

Abstract

Osteoclasts play a central role in the formation, growth and remodeling of the vertebrate skeleton. For their bone-resorbing activity, osteoclasts differentiate from bone marrow precursors, proliferate, fuse, and create specific adhesion structures known as sealing zones (SZ), assembled from podosomes. Podosomes consist of a core of actin filaments growing perpendicular to the cell membrane, and anchored by a surrounding ring of adhesion proteins. When podosomes form the SZ actin cores are compacted into a belt, the podosome belt is flanked by inner and outer regions containing integrins and associated adhesion proteins. Live-cell analyses indicate that the SZ is a highly dynamic structure, characterized by continuous assembly and disassembly. Although osteoclast differentiation and function have been extensively studied, the mechanisms that control SZ formation and turnover have not been resolved.

In this work, we studied the morphological and molecular features of stable and unstable regions of the SZ. The unstable regions are commonly characterized by a convex shape, fast displacement, and lower actin intensity, while stable regions are concave, and contain higher actin content. When we analyzed time-lapse movies of actin and other plaque proteins (i.e., vinculin), we found that cycles of stable and unstable regions are not synchronized along the SZ of a given cell, indicating local rather than global control. Such movies also showed that assembly of actin in the SZ precedes vinculin, and that vinculin assembly as well as disassembly rates are faster than those of actin, implying that actin assembly drives SZ formation.

To identify structural and control components in the osteoclast that play crucial roles in SZ stability, we treated osteoclasts with drugs perturbing microtubules (MT), actin, src, ROCK, PI3K, and myosin, and found that modulation of MT and src strongly affects SZ stability.

Treatment by the src inhibitor pp2 caused SZ disruption, and SZ recovery after washout. We found that pp2 first disperses podosomes within the SZ, then also dissolves the podosomes themselves. We conclude that src activity is essential for both podosome integrity and SZ stability. We then disrupted MTs by means of nocodazole, and studied the effects of this treatment on SZ stability. Live-cell microscopy of treated osteoclasts indicated that although podosome compaction in the SZ was completely lost upon treatment, individual podosomes appeared rather stable, and were scattered throughout the cell. This behavior suggests that MTs

are primarily involved in regulating podosome compaction in the SZ.

To further characterize the interrelation between actin and MT in live osteoclasts, we examined stable and unstable regions along the SZ, and recorded the 3D distribution of tubulin and actin in them. We found that in stable parts of the SZ, MTs reach out toward the actin belt, but are somewhat confined by the actin belt to the central areas of the cell. In contrast, in unstable regions, the MTs extend radially, beyond the actin belt. In time-lapse movies, we saw that MTs are as dynamic as actin, and rapidly respond to changes, implying that the interplay between the MT system and actin is crucial for SZ stabilization.

To understand the mechanism underlying this interplay, we searched for molecules that could mediate the cross-talk between the two cytoskeletal systems. One potential mediator of actin-MT interaction is dynamin2, which was found to be associated with the podosomes belt. We acquired super-resolution images of osteoclasts triple-labeled with actin, MT, and dynamin2, and indeed found considerable co-localization of dynamin2 with actin and MTs. Time-lapse movies of actin-MT double-transfected cells treated with the dynamin2 inhibitor Dyngo-3a, led to podosome dispersal in a manner similar to that caused by nocodazole, while it had no apparent effect on the MTs themselves. This finding suggested that MT-dependent stabilization of the SZ requires active dynamin.