SH3BP2 Mutations in Cherubism Potentiate sRANKL-Induced Osteoclastogenesis via Phosphorylation of PLCγ2

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Introduction: Cherubism is an autosomal dominant disorder characterized by extensive bone resorptive giant cell lesions in the maxilla and mandible, substantial facial swelling and cervical lymphadenopathy. Age of onset is 2-5 years of age. The localized nature of the bone lesions in patients with cherubism is unexpected as the disorder is associated with heterozygous germline mutations in the SH3BP2 gene (4p16.3) and because SH3BP2 is widely expressed throughout the osteoimmune system. SH3BP2 is a complex adaptor protein, with a ten amino acid Src Homology 3 (SH3) binding domain, and a Src Homology 2 (SH2) domain. In the present study we evaluated the effect of forced expression of mutant compared to wild type SH3BP2.

Materials and Methods: RAW 264.7 cells were cultured to 40-70% confluence and transiently transfected (Nucleofector, Amaxa). 24 hours after transfection, sRANKL or vehicle was added, and after an additional 24 hours of culture the Dual-Luciferase® Reporter Assay System (Promega) was used to measure luminescence. Osteoclast differentiation was assessed by measuring enzymatic activity of TRAP. For immunoblots, whole cell lysates were electrophoresed, transferred to a PVDF membrane. Antibody binding was detected by enhanced chemiluminescence (ECL, Amersham).

Results: Transfection of RAW 264.7 cells with a low dose (0.5 μg cDNA/well of a six well dish) of SH3BP2 cDNA showed that mutant SH3BP2 isoforms activated significantly more NFAT than wild type SH3BP2 (Image 1, p<0.05). Moreover, cells that had been incubated with 100 ng/ml sRANKL for one week showed a significant increase in TRAP activity (Image 2, a1-5 versus b1-5), with mutant forms of SH3BP2 (Image 2, b1-4) producing significantly greater TRAP activity than cells that had been transfected with the wild type SH3BP2 (Image 2, b5), (p<0.05). Also, the level of phospho-Tyr1217PLCγ2 was increased in cells that had been transfected with all mutant SH3BP2 isoforms compared to wild type SH3BP2 (2-4 fold) by ImageJ analysis (Image 3).

Discussion: In conclusion, we show for the first time that mutant forms of SH3BP2 that occur in patients with cherubism have a specific activating effect that further potentiates RANKL-induced phosphorylation of PLCγ2 and osteoclastogenesis. The results of the current study suggest that SH3BP2, as well as PLCγ2, will be interesting targets for the development of novel therapies for disorders that are characterized by excessive osteoclastic development and bone resorption.

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Image 1. SH3BP2 mutations increase NFAT luciferase activity in a dose dependent manner. Mutant and wild type SH3BP2 were transfected at different doses (0.5 and 2.0 μg/ml). Mutant SH3BP2 plasmid transfected at a dose of 0.5 μg/ml increased NFAT activation compared to the wild type. a1-R415Q, a2-G420E, a3-P418R, a4-D419N, a5= Wild Type, all with no RANKL added and b1-R415Q, b2-G420E, b3-P418R, b4-D419N, b5= Wild Type all with RANKL (100 ng/ml) added. This figure demonstrates that in cells transiently transfected with mutant SH3BP2 cDNA there is significantly increased TRAP activity compared to the wild type control (p<0.05).

Image 2. TRAP activity is increased in the presence of SH3BP2. RAW 264.7 cells were transiently transfected with mutant or wild type SH3BP2 and treated with sRANKL (100ng/ml). a1-R415Q, a2-G420E, a3-P418R, a4-D419N, a5= Wild Type all with no RANKL added and b1-R415Q, b2-G420E, b3-P418R, b4-D419N, b5= Wild Type all with RANKL (100 ng/ml) added. This figure demonstrates that in cells transiently transfected with mutant SH3BP2 cDNA there is significantly increased TRAP activity compared to the wild type control (p<0.05).

Image 3. Mutant SH3BP2 increases PLCγ2 phosphorylation by immunoblot. Levels of PLCγ2 Tyr1217 phosphorylation and total PLCγ2 were examined in response to transient transfection of mutant or wild type SH3BP2 in RAW 264.7 cells β-actin is used as a control to ensure that equal protein was loaded in each lane. VO represents cells transiently transfected with vector only and WT represents cells transiently transfected with wild type.