Effects of Rac1 on the Production of MMP-3 by TNF-α

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Abstract

Purpose: Inflammatory cytokines such as tumor necrosis factor-α (TNF-α) were previously shown to be secreted in pulptic tissue during the caries process. Matrix metalloproteinases (MMPs) such as MMP-1, 2, 3, and 14 were also shown to be expressed in inflamed dental pulp. MMP-3 can degrade the extracellular matrix (ECM) and activate other MMPs. MMP-3 is considered to be involved in wound healing, inflammation, and tumor initiation. Dental pulp destruction may be regulated, in part, by MMP-3, and other MMPs activated by MMP-3 have been shown to regulate the degradation and regeneration of dental pulp. Ras-related C3 botulinum toxin substrate 1 (Rac1) is a pleiotropic regulator of many cellular processes, including cell growth, cytoskeletal reorganization, and the activation of protein kinases. We hypothesized that Rac1 may negatively regulate the production of MMP-3 from human pulp fibroblasts (HPFs). To test this hypothesis, we isolated and purified HPFs from healthy donors and stimulated them with TNF-α.

Methods: HPFs were incubated in serum-free α-MEM containing TNF-α (0, 10, 20, 50, or 100 ng/ml) for 24 h with or without the Rac1 inhibitor, NSC23766. The production of MMP-3 and activation of Rac1 by TNF-α were evaluated by the phosphorylation of Rac1 and MMP-3 antibodies using Western blot analysis.

Results: We demonstrated that MMP-3 was produced from HPFs in response to TNF-α in a Rac1-dependent manner. TNF-α-induced production of MMP-3 without affecting the total production of MMP-2. Blocking Rac1 activation with NSC23766 significantly enhanced the TNF-α-induced production of MMP-3 without affecting the total production of MMP-2.

Conclusion: These results suggest that Rac1 prevents pulpitis by negatively regulating the production of MMP-3 in HPFs.

Key words: Matrix metalloproteinase-3, Ras-related C3 botulinum toxin substrate 1, Human pulp fibroblasts