2-METHOXYESTRADIOL INDUCE APOPTOSIS AND CELL CYCLE ARREST IN HUMAN CHONDROSARCOMA CELL

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ABSTRACT INTRODUCTION:
Surgical resection remains the primary mode of therapy for chondrosarcoma, this mesenchymal malignancies have a poor prognosis due to the absence of an effective adjuvant therapy. It is important to explore a new approach to therapy for this tumor. 2-Methoxyestradiol (2ME) is an endogenous metabolite of estradiol with estrogen-receptor-independent antitumor and antiangiogenic activity. 2ME has recently emerged as a promising candidate for systemic therapy for many types of cancer. It has been demonstrated that 2ME induces apoptosis in many different tumor cell lines. In light of the lack of an effective systemic treatment for chondrosarcoma, we embark on a study of the effects of 2ME in chondrosarcoma cell lines and further investigate the possible molecular mechanism of antitumor activity.

METHODS:
Chondrosarcoma cell line (JJ012) were cultured in DMEM/F12 supplemented with 10% FBS and treated with 2ME in different concentrations. MTT assay was used to detect growth inhibition. Flow cytometry and DAPI stain were used to detect apoptosis. Western blotting was used to observe the expression of p53, p21, Bax, and Bcl-2 protein. Significant differences between groups were determined by Fisher’s protected least significant difference post hoc test for multiple-group comparisons following detection of significance by one-way analysis of variance (ANOVA). The value of p < 0.05 was considered statistically significant.

RESULTS SECTION:
Treatment with 2ME caused a significant time- and dose-dependent cytotoxicity in JJ cells by MTT assay. The cell viability was reduced to 49.8% after incubation with 5uM at 72 hours (Fig 1). The cell viability was reduced at concentration above 1uM at 48 hrs. Importantly, human articular chondrocytes display a lower sensitivity toward 2ME: incubation with 10uM 2ME for 72hrs causing a reduction in cell viability of only 21.8% (Fig 2). Cell proliferation was inhibited by inducing apoptosis and detected the apoptotic cell death by DAPI stain presents the cell shrinking and nuclear fragmentation (Fig 3). The results of flow cytometry showed an accumulation of JJ cells in G0/G1 phase in response to 2ME (Fig 4). The expression levels of protein Bax and caspase-3 increased following 2ME treatment in JJ cells, whereas Bcl-2 and p53 protein expression were unaffected after treatment with 2-methoxyestradiol (Fig 5).

DISCUSSION:
These results suggest that 2ME inhibits proliferation by inducing cell cycle arrest at G0/G1 phase and initiating apoptosis by upregulating Bax synthesis. The several possible mechanisms for antineoplastic effects include: the inhibition of endothelial cell proliferation, antiangiogenesis, inhibition of tubulin polymerization, cell cycle interruption, and induction of apoptosis. The number of different tumors examined for a growth-inhibiting effect is increasing, and new mechanisms are continuously described; but no related results of chondrosarcoma are reported. The effectors of apoptotic signaling through 2ME are cell type-dependent. With an understanding of the underlying mechanism, 2-Methoxyestradiol may have a potential role as for the systemic therapy of chondrosarcomas, as well as other malignant diseases.

Fig. 1 The data shows a significant decrease in cell viability of 2ME-treated chondrosarcoma cell (JJ cell) in a dose- and time-dependent manner by MTT assay.

Fig. 2 The data shows a very weak sensitivity and cytotoxicity in 2ME-treated human chondrocyte by MTT assay.

Fig. 3 Detection of apoptosis by DAPI stain in the absence (control: A, C) and presence (B, D) of 2ME-treated JJ cell 5uM for 24hrs. White arrows showed cell shrinking and nuclear fragmentation.

Fig. 4 The results of flow cytometry showed an accumulation of JJ cells in G0/G1 phase in response to 2ME.

Fig. 5 Effect of 2ME on Bax protein levels in JJ cells, the levels increased in a dose-dependent manner.