INTRODUCTION
The collagenase MMP-13 is produced by osteoarthritic chondrocytes and is thought to play a key role in cartilage destruction in OA. Matrix fragments, including fibronectin fragments (FN-f), accumulate in the matrix during the development of OA and signal through integrins to upregulate MMP-13 expression. This signaling requires endogenous reactive oxygen species as secondary messengers in the integrin signaling pathway (1). The small GTPase Rac controls production of ROS through regulation of the NADPH oxidase complex and is known to be activated by integrin signaling in various cell types. The objective of this study was to test the hypothesis that Rac activity is required for FN-f stimulation of MMP-13 expression by articular chondrocytes and to determine if Rac contributes to MMP-13 production by OA chondrocytes.

METHODS
Human articular chondrocytes were isolated from normal ankle cartilage obtained from adult tissue donors or from OA knee cartilage removed during joint replacement. Cells were cultured in primary high density monolayers or in alginate beads. Cultures were changed to serum-free media overnight prior to experiments. Normal chondrocytes were pretreated with 1μM of a recombinant FN-f that binds and activates the α5β1 integrin. Rac1 was inhibited with the specific inhibitor NSC23766 (Rac1i) at 100μM which has been shown to be a Rac inhibitor. Cell cultures were transfected with Rac1 siRNA or constitutively active Rac1 siRNA. FN-f fragment treatment of normal chondrocytes stimulated ROS production (Fig.1). Inhibition of Rac1 with NSC23766 or knock-down with Rac1 siRNA also inhibited MMP-13 production stimulated by FN-f (Fig.2).

Inhibition of Rac1 with NSC23766 or knock-down with Rac1 siRNA also inhibited MMP-13 production stimulated by FN-f (Fig.2).

DISCUSSION
To our knowledge, this is the first study to demonstrate a role for Rac1 in the regulation of MMP-13 expression by adult articular chondrocytes. Inhibition of Rac1 blocked FN-f stimulated MMP-13 production by normal chondrocytes as well as unstimulated basal MMP-13 production by OA cells. ROS can contribute to activation of signaling pathways that upregulate MMP expression and we found that inhibition of Rac1 reduced ROS produced by cultured chondrocytes. Although ROS can activate certain MAP kinases, we did not find a connection between Rac1 and MAP kinase activation in response to FN-f. We and others have shown a role for NFκB in MMP-13 expression including that Rac1 regulates MMP-13 expression. Further studies are needed to better define this mechanism and to study the role of Rac1 in the development of OA in vivo. This work could lead to targeting Rac1 as a novel therapeutic approach in OA.

REFERENCES
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