

**P002** The serine protease HtrA1 regulates physiological and pathological matrix mineralisation

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HtrA1 is a secreted multi-domain protein with proven serine protease activity. There is increasing evidence that this protease regulates skeletal development and pathology. The purpose of this study was to test the hypothesis that HtrA1 regulates matrix mineralisation and to identify the domains essential for its activity. We demonstrate that HtrA1 is localised at sites of physiological bone formation and at sites of pathological calcification of human arteries. HtrA1 expression is markedly up-regulated when vascular smooth muscle cells (VSMC), pericytes and osteoblasts undergo osteogenic differentiation and is then down-regulated when their matrix is mineralised. Mineralisation is significantly inhibited when osteoblasts are induced to over-express HtrA1 and is enhanced when the expression of HtrA1 is knocked-down by >90% using siRNA. Recombinant HtrA1 inhibits mineral deposition by VSMC and osteoblasts and the protease domain is required for this effect. Over-expression of HtrA1 prevents BMP-2-induced mineralisation of osteoblasts. Recombinant HtrA1 cleaves matrix Gla protein and decorin, which have previously been shown to regulate mineralisation directly and indirectly by regulating BMP-2/TGF $\beta$  bioavailability and activity. Together, these studies suggest that HtrA1 regulates physiological and pathological matrix mineralisation by osteoblasts and VSMC by inhibiting growth factor signalling and/or by cleaving specific matrix proteins.