CDK9 Inhibition Prevents Injury-Induced Subchondral Bone Resorption by Targeting Osteoclast Precursors.

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INTRODUCTION

Common joint injuries such as an ACL or meniscal tear substantially increase the risk of osteoarthritis (OA). Ample evidence indicates that the acute inflammatory response to injury causes further catabolic degradation of cartilage matrix and subchondral bone resorption. These secondary joint tissue damages occur within the first week of injury and could potentiate the pathogenesis of future post-traumatic OA (PTOA). Current clinical treatments of joint injury do not address this issue and cannot prevent the development of PTOA in the future. Therefore, a strategy to modulate the acute inflammatory response to injury may limit secondary joint damage and prevent PTOA. We have previously shown that small molecule inhibitors of CDK9, which centrally controls the rate-limiting step of most primary response gene transactivation, effectively prevent secondary joint tissue damage in our non-invasive ACL rupture mouse model of PTOA. In particular, the subchondral bone resorption in joints of the injured mice is effectively prevented by CDK9 inhibitor treatment. We hypothesize that CDK9 inhibitors may target the osteoclast-mediated bone resorption pathways. In this study, we investigate the effects of CDK9 inhibitors on the proliferation and differentiation of the osteoclast precursor cell line RAW in order to gain mechanistic insight into the suppressive effects of CDK9 inhibitors on bone resorption.

METHODS

Proliferation of RAW cells. The monocytic mouse cell line RAW (ATCC) was maintained in DMEM supplemented with 10% FBS. Cells were seeded as triplicates at a density of 62,500 cells/cm^2 in 24-well plate with 1 ml media. Cells were then treated with the drug flavopiridol (Sigma-Aldrich), which inhibits CDK9 kinase activity, and/or JQ1 (Tocris Biosciences), which inhibits recruitment of CDK9 to promoters, at various concentrations. Cells were trypsinized and counted in a hemocytometer after 3 and 5 days. Data with standard deviation represent averages of measurements done in triplicates.

Differentiation of RAW cells. RAW cells were seeded at a density of 62,500 cells/cm2 in 8-well slide chamber (Millipore) containing 0.5 ml media. The differentiation inducer RANKL (R&D Systems) was then added to a final concentration of 35 ng/ml, with or without flavopiridol and/or JQ1 at various concentrations. After 5 days, osteoclasts were stained with a TRAP staining kit (Sigma-Aldrich) according to the manufacturer's instructions. The stained slides were examined under a light microscope with a 10X objective, and TRAP positive cells in each sample were determined from 3 random fields.

RESULTS

CDK9 inhibitors suppress RAW cell proliferation at high doses. Our data indicate that treatment with flavopiridol/JQ1alone or in combination at dosages between 15-30 nM slightly reduced the growth rates of RAW cells (Fig 1). However, combination of flavopiridol and JQ1 at 60 nM each significantly reduced cell numbers at day 5, whereas 60 nM flavopiridol/ 250 nM JQ1 was apparently cytotoxic at day 3 and by day 5, almost no surviving cell were detected. These results show that doses of 30 nM or more of both drugs are cytotoxic to RAW cells under proliferation conditions.

CDK9 inhibitors suppress osteoclastic differentiation of RAW cells. We next examined the effects of CDK9 inhibitors on RAW cells induced to differentiate into osteoclast by addition of RNAKL. The data show that in all single and combination drug dosages tested, the numbers of TRAP positive osteoclast were significantly reduced, compared to control with no treatment (Fig 2). These results indicate that CDK9 inhibitors suppress osteoclast differentiation.

DISCUSSION

The CDK9 inhibitors flavoipridol and JQ1 can inhibit cell cycle progression in a variety of actively proliferating cells. This is the first study to examine the in vitro effects of these CDK9 inhibitors on osteoclast precursor cells proliferation and differentiation. Previously we showed a synergistic effect of combined treatment of flavopiridol and JQ1to effectively inhibit CDK9 activity at much reduced individual drug dosages. This enables us to distinguish the effects of these inhibitors on proliferation and differentiation of RAW cells. Our results indicate that CDK9 inhibitor at relatively low dosages significantly suppress osteoclast differentiation (Fig 2), even though cellular proliferation is not greatly affected (Fig 1). This could in turn lead to less bone resorption activity in vivo.

SIGNIFICANCE

Osteoclast is responsible for bone resorption that leads to secondary joint damage as seen in our ACL rupture mouse model of PTOA. Our results thus provide a possible mechanistic explanation for the effects of CDK9 inhibitors on preventing injury-induced bone resorption in our mouse model. Since changes in subchondral bone homeostasis following joint injury could potentially contribute to OA pathogenesis, targeting CDK9 shortly after joint injury could be a viable strategy to prevent future PTOA development.

ACKNOWLEDGEMENTS

This work was funded by NIH/NIAMS grant AR063348 to Dominik R. Haudenschild. The authors declare that they have no competing interests.

Fig 1

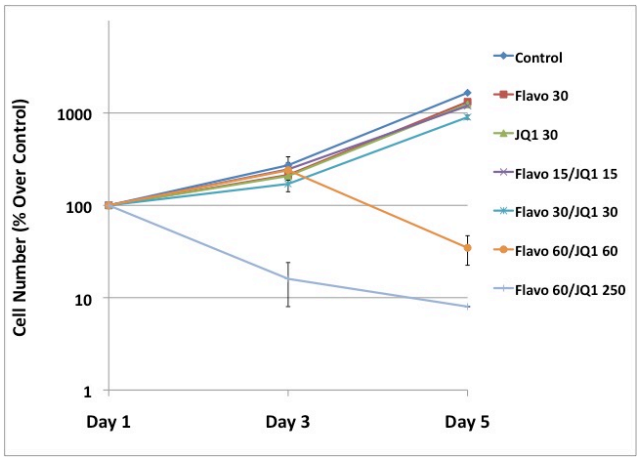

Fig 2