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Back to the future: Evaluation of the role of glutamate in bone cells

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The recent re-examination of all things osteogenic, in particular those associated with the osteocyte has led to a revolution in efforts to understand this long ignored cell [1]. Derived from mesenchymal progenitors and doomed to a life entrapped in the mineralized skeleton, the osteocyte has emerged as a major regulator controlling much of bone resorption and formation. If there is any truth to the concept that form follows function, then it is perhaps not surprising that the morphology of the osteocyte, with long and interconnected canaliculi reaching into virtually all aspects of the bone microenvironment, is positioned to be so important. In the late 1990's, multiple investigators identified the expression and function of the ionotropic glutamate receptor NMDA receptor (NMDAR) in osteocyte-enriched bone [2–4], suggesting that glutamate may also act as a signaling molecule in bone and regulate bone cell metabolism. Indeed, these original studies were soon followed by others that demonstrated NMDAR and glutamate transporter expression in both osteoblasts and osteoclasts [3,5] as well as regulation [6–8].

The NMDAR is a cation channel and a member of a heterogeneous and widely-recognized family of ionotropic glutamate receptors that have been studied extensively in the CNS [9]. NMDAR is a heteromeric protein complex composed of different subunits from two separate protein families, termed as NMDAR1 (GluN1) and NMDAR2 (GluN2) [10]. An essential feature of NMDAR is that receptor activation and the subsequent influx of calcium ions can trigger a series of calcium-mediated intracellular events. These critically important events, mediated in the CNS primarily by these receptors and their associated transporters are highly dependent on the subunit composition of NMDAR [11]. The most rapid excitatory synaptic transmission in the CNS is mediated by GluR channels that are classified into two major groups, termed ionotropic and metabotropic glutamate receptor [11].

In the accompanying article, Xie et al. (*Calcified Tissue International and Musculoskeletal Research*, this issue) provide an elegant and quantitative assessment of the expression and function of glutamate receptors and transporters in primary mouse osteoblast lineage cells isolated from the calvariae of one- to two-day-old C57/B6J mice. Their investigation is the first to define the broader expression profile of ionotropic glutamate receptor family members and confirmed expression of five glutamate transporters, four ionotropic GluRs (AMPA, NMDA), and seven metabotropic GluRs. These efforts re-address the role of these

important regulatory mediators in this area for many years and re-sharpens the focus on the impact of these molecules on osteoblast development and activity. They evaluated gene expression by targeted low density gene arrays, representing a major proportion of the transporter-related genes in the genome. For completion, they validated any changes in independent biologic replicates using PCR, examined levels of protein expression of the transporters by Western blot and functionally tested the activity of the identified receptors and transporters *in vitro*. Using these analytic techniques, they demonstrated that the inhibition of glutamate transport increases extracellular glutamate concentration that is accompanied by a decrease in osteoblast proliferation, the stimulation of alkaline phosphatase, and the expression of transcripts encoding osteocalcin, collectively suggesting the regulation of osteoblast differentiation. Importantly, these effects are abolished by enzymatic removal of extracellular glutamate. In addition, the downstream mediators of the effects of glutamate on murine osteoblastogenesis were revealed in primary calvaria by the similar effects elicited by the inhibition of PKC and Erk1/2 signaling, supporting even earlier work [12].

The current study is valuable for several reasons. First, Xie et al independently validated gene expression profiling, which should be noted and commended. Next, examination of the effects of glutamate transport inhibitors along with enzymatic degradation with glutamate pyruvate transaminase robustly confirmed the importance of the pathway during osteoblastogenesis *in vitro*. Specifically, the studies demonstrated that glutamate can regulate recruitment into and transition down the osteoblastic lineage, an important point to note given that the NMDAR system and transporters are also highly expressed in osteocytes [2–4].

As with all high quality studies, more questions are generated than answered. Does the release of glutamate by osteoblast lineage cells and the expression of transporters and receptors for glutamate provide new therapeutic targets for the regulation of bone metabolism? Where along the osteoblast (and potentially osteocyte) cell membrane are these different glutamate receptors and transporters localized? Can the local release of glutamate in the multicellular unit contribute to the control of bone cell function, which is so intricately controlled at this anatomic site? Is osteocyte glutamate release a component of the mechanostat? The role of the NMDARs *in vivo* is extremely complex. Indeed, Gray et al. [13] reported no effect of NMDARs on bone *in vivo* using mice lacking the glutamate aspartate transporter. These animals exhibit no bone phenotype, but also lack any CNS abnormalities, suggesting that redundant pathways converge on glutamate transport. The answers to these and many other intriguing and challenging questions will hopefully be addressed in future studies.

It seems clear that GluRs and glutamate play a role in many aspects of bone biology. The continuous remodeling of the skeleton, which is controlled by the integrated response of systemic and local growth factors, as well as hormones and cytokines, is likely also modulated by changes in local glutamate release. The presence of GluRs in the skeleton and in the cells responsible for bone formation points to an important role of glutamate in regulating calcium and perhaps phosphate levels. In sum, Xie et al provide the field with a robust reminder that goes back to the future by reminding us of the important interactions

between cellular transporters in bone and provocatively stimulating further investigation of the NMDAR family in the regulation of bone mass. It remains to be seen whether these data will expand the focus of the field to consider the structural and functional assessment of these molecules in the development of a bone phenotype.

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