Is There a Role for NFAT Inhibitors in the Prevention of Bone Destruction?

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Abstract Pathologic conditions resulting from excessive bone destruction include osteoporosis, rheumatoid arthritis, metastases, periprosthetic osteolysis, cherubism, and others. A scarcity of molecular targets in bone has thwarted the development of drugs to combat these conditions. Nuclear factor of activated T-cells (NFAT) is a master regulator of osteoclastogenesis and is induced by RANKL. The immunosuppressive drugs, Cyclosporin A and Tacrolimus, inhibit osteoclast formation by targeting the NFAT/calcineurin pathway. These NFAT inhibitors should be considered in the treatment of osteoclastic hyper-resorptive syndromes.

Keywords osteoclast · NFAT · Cyclosporin A · Tacrolimus · calcineurin

Nuclear factor of activated T-cells (NFAT) is a family of transcription factors originally identified in T-cells [1]. NFAT plays a critical role in osteoclast development [2]. NFAT molecules act on osteoclast DNA, where they regulate expression of diverse genes [3]. These include interleukin and TNF- α which are also known to be expressed in rheumatoid synovial tissue [4]. NFAT activation requires calcium signaling.

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Embryonic stem cells deficient in NFAT were unable to differentiate into osteoclasts in vivo [5]. Forced expression of NFAT-induced osteoclast precursor cells to differentiate into mature osteoclasts [6]. Genetically altered mice in which NFAT is ablated do not generate osteoclasts and develop osteopetrosis [7].

Osteoclasts differentiate after the binding of RANKL to RANK. Takayanagi [5] and Ishida [8] demonstrated that NFAT is induced in osteoclast precursors by RANKL stimulation. The expression of NFAT is almost undetectable before RANKL stimulation, but abrupt induction is detected on day 1 [8]. Activation of NFAT by RANKL occurs via stimulation of a calcium-dependent protein, calcineurin [9]. NFAT is activated by calcineurin after calcium signaling [1]. Calcineurin is a protein phosphatase which activates NFAT by dephosphorylating it. Calcineurin is required for the genesis of bone-resorbing osteoclasts [11].

In unstimulated cells, NFAT is localized in the cytoplasm, and once calcineurin is activated by extracellular stimuli and following calcium signaling, NFAT forms a complex with calcineurin, resulting in translocation into nuclei to become the active form of NFAT [8]. A recent study by Yeo et al. [10] has shown that the inhibition of calcineurin in osteoblasts increases bone mass by directly increasing osteoblast differentiation and indirectly decreasing osteoclastogenesis.

Cyclosporin A and Tacrolimus [12] inhibit osteoclast formation by targeting the calcineurin-NFAT pathway [13]. Cyclosporin A and Tacrolimus are immunosuppressant drugs frequently used in organ transplantation to reduce the activity of the patient's immune system and thus the risk of organ rejection. Both drugs are also used in a topical preparation in the treatment of eczema, psoriasis, and vitiligo. In addition, they are used in the treatment of rheumatoid arthritis, ulcerative colitis, and Crohn's disease. Adverse drug reactions include vulnerability to opportunistic fungal and viral infections, increased risk of lymphoma, nephrotoxicity, and neurotoxicity.

Treatment with Cyclosporin A results in a blockade of osteoclast formation to less than 1% of normal conditions [14] with a downregulation of more than 30-fold [15]. Cyclosporin A reduces the nuclear localization of NFAT by inhibiting calcineurin [8]. When calcineurin is inhibited by Cyclosporin A, NFAT is rapidly exported from the nucleus to the cytoplasm [14]. Under normal conditions, NFAT was localized mainly in the nuclei at 48–72 h after RANKL stimulation. In contrast, when Cyclosporin A was added together with RANKL, this nuclear transport was largely suppressed and most NFAT was found in the cytoplasm [14].

Deletion of NFAT in young mice resulted in osteopetrosis, growth plate dysplasia, and blocked osteoclastogenesis [7]. Remarkably, osteoclast progenitors made osteoprotegerin, thought previously to be only an osteoblast-derived inhibitor of bone resorption. NFAT deficient mice also have cardiac valve defects. NFAT is expressed in most cells of the immune system and is also involved in the development of skeletal muscle and the nervous system. Immunosuppressive drugs are believed to contribute to the osteoporosis often seen in patients receiving organ transplants, although the incidence of bone disease depends on which organ is transplanted [16].

Recently, polymethylmethacrylate particles were shown to stimulate nuclear translocation of NFAT [17]. Moreover, induction of osteoclastogenesis was selectively blocked by Cyclosporin A and Tacrolimus [17]. The study investigated the effect of PMMA particles on the NFAT signaling pathway in osteoclast precursor cells. Particle-induced periprosthetic osteolysis is the major cause for orthopedic implant failure. The failure is mediated mainly by the action of osteoclasts.

In genetically engineered “cherubism mice”, Cyclosporin A inhibited calcineurin/NFAT by 65% [2], indicating that patients with cherubism who suffer from jaw bone loss may benefit from treatment with Cyclosporin A or Tacrolimus. Cherubism mice carry a gain of function missense mutation in the adapter molecule *Sh3bp2* involving a proline to arginine substitution. SH3BP2 was shown to be acting in the signaling pathway that led to activation of calcineurin.

In summary, the metabolic pathway is: RANKL–RANK–calcineurin–NFAT–calcineurin/NFAT complex enters nucleus—activates osteoclast DNA. In treated cells, the pathway becomes: RANKL–RANK–calcineurin–Cyclosporin A inhibits calcineurin–NFAT–calcineurin/NFAT complexes do not form—NFAT remains in cytoplasm—osteoclast DNA not activated.

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