

ELUCIDATION OF MECHANICALLY-REGULATED SIGNALLING PATHWAYS IN BONE AND THEIR APPLICATION TO BONE TISSUE ENGINEERING

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INTRODUCTION: It has long been established that mechanical loading is an important stimulus in maintaining bone mass *in vivo*. The removal of normal mechanical forces applied to the skeleton causes bone loss however, very short periods of defined mechanical stimuli are all that is required to prevent this loss or even to induce bone formation. The mechanisms by which mechanical signals are propagated in bone are not fully understood but the identification of mediators of mechanical signal transduction may allow the mechanical component of the osteogenic signal to be bypassed. This approach is clearly of benefit both to bone pathologies where the skeleton is too weak to resist osteogenic forces and to bone tissue engineering where the mechanical environment of the bone is compromised by culture conditions. Previously we have used osteogenic stimuli to identify genes regulated in osteocytes by mechanical loading *in vivo*¹. One gene found to be down regulated by mechanical loading was the glutamate/aspartate transporter, GLAST-1. This led to the intriguing possibility that excitatory amino acids such as glutamate, known to be important signalling molecules in the CNS, may mediate mechano-responsive pathways in bone.

In the central nervous system (CNS) glutamate is the major neurotransmitter at excitatory synapses. Excitation of presynaptic neurons causes release of glutamate into the synaptic cleft where it binds to receptors on the postsynaptic neuron to propagate the signal. GLAST-1 is expressed within the plasma membranes of glial cells surrounding these synapses and rapidly binds and transports glutamate into the cells causing termination of the excitatory signal. Whilst other workers have shown that various classes of glutamate receptors are expressed and functional in osteoblasts and osteoclasts, we aimed to determine what the function of GLAST-1 might be in bone and whether it represents a suitable target for modulation of bone cell phenotype.

METHODS & RESULTS: We have cloned the entire open reading frame (ORF) of GLAST-1 from bone and shown that the bone-derived mRNA encodes a protein identical to that expressed in brain². Immunohistochemistry demonstrated that GLAST-1 protein is expressed in osteocytes and osteoblasts and western blotting has revealed a protein of ~70kDa expressed in bone and brain indicating that GLAST-1 mRNA is translated in bone and glycosylated similarly in both tissues². Northern blots have revealed GLAST mRNAs of various sizes indicating that splicing of this gene may occur¹. We have cloned a novel splice variant of this gene, called GLAST-1a, in which exon 3 is excised². Exon 3 encodes 46 amino acids and GLAST-1a is expressed both in bone and brain *in vivo*.

To determine whether GLAST-1 and GLAST-1a may operate as transporters in bone cells we have investigated their intracellular localisation by transfecting MLOY4 osteocytes and SaOS-2 osteoblast-like cells with GFP-tagged GLAST-1 or GLAST-1a. Scanning confocal microscopy has revealed that GLAST-1 is expressed within the plasma membrane consistent with a glutamate uptake function³. Transfection of MLOY4 osteocyte-like cells with GFP-tagged GLAST-1a has revealed a more internalised expression pattern of this variant when compared with GLAST-1³. Interestingly both the expression pattern and relative abundance of GLAST-1 and GLAST-1a appeared to be responsive to extracellular glutamate concentration³.

Recently we have been investigating the function of GLAST-1a by microinjecting *Xenopus* oocytes with cRNA encoding the ORF of GLAST-1a. These experiments have revealed that GLAST-1a can also transport glutamate (Mason, Huggett and Daniels unpublished data).

DISCUSSION & CONCLUSIONS: Other workers have shown that osteoblasts constitutively release glutamate by exocytosis and respond to

glutamate through activation of various ionotropic and metabotropic receptors⁴. Inhibition of glutamate signalling using receptor antagonists can modulate both osteoblast and osteoclast phenotype⁴. In the CNS glutamate transporters such as GLAST-1 are critical regulators of extracellular glutamate concentrations and their activity is controlled by post-translational modifications (oxidation, phosphorylation) as well as by protein trafficking and gene expression. A better understanding of how the activity of these transporters is controlled in bone cells may allow us to mimic the osteogenic effect of mechanical stimuli. This is particularly relevant to bone tissue engineering where modulation of glutamate signalling may ultimately be used to enhance the bone forming capacity of osteoblasts and improve the quality of the matrix they produce.

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