Historically, the aims of anti-cancer chemotherapy have been to induce apoptosis, growth arrest, or differentiation to promote cell death. Since chondrosarcomas are highly resistant to conventional chemotherapy and radiotherapy, surgery is the only effective form of treatment. This tumor type is propagated by neoplastic chondrocytes involved in producing the cartilaginous extracellular matrix and it has a spectrum of histological differentiation. Histochemical and immunohistochemical analyses have demonstrated that chondrosarcomas showing mainly mature and terminally differentiated hypertrophic chondrocyte phenotypes display only scant proliferation, whereas less differentiated chondrosarcomas with the phenotype of dedifferentiated chondrocytes show significantly higher proliferative activity. These features are highly correlated with prognosis (1). The findings suggest the possibility that the induction of differentiation in chondrosarcoma cells could lead to a novel therapeutic strategy.

Recently, several studies have shown that the transcriptional regulating effects of histone acetylation or deacetylation play important roles in both specific gene expressions and the differentiation of chondrocytes. Histone Deacetylase (HDAC) inhibitors represent a novel class of anti-neoplastic agents and several HDAC inhibitors are currently under clinical trial. A number of studies have demonstrated that HDAC inhibitors cause a variety of phenotypic changes, such as cell-cycle arrest, morphological reversion of transformed cells, apoptosis and differentiation. In this study, we report the anti-tumor activity of HDAC inhibitors as differentiating agents for chondrosarcomas both in vitro and in vivo.

Materials and methods

Human chondrosarcoma cell lines, SW1353 and OUMS-27, and the Swarm rat chondrosarcoma (RCS) cells were cultured. Depsipeptide (FK228) was generously provided by Gloucester Pharmaceuticals Inc. (Cambridge, MA). The cell viability assay was carried out by using a CellTiter-Glo Luminescence Cell Viability Assay Kit (Promega, Madison, WI). In Western blot analyses, antibodies against human p21 (Upstate Biotechnology, Waltham, MA) were used. Total RNA was isolated from SW1353 and OUMS-27 cells treated with depsipeptide, Apicidin, or TSA using an RNA-easy kit (Qiagen, Hilden, Germany). Quantitative real-time RT-PCR (TaqMan PCR) was carried out using a PE Applied Biosystems 7700 Sequence Detector. Transfection was carried out using FuGENE 6 (Roche Molecular Biochemicals, Indianapolis, IN). Then, the luciferase activity was assayed using Dual-Luciferase Reporter Assay Systems (Promega). Chromatin immunoprecipitation (ChIP) assays were carried out using an Acetyl-Histone H3 ImmunoPrecipitation (ChIP) Assay Kit (Upstate Biotechnology), according to the manufacturer’s protocol. For in vivo assay, RCS cells were subcutaneously inoculated into 5-week-old female athymic nu/nu mice (1 x 10⁷ viable cells/mouse). Depsipeptide suspended in PBS was administered intraperitoneally at a total dose of 0, 5 or 10 mg/kg in six evenly divided doses on days 0, 4, 8, 14, 18 and 22. Six mice were used in each group and were followed up every 3-4 days by measurement of the body mass, as well as the length (A mm) and width (B mm) of each tumor. On day 25, the mice were killed and the tumors were resected and subjected to hematoxylin and eosin (H&E), safranin O, alkaline phosphatase (ALP), Alizarin red and TUNEL stains.

Results

Depsipeptide, an HDAC inhibitor, inhibited the growth of chondrosarcoma cells by inducing cell-cycle arrest and/or apoptosis. We demonstrated that treatment with the HDAC inhibitors, depsipeptide, Apicidin and TSA, induced the chain of type II collagen (COL2A1) mRNA expression in chondrosarcoma cells. Moreover, the luciferase assay and the ChIP assay indicated that depsipeptide caused histone acetylation in the COL2A1 promoter and/or enhancer, resulting in the enhancement of COL2A1 transcriptional activity in chondrosarcoma cells. Quantitative real-time RT-PCR analyses showed that HDAC inhibitors also up-regulated the expressions of Aggrecan and the α2 chain of type XI collagen (COL11A2) mRNA, which indicated that HDAC inhibitors may induce chondrocytic maturation in chondrosarcoma cells. Moreover, long-term treatment with a low dose of depsipeptide resulted in the induction of differentiation into hypertrophic phenotype, as demonstrated by the increment of the α1 chain of type X collagen (COL10A1) expression, in chondrosarcoma cells. Using the xenograft chondrosarcoma model, we confirmed that depsipeptide significantly inhibited tumor growth. Histological analyses and TUNEL staining demonstrated that depsipeptide induced differentiation into the hypertrophic and mineralized state as well as apoptosis in chondrosarcoma cells in vivo (Fig. 1). These results suggest that HDAC inhibitors may be promising reagents for a differentiating chemotherapy against chondrosarcomas.

Discussion

Although a variety of agents including HDAC inhibitors have been identified as differentiation-promoting agents, the concept of differentiation therapy has, to date, been mainly limited to the treatment of hematological malignancies. Recent advances in the understanding of chondrosarcoma development have suggested several molecular targets for novel adjuvant therapies for the tumor. However, there have been quite a few in vivo experiments using the xenograft chondrosarcoma model. In the present study, we report the anti-tumor activity of HDAC inhibitors as differentiating agents for chondrosarcomas both in vitro and in vivo. This is the first report demonstrating the potential clinical utility of HDAC inhibitors in the treatment for chondrosarcomas.

Reference


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