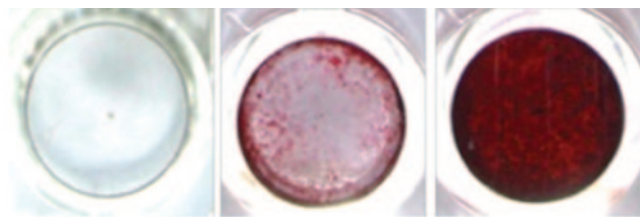


Fig. 1 BMSCs isolated from osteoporotic patients were incubated in growth medium (left), osteogenic medium (middle) or osteogenic medium plus bisphosphonate (zoledronate) (right) and stained with Alizarin red. The red colour indicates mineralisation

potential for osteogenic differentiation and thus play a central role in the overall maintenance of bone quality. However, their general fitness appears to decline with donor age and passage number in culture as evidenced by increases in cellular ageing markers and an inability to maintain osteogenic potential under normal conditions and following exposure to stress.

Such observations have therefore led to speculation that deficiencies in resident BMSC differentiation may play a significant role in the development of age-related osteoporotic phenotypes. This is supported by the discovery that BMSCs isolated from aged osteoporotic patients have a higher propensity towards fat formation (adipogenesis) than bone formation (osteogenesis) and therefore implies that the structural abnormalities associated with osteoporotic bone maybe as a consequence of inadequacies in bone cell differentiation. As such, it is imperative to have a full understanding of the mechanisms governing BMSC osteogenesis in order to design effective therapeutic interventions to enhance, and possibly even normalise, bone quality in osteoporotic individuals.

The Bone and Stem Cell Research Group

To this end, one of our major research aims within the Bone and Stem Cell Research Group is to characterise the functional role played by BMSCs in the development of osteoporotic bone through the use of molecular, biochemical and histological techniques. As such, part of our work is focused on elucidating novel pathways involved in regulating osteogenic differentiation and how these may contribute to the onset and progression of osteoporosis. We are currently utilising stem cells isolated from both human patients and experimental models and have developed the necessary techniques with which to successfully harvest and expand cells from specific stem cell niches. A better understanding of how osteoporosis is regulated at the cellular level could lead to the development of more effective therapeutic strategies to treat age-related bone loss. Furthermore, the potential benefits of having identified new regulators of bone formation could easily be extended to other orthopaedic-related areas including fracture healing, where there exists a growing need for more effective inducers of bone regeneration, especially where bone healing is severely impaired as is the case with critically sized defect fractures and non-unions.

Do bisphosphonates influence stem cells?

The majority of current treatments available for osteoporosis are directed towards preventing bone resorption, with bisphosphonate therapy being the most widely used anti-resorptive approach to enhance bone strength in osteoporotic patients. Bisphosphonates are a well-characterised class of synthetic compounds structurally related to pyrophosphate, and are thought to mediate their anti-resorptive actions primarily through inhibition of osteoclast activity. However, there are now an increasing number of reports alluding to the idea that the preventative effects of bisphosphonates on bone loss, may be additionally mediated through their anabolic effects on cells of the osteoblastic lineage.

Thanks to the CABMM research network and the efforts of Professor Michael Blauth and his research team (University Hospital of Innsbruck), we were able to gain access to BMSCs isolated from human osteoporotic patients. We could show that, through the collaborative efforts of both research groups, BMSCs isolated from osteoporotic patients were defective in terms of their ability to differentiate to osteoblasts and produce mineralised tissue *in vitro*, and that these effects could be overcome through the addition of bisphosphonates (Fig. 1).

Furthermore, we demonstrated that BMSCs isolated from osteoporotic patients receiving bisphosphonate treatment had a greater propensity to form osteoblasts than BMSCs harvested from non-treated patients. At the molecular level, we identified osteopontin as being a candidate gene through which the osteoanabolic effects of bisphosphonates could be regulated. Indeed, the silencing of osteopontin in BMSCs isolated from osteoporotic patients led to an increase in their osteogenic potential. These findings thus further support the concept of BMSCs as being important regulators of bone quality, and could also represent an alternative means by which bisphosphonates increase bone mineral density in osteoporotic patients. It is expected that further investigations into how bisphosphonates influence BMSC function will better our understanding of how these potent synthetic compounds act to mediate bone quality and turnover in osteoporotic patients, and that these insights may ultimately lead to the generation of new and novel approaches to treat age-related osteoporotic bone loss.

Enzyme linked to age-related bone loss

Our research into the biological role of serine protease HTRA1 has implicated it as a detrimental factor in some diseases, while at the same time being shown to have a positive impact in others. Initially, our research started out as an investigation into the role of HTRA1 in the regulation of stem cells isolated from the bone marrow in terms of their ability to differentiate. Stem cells are multipotent, meaning they have the potential to turn into various different cell types.

In osteoporosis, one of the theories is that the stem cells within the bone marrow are turning into fat cells rather than bone cells. This presents many problems such as loss of bone stability, inflammation and dysregulation of processes within the bone. Our

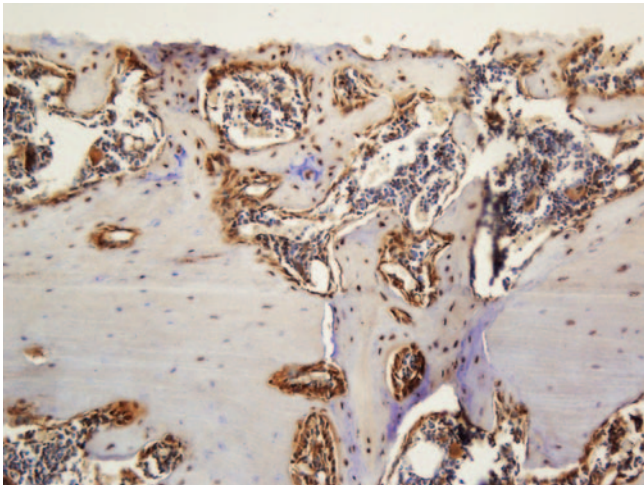


Fig. 2 HTRA1 protein (brown) localised to bone-forming cells within newly formed cortical bone of an experimental fracture model

first hints of HTRA1 as a potential mediator of bone formation came from studies using an experimental fracture model, where increased levels of HTRA1 protein were identified in close proximity to newly regenerated bone in association with bone-forming cells (Fig. 2).

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In vitro analysis later confirmed that when the gene encoding for HTRA1 was completely eliminated from human BMSCs, their osteogenic differentiation potential was dramatically impaired. By contrast, these same cells demonstrated an increased ability to turn into fat, a similar state of dysregulation one would expect to find in an osteoporotic patient. Interestingly, subsequent tests showed that by adding more HTRA1, we could achieve the

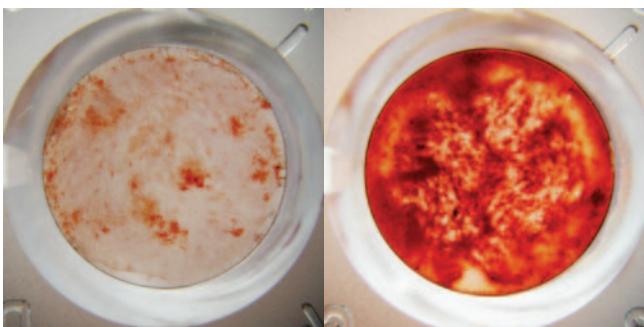


Fig. 3 Alizarin red staining of untreated (left) or HTRA1-treated (right) osteogenic BMSCs. Red colour indicates mineral formation

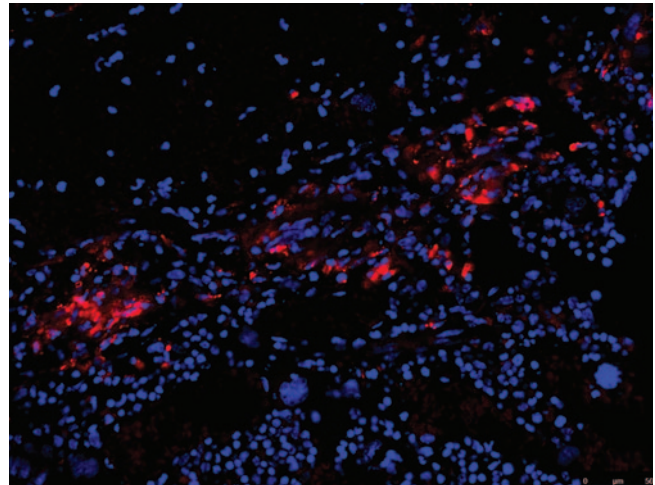


Fig. 4 Paraffin wax tissue section of treated mouse tibia demonstrating accumulation of fluorescently labelled donor ASCs (red) within the bone marrow. Dapi (blue) was used as a nuclear stain to allow for visualisation of cells within the bone sections

opposite effect (Fig. 3). Therefore, due to its effects on the differentiation potential of mesenchymal stem cells, HTRA1 may represent a possible therapeutic target or even a biomarker in treating age-related bone loss. As such, we are now beginning tests on osteoporotic subjects to investigate whether alterations in HTRA1 levels are indeed linked to changes in human bone quality.

Therapeutic potential of stem cells

In an alternative approach, we have also considered the possibility of using stem cells, other than those isolated from bone marrow, for the purposes of restoring bone quality. These studies have been focused predominantly on the use of stem cells harvested from fat tissue, termed adipose-derived stromal cells (ASCs). Our findings to date have confirmed that ASCs isolated from experimental models of osteoporosis maintain telomere length, show no signs of premature cellular senescence and retain a high capacity for osteogenic differentiation. Such observations therefore support the notion of adipose tissue being a valuable source of osteoprogenitor cells and that, unlike osteoporotic BMSCs, ASC proliferation and differentiation does not appear to be adversely affected by the age or osteoporotic status of the donor from which they were isolated.

These findings are currently being utilised in our most recent studies where ASCs isolated from aged osteoporotic mice have been reintroduced back into the bone marrow of the same animal with an aim to stimulating new bone growth and improving the overall level of bone quality (Fig. 4). In order to help facilitate this process, we have also introduced the concept of using microtissue spheroids as scaffold-free vehicles for the delivery of ASCs into the bone cavity. ASCs grown in hanging drop cultures not only have the ability to form tissue spheroids, but also demonstrate an increased capacity to undergo osteogenic differentiation and may therefore represent a means by which to optimise their bone-forming potential *in vivo* (Fig. 5). Therefore, if successful, we would consider autologous mesenchymal stem cells isolated from fat tissue as

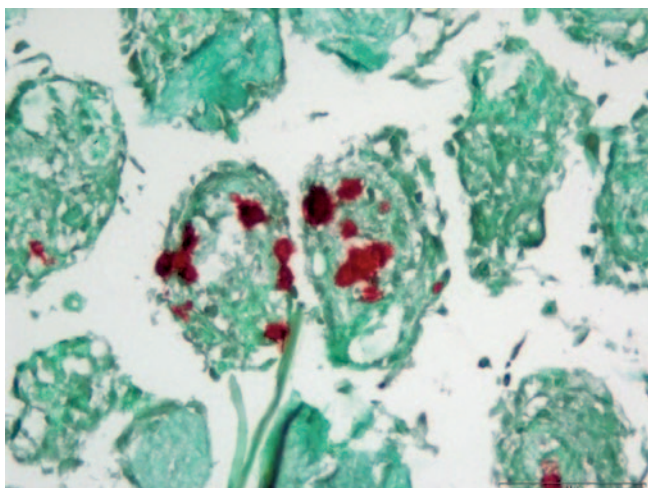


Fig. 5 Alizarin red and fast green stained paraffin wax sections of osteogenic ASC spheroids demonstrating mineral deposition (red)

being an alternative bone-regenerative therapeutic strategy for the treatment of age-related osteoporosis.

Conclusions and outlook

We envisage that a better understanding of the mechanisms governing BMSC differentiation and how these can be manipulated *in vivo* will be of paramount importance in the development of more effective therapeutic strategies for the treatment of bone loss in the elderly. The use of mesenchymal stem cells isolated from sources other than bone marrow may prove to be an alternative cell-based approach by which to improve bone quality in osteoporotic patients. Improvements in stem cell differentiation capacity and delivery methods will no doubt enhance their regeneration potential and result in a more favourable clinical outcome.

Dr Richards' interest in the field of musculoskeletal research and disease was first realised during his training as a Medical Laboratory Service Officer in the Rheumatology Department, University of Wales College of Medicine. The insights gained from this initial experience proved invaluable in helping him to successfully complete his PhD thesis in 1999, where he was given the opportunity to develop his fascination with both basic and applied science in the area of musculoskeletal biology and disease. This scientific work was expanded further, when he undertook a three-year postdoc position in the group of Prof Simon A Jones, Cardiff University, investigating the underlying molecular mechanisms regulating arthritis. It was during this time that he became interested in research relating to the role of serine protease HTRA1 in disease, and subsequently managed to form a fruitful collaboration with Prof Michael Ehrmann, which remains on-going to this day.

His relocation to Zurich University Hospital in 2005 offered him the chance to utilise his knowledge of basic and applied research in a closely associated field of scientific study. He took over managing a small research group within the Trauma Surgery Department, where he became introduced to the concept of

The CABMM's mission, vision and values

The CABMM is dedicated to fostering advances in clinically oriented research in the fields of regenerative medicine, experimental medicine and surgery, applied biotechnology, and molecular medicine. Embedded within both the Vetsuisse and Medical Faculties of Zurich University, it provides an interdisciplinary research platform on which basic scientists and clinicians are able to exchange scientific information and create collaborations for the purpose of developing novel therapeutic approaches for the treatment of dysfunctional and diseased tissues.

In addition, the CABMM takes responsibility for training and mentoring junior scientists and newly founded groups. Based on these concepts, it also aims to establish relevant research activities at the University of Zurich and to strengthen already existing bonds, especially between the Vetsuisse Faculty and the Medical Faculty for Clinical Research. Uniting clinically oriented research activities not only creates a solid basis for core competencies, but also optimises the use of available infrastructure. It is envisaged that through the unique collaborative network provided by the CABMM, new and important advances can be made in our ability to understand, treat and manage human disease.

stem cell involvement in bone disease, which has since become one of the main focuses of his research.

Following the successful completion of this project, he was given the opportunity to join Kuros Biosurgery AG as a project manager. During these two years of industrial employment, he was responsible for organising and overseeing both the preclinical and clinical phases of orthopaedic research-based projects. In 2008, he was fortunate enough to be given the chance to return back to academia, and has since established his own research group within the framework of the Center for Applied Biotechnology and Molecular Medicine, Zurich University, under the guidance of Professor Brigitte von Rechenberg.



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