Roles of Intercellular Gap Junctions and Extracellular ATP in Calcium Signaling in Bone Cell Network

INTRODUCTION: Intracellular calcium ([Ca\textsuperscript{2+}]\textsubscript{i}) elevations in response to mechanical stimulation can be transmitted to neighboring cells in a bone cell network [1]. Diffusion of intracellular second messengers (e.g., Ca\textsuperscript{2+}) through gap junctions and activation of surface membrane receptors by extracellular messengers (e.g., ATP) provide the two major pathways for intercellular Ca\textsuperscript{2+} signal propagation [2]. However, little is known about the relative contribution by these two pathways. A rational step to investigate the role of intercellular gap junction (Cx43) signaling is to build a cell network of bone cells from connexin43 (Cxs43) knockout (KO) mice, and to compare its [Ca\textsuperscript{2+}]\textsubscript{i} responses with those from wild-type (WT) cells. Interestingly, previous studies have revealed that in murine neural cells, the expression of P2Y receptors (G protein-coupled receptors stimulated by nucleotides such as ATP) were altered when Cxs43 is downregulated, while P2X7 receptors (ligand gated ion channels activated by ATP) are the prevalent P2R subtype [3]. Both of these ATP receptors are believed to be significantly involved in the generation and propagation of osteoblast [Ca\textsuperscript{2+}]\textsubscript{i} signaling [2]. In the present study, we subjected bone cell networks, constructed using osteoblasts from either KO or WT mice, to fluid shear stimulation and compared the spatiotemporal characteristics of the [Ca\textsuperscript{2+}]\textsubscript{i} responses from the two different types of cells. Furthermore, the role of extracellular ATP and different P2 receptor in [Ca\textsuperscript{2+}]\textsubscript{i} signaling in both cell networks was also examined and compared by using a P2 receptor antagonist.

METHODS: Primary osteoblasts were obtained from the calvaria of neonatal wild-type (Gja1\textsuperscript{+/+}) or Cxs43-null (Gja1\textsuperscript{−/−}) mice [4]. Cells were later seeded and cultured on patterned slides prepared using microcontact printing and self assembled monolayer technologies [1], forming a controlled bone cell network (Fig. 1). To monitor [Ca\textsuperscript{2+}]\textsubscript{i}, the cells were loaded with Fura-2 AM. During the shear flow test, the slide was mounted in a parallel flow chamber, which was connected to a magnetic gear pump to generate steady fluid flow with a constant 40 dyne/cm\textsuperscript{2} shear stress on the cell surface. The [Ca\textsuperscript{2+}]\textsubscript{i} responses of the bone cell network were recorded by a high speed CCD camera for a period of 10 minutes: one minute for baseline and 9 minutes after the onset of flow. Five different groups were tested: (1) WT cell, untreated (n=17); (2) KO cell, untreated (n=18); (3) WT cell, suramin treated for 0.5 hr before test (n=14); (4) KO cell, suramin treated for 0.5 hr (n=13); (5) KO cell, suramin treated for 4 hrs (n=15). Suramin is considered to be a nonselective antagonist for all P2 receptors. However, its affinity at the P2Y-receptor appears to be much faster and stronger than that found at P2X7 receptors [5]. To quantitatively analyze the spatiotemporal characteristics of the recorded [Ca\textsuperscript{2+}]\textsubscript{i} responses, a set of parameters were defined and illustrated in Fig. 2, including the total number of responsive calcium peaks, the time to reach the first calcium peak, the relaxation time, and the time in-between successive calcium peaks.

RESULTS: In the WT untreated group, over 82% of cells in the network were able to respond two or more times (mean = 2.53) (Fig. 3), while this number decreased to 36% for the WT suramin-treated/ATP blocked group. This suggests extracellular ATP plays a significant role in the multiple [Ca\textsuperscript{2+}]\textsubscript{i} responses, which is consistent with our previous findings using MC3T3-E1 cells [6]. However, there is no significant difference of the number of responsive peaks between KO untreated and WT untreated groups, which implies that gap junctions formed by Cxs43 have a minor effect on the multiple calcium responses in osteoblast networks. More interestingly, the half hour suramin-treated KO group (mainly P2Y receptors blocked) failed to show a decrease in the number of multiple responses compared to the untreated groups. Also, the 4-hour-suramin-treated KO group (all P2 receptor blocked) showed significantly less responsive peaks than the two untreated groups (Fig. 3). This suggests that in addition to neural cells, osteoblasts may have attenuated expression of the P2Y receptor, and P2X7 receptor is yet to be unattenuated in the Cxs43-null mouse bone cells at the same concentration.

REFERENCE: