Understanding the nuances of the fiendishly complex systems of biochemistry can be frustrating and counterintuitive, but at the same time fascinating – ask anyone studying them. Research into the serine protease HTRA1 has implicated it as a factor in a number of diseases in both a positive and negative light. New research from the CABMM, University of Zurich, has been exploring the role of this enigmatic protease in age related bone disease.

Enzyme linked to age-related bone disease

"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," says Dr Peter Richards. "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."

Richards and his team first discovered the ability of HTRA1 to alter the differentiation of stem cells quite by accident while playing around with its expression levels in isolated human stem cells. When the gene coding for HTRA1 was completely eliminated, the stem cells showed reduced ability to turn into bone and an increased ability to turn into fat, a similar state of dysregulation that you would expect to find in an osteoporotic patient. "This caught our attention, and subsequent tests showed that by adding more HTRA1, we could achieve the opposite effect.

"We then took things further and started having a look at some of the other proteins involved in osteogenesis. There are a number of these proteins found in the matrix of bone which help the process of mineralisation. We found through various expression assays that when we added HTRA1, we actually enhanced the expression of one of these proteins, BSP2. We then consulted the literature and found that BSP2 is in some cases thought to stimulate the differentiation of stem cells into bone."

From the face of it this seemed like a perfectly logical scenario; the HTRA1 was enhancing the BSP2 and that was in turn enhancing the formation of bone. However, the next step of the investigation threw something of a curveball to the researchers. Going back to the culture system, they first allowed the cells to differentiate into bone cells, added HTRA1 and then used immunofluorescence labeling to track what was happening to

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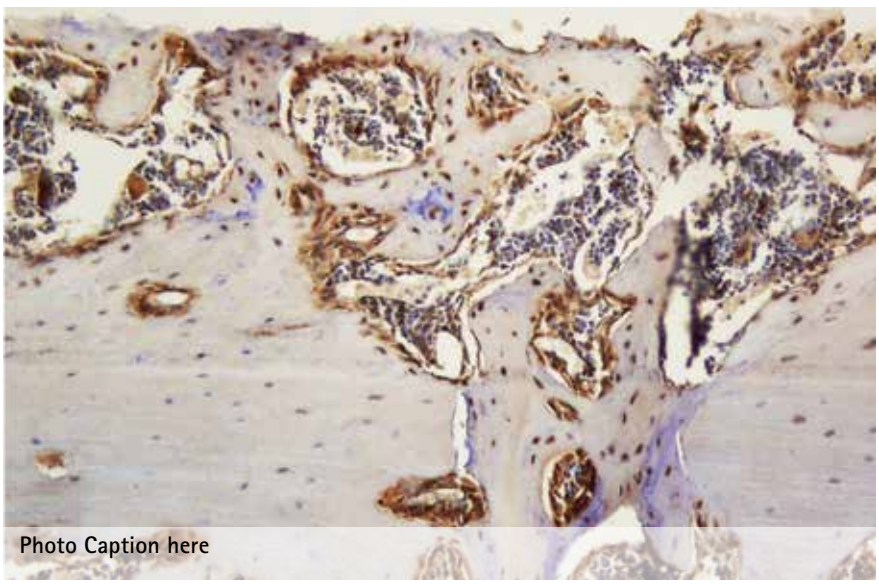

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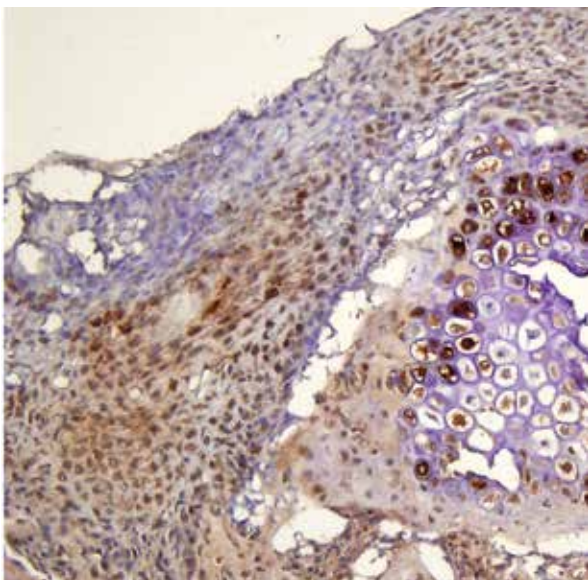


the BSP2 protein. To their great surprise, it appeared that the HRTA1 had completely eradicated any trace of the BSP2 protein.

After running extensive further tests, Richards and his team confirmed that the HTRA1 was indeed digesting the BSP2 protein. "We were a bit stumped at this point, because the HTRA1 was simultaneously enhancing the expression of BSP2 while also completely destroying it. We decided to look deeper into the literature, and eventually found that BSP2 has also been shown to inhibit mineralisation."

Using tissue sections from a mouse femur in which a fracture had been deliberately induced and then allowed to heal, Richards found through immunostaining techniques that the HTRA1 protease is found in the bone only when it is healing, suggesting a functional role in bone regeneration. Further staining showed that HRTA1 and BSP2 were found colocalised in areas of newly regenerated bone, suggesting a functional interaction.

"The scenario we came up with was that the HTRA1 was removing the BSP2 protein, thereby allowing various processes to become enhanced, but at the same time causing the cells to upregulate BSP2 expression in order to replace it.

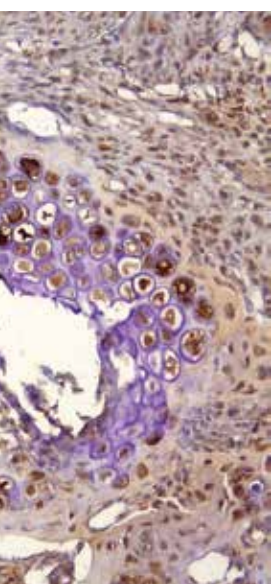
"This is as far as we have got with the mechanism, but of course there are a number of other proteins which may be involved. BSP2 is a member of a family of specialised matrix proteins called SIBLINGs, a group of five proteins which show very similar structural qualities and functional roles. It seems that HTRA1 may be targeting these particular proteins, and so we are now starting to look at some of the others aside from BSP2."

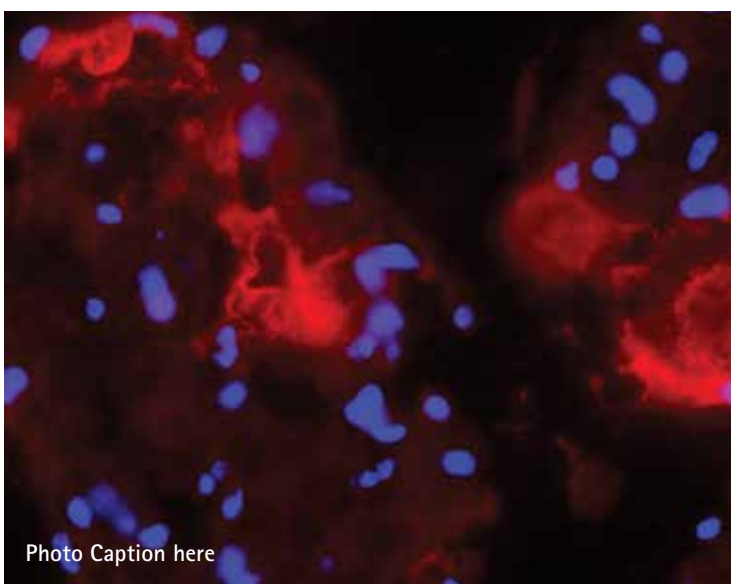
Osteoporosis is a bone disease in which the bone mineral density is reduced, bone microarchitecture deteriorates and the amount and variety of proteins in bone are altered. In mice, it has been shown to be reliant on stem cells in the bone marrow. Due to the effect that HTRA1 has on the differentiation of these stem cells, it has potential use in the future as a therapeutic target or even a biomarker. Richards and his colleagues are now beginning tests on osteoporotic patients, analysing their stem cells and blood serum in the hope of identifying potential targets for inhibition of the disease.

HTRA1 continues to bewilder researchers all over the world with its ambiguous role within the body. "There has always been a fair amount of interest in HTRA1 in the medical world," says Richards. "Previously, it was found that if it was upregulated within tumour cells then they would stop dividing. This caused a huge amount of excitement within the research world and caused a flood of papers to be published on the possible ways that HTRA1 could be regulated within the tumour cells.

"However, more recently the focus has turned towards its role in age-related macular degeneration, the leading cause of blindness within the Western world. A lot of research from Japan has been suggesting that it is related to the disease in a negative way. It is already known that it has a negative effect on rheumatoid arthritis and osteoarthritis, but at the same time it has been shown to have positive potential in cancer and Alzheimer's.

"I think this is the reason why it is such an interesting topic of study; nothing is black or white in the world of HTRA1!" ★





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AT A GLANCE

Project Information

Project Title:
Regulation of Osteogenesis by HTRA1

Project Objective:
Human serine protease HTRA1 positively regulates osteogenesis of human bone marrow-derived mesenchymal stem cells and mineralization of differentiating bone-forming cells through the modification of extracellular matrix protein. To examine the effects of recombinant HTRA1 on the osteogenesis of human BMSCs (hBMSCs) and matrix mineralization by differentiating bone-forming cells with an aim to establishing a role for HTRA1 in bone formation.

Project Duration and Timing:
3 years; 2009 - 2012

Project Funding:
Swiss National Science Foundation; CABMM Start-up Grant; Forschungskredit of the University of Zurich; Novartis Foundation, formerly Ciba-Geigy-Jubilee-Foundation; Uniscientia Foundation.

Project Partners:
Prof. Michael Ehrmann

MAIN CONTACT

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Peter J. Richards was born in Australia, and studied immunology at the University of Wales College of Medicine, UK where he received his doctoral degree in 1998. He is currently Head of the Bone and Stem Cell Research Group and also Scientific Director of the CABMM in Zurich University, Switzerland.

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