Induction of apoptosis by anti-CD44 antibody in human chondrosarcoma cell line SW1353

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ABSTRACT

Because chondrosarcoma is resistant to chemotherapy and ionizing radiation, the primary treatment of chondrosarcoma is surgical resection. Effective chemotherapeutic agents for chondrosarcoma are necessary. Although there is evidence that CD44 is involved in apoptosis susceptibility in several cell types, the effectiveness of anti-CD44 treatment on chondrosarcoma has never been studied. This study was aimed to clarify whether anti-CD44 monoclonal antibody induces apoptosis in human chondrosarcoma cell line SW1353. Confocal microscopy revealed that the SW1353 cells expressed CD44 that bound the anti-CD44 antibody IM7. Treatment of the cells with IM7 resulted in a significant decrease in cell viability, compared with that with control IgG. In contrast, IM7 failed to reduce cell viability in human chondrocytes. In SW1353 cells, IM7 induced chromatin condensation, nuclear fragmentation, and apoptotic body formation while control IgG had marginal effect. These data indicate that anti-CD44 treatment could induce apoptosis in chondrosarcoma cells.

Chondrosarcoma is the second most frequent malignant primary bone tumor in human. Because chondrosarcoma is usually resistant to ionizing radiation and chemotherapy, only curative therapy is wide surgical resection. The prognosis of the disease depends on the histologic grade of chondrosarcoma which indicates the differentiation status of tumor cells (5). The surgical treatment of chondrosarcoma with adequate marginal resection is effective for low-grade chondrosarcoma with better clinical outcomes (19), while the prognosis is poor even after adequate surgery for high-grade chondrosarcoma (22). Thus, the development of effective adjuvant therapy is required for the treatment of high-grade chondrosarcoma.

CD44 is a transmembrane glycoprotein that is known as the principal receptor for hyaluronan (1). CD44 mediates cell adhesion and migration in a variety of physiologic and pathophysiologic processes (2). The biological properties of CD44 are also associated with the pathologic activities of malignant cells. CD44 is highly expressed in many tumors (20). CD44 has been shown to promote tumor cell adhesion, migration, invasion, and angiogenesis, which are crucial steps in the process of metastasis (17). Earlier studies have indicated that CD44 ligation with anti-CD44 antibody can induce apoptosis in several cells such as fibroblasts (11, 24). A contribution of CD44 to apoptosis blockade of tumor cells has also been shown in myeloid leukemia cells (3) and colon carcinoma cells (9). At present, however, there is little information about anti-CD44 treatment on human chondrosarcoma cells. This study was aimed to investigate the effect of anti-CD44 antibody IM7 on human chondrosarcoma cell line.

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SW1353. We have shown herein the induction of apoptosis by anti-CD44 antibody in SW1353 cells.

MATERIALS AND METHODS

Antibodies. IM7 and mouse IgG2b-κ isotype control were obtained from eBioscience (SanDiego, CA, USA). Fluorescein isothiocyanate (FITC)-conjugated IM7 and FITC-conjugated mouse IgG2b-κ were also from eBioscience.

Cell culture. The human chondrosarcoma cell line, SW1353, was obtained from the American Type Culture Collection (Manassas, VA, USA). SW1353 has been used in the studies that investigated the therapeutic efficacy of anti-tumor agent for chondrosarcoma (14, 16). Osteoarthritis (OA) cartilage was obtained from the distal femur and proximal tibia at the time of total knee replacement surgery from patients who were diagnosed as having OA based on the criteria developed by the American College of Rheumatology Diagnostic Subcommittee for OA. The cartilage tissues were minced into small pieces and incubated at 37°C for 1 h with trypsin. After washing with phosphate buffered saline (PBS) three times, tissues were digested with 4 mg/mL collagenase (Wako Pure Chemical, Osaka, Japan) in Dulbecco’s modified Eagle’s medium (DMEM; Gibco BRL, Life Technologies, Grand Island, NY, USA) containing heat-inactivated 10% fetal bovine serum (FBS; ICN, Aurora, OH, USA) at 37°C for 12 h. The cell suspension was poured through nylon mesh and centrifuged for 5 min at 1500 rpm. SW1353 cells and chondrocytes were individually cultured in DMEM with 10% fetal bovine serum (FBS) in monolayer in 96-well plates (Corning Ltd, Corning, NY, USA) at 37°C in a humidified atmosphere of 5% CO₂/95% air. At confluence, cells were washed with PBS, and kept in serum-free DMEM for 24 h, and thereafter incubated with IM7 or non-specific IgG.

Immunofluorescein cytochemistry for CD44. SW1353 cells were cultured on collagen-coated cover glasses (Iwaki) in 24-well plates (Corning) with DMEM containing 10% FBS. The experiment was carried out with subconfluent cells. To detect the expression of CD44, after extensive washing with 50 mM Tris buffered saline (TBS, pH 7.6), the cells were fixed with 4% paraformaldehyde in PBS for 30 min. Blocking was performed in PBS with 1% bovine serum albumin (BSA) at 4°C for 30 min. The samples were incubated with FITC-conjugated IM7 or subclass-matched FITC-conjugated non-specific mouse IgG2b-κ at 5 μg/mL for 12 h at 4°C. After washing with TBS, SW1353 were counterstained with 0.08 μg/mL propidium iodide (KPL) for 5 min. The samples were mounted on glass slides with glycerol and subjected to confocal microscopic analysis (Olympus, Tokyo, Japan).

Cell viability. SW1353 cells or OA chondrocytes were plated in monolayer in 96-well flat bottom culture plates. At confluence, the cells were incubated in the presence or absence of IM7 or isotype-matched control IgG at 50 μg/mL in serum-free DMEM for 72 h. According to the manufacturer’s instruction, cell viability was assessed by measuring mitochondrial NADH-dependent dehydrogenase activity with Cell Counting Kit (Dojindo Molecular Technologies, Kumamoto, Japan) using a sulfonated tetrazolium salt, 2-(4-iodophenyl)-3-(4-nitrophynyl)-5-(2, 4-disulfophenyl)-2H-tetrazolium monosodium salt (WST-1). Optical density (OD) at 405 nm against OD at 690 nm of formazan products was measured by microtitre plate reader (Titertek Multiskan MCC, Huntsville, Alabama, USA).

Morphologic evaluation of SW1353 and chondrocytes. SW1353 cells and chondrocytes were grown to confluence in 6-well plates (Corning) and treated with IM7 or non-specific IgG at 50 μg/mL for 72 h. The cells were scraped, collected by centrifugation, and fixed in 1% glutaraldehyde in 0.1 M phosphate buffer (PB) overnight at 4°C. They were then washed twice in 0.15 M PB. The cells were then fixed with 2% osmium tetroxide, dehydrated to absolute ethanol, and embedded in Epon. Semi-thin sections were first stained by 0.1% toluidine blue and examined by light microscopy. Adjacent ultrathin sections were then contrasted with uranyl acetate and lead citrate, and examined by a transmission electron microscope (TEM) (type H-300; Hitachi, Tokyo, Japan). In a visual field of light microscope with the fixed number of total cells, the number of cells with chromatin condensation and/or apoptotic body was counted.

Data Analysis. Comparisons between two groups were statistically performed by Student’s t test. P values less than 0.05 were considered significant.

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Data Analysis. Comparisons between two groups were statistically performed by Student’s t test. P values less than 0.05 were considered significant.
RESULTS

Association of Anti-CD44 antibody with CD44 on SW1353 cells

The presence of functional CD44 on human chondrosarcoma cell line SW1353 cells was investigated using FITC-conjugated anti-CD44 antibody. Immunofluorescence cytochemistry showed that CD44 on the plasma membrane was intensely stained with anti-CD44 monoclonal antibody IM7. We found that almost every SW1353 cell constitutively expressed CD44 that can bind the anti-CD44 monoclonal antibody IM7 (Fig. 1A). When we used subclass-matched FITC-conjugated mouse IgG2b-κ, fluorescein signals were scarcely seen (Fig. 1B). As already shown in our previous studies (13), almost every OA chondrocyte expressed CD44 on the plasma membrane (data not shown).

Effect of anti-CD44 antibody on cell viability

In order to evaluate the effect of anti-CD44 antibody on cell viability, SW1353 cells were incubated with or without IM7 or control IgG for 72 h. Treatment with the anti-CD44 antibody resulted in a decrease in cell viability in time- and dose-dependent manners. Compared with non-specific IgG-treated or non-treated cultures, the viability was significantly reduced in 50 µg/mL IM7-treated SW1353 cells (Fig. 2). In contrast, the anti-CD44 antibody had no significant effect on the viability of human OA chondrocytes, compared with non-specific IgG (Fig. 2). The effective concentration of anti-CD44 antibody (50 µg/mL) was compatible with the levels that have previously caused apoptosis of fibroblasts (11).

Morphologic changes consistent with apoptosis following anti-CD44 antibody treatment in SW1353 cells

SW1353 cells after treatment with anti-CD44 antibody were examined using TEM to determine whether this treatment induced the morphologic alterations of apoptosis. The TEM studies revealed chromatin condensation in the nucleus and nuclear fragmentation in IM7-treated SW1353 cells (Fig. 3). Under light microscopy, chromatin condensation, nuclear fragmentation, and apoptotic body formation were observed in IM7-treated SW1353 cells (Fig. 4). The number of SW1353 cells containing chromatin condensation and apoptotic body per light microscopic field significantly increased with treatment with IM7 compared with that with non-specific IgG.
nant tumors. One of these potential targets is CD44. The cell surface transmembrane glycoprotein has previously been implicated in apoptosis susceptibility (10). Ligation of CD44 with its ligand hyaluronan or CD44-specific antibodies has been shown to be proapoptotic in several cell types including fibroblasts (11, 24), neutrophils (23), T cells (6), and synovial cells (8). In addition, CD44 contribution to apoptosis has been indicated in leukemia cells (3) and colon carcinoma cells (9). In the present study, we have demonstrated for the first time that engagement of CD44 with anti-CD44 antibody can lead to apoptosis in chondrosarcoma cells. Anti-CD44 treat-

![Fig. 3](image1) Transmission electron microscopy of SW1353 cells with treatment with 50 µg/mL anti-CD44 antibody for 72 h. Dense chromatin in the nucleus (A) and nuclear fragmentation (B) are observed. (original magnification × 2000)

![Fig. 4](image2) Light microscopy of SW1353 cells with treatment with 50 µg/mL anti-CD44 antibody or non-specific IgG for 72 h. Cells were stained with toluidine blue. Chromatin condensations with nuclear fragmentation (arrows) and apoptotic body formation (open arrowheads) are observed. (original magnification × 600)

(Table 1). In human OA chondrocytes, however, IM7 caused no significant alteration in morphology (data not shown).

**DISCUSSION**

Evading apoptosis is a fundamental pathophysiologic property of malignant cells that mediate their resistance to anti-cancer drugs (21). Chemotherapeutic killing of tumor cells is mainly induced through apoptosis (12). Thus, identification of cellular proteins that mediate apoptosis is a critical step toward the development of effective therapeutics for malignant tumors. One of these potential targets is CD44. The cell surface transmembrane glycoprotein has previously been implicated in apoptosis susceptibility (10). Ligation of CD44 with its ligand hyaluronan or CD44-specific antibodies has been shown to be proapoptotic in several cell types including fibroblasts (11, 24), neutrophils (23), T cells (6), and synovial cells (8). In addition, CD44 contribution to apoptosis has been indicated in leukemia cells (3) and colon carcinoma cells (9). In the present study, we have demonstrated for the first time that engagement of CD44 with anti-CD44 antibody can lead to apoptosis in chondrosarcoma cells. Anti-CD44 treat-
Apoptosis by anti-CD44 in SW1353

Table 1  Induction of apoptosis by anti-CD44 antibody in SW1353 cells

<table>
<thead>
<tr>
<th>Sample</th>
<th>chromatin condensation</th>
<th>apoptotic body</th>
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<tbody>
<tr>
<td></td>
<td>anti-CD44 IgG</td>
<td>anti-CD44 IgG</td>
</tr>
<tr>
<td>1</td>
<td>3 0</td>
<td>2 0</td>
</tr>
<tr>
<td>2</td>
<td>4 1</td>
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<td>4</td>
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p value 0.017 0.024

After treatment with anti-CD44 antibody or non-specific IgG for 72 h, morphologic features of apoptosis were observed under light microscopy. The number of cells containing chromatin condensation and/or apoptotic body per field with the fixed number of total cells is indicated for the four separate samples (designated as samples 1, 2, 3, and 4). Induction of chromatin condensation and apoptotic body formation was individually compared between anti-CD44 antibody- and non-specific IgG-treated cells by t test. Three separate experiments were performed with similar results.

In general, apoptotic signaling proceeds through the extrinsic and intrinsic pathways (4). The extrinsic pathway transmits the signal through Fas/CD95 on the binding to Fas ligand. Recent studies (10, 18) have indicated that CD44 could sequester Fas and prevent its trimerization, which is an initial step to Fas ligand binding and Fas signaling. Up-regulation of CD44 commonly observed in malignant cells (20) may, therefore, reduce the availability of Fas to activate apoptotic signaling and thereby enhance apoptotic resistance. A similar mechanism has been shown that Fas is sequestrated by c-Met, a tyrosine kinase receptor (25). Of interest, CD44 induces c-Met activation in response to fragmented hyaluronan in human chondrosarcoma cell line HCS-2/8 (15). Further studies are underway to clarify the underlying molecular mechanisms of apoptosis induction by anti-CD44 treatment in chondrosarcoma cells.

Because of insufficient response of chondrosarcoma to chemotherapy and ionizing radiation, the primary therapy of the tumor is wide surgical resection. The development of effective adjuvant treatment may overcome such therapeutic limitations of chondrosarcoma. Our results of the current study suggest that anti-CD44 treatment results in the induction of apoptosis in chondrosarcoma cells. Importantly, anti-CD44 treatment lacks appreciable effects on the viability of human chondrocytes. Other potential anti-chondrosarcoma agents have recently been described. The third-generation bisphosphonate minodronate and 2-methoxyestradiol can induce apoptosis of SW1353 cells (16) and another chondrosarcoma cell line JJ012 (7), respectively. Furthermore, the tyrosine kinase inhibitor SU6668 can suppress SW1353 cell growth via anti-angiogenesis in vivo (14). Comparable studies should be performed to elucidate which is a promising agent for the effective systemic therapy of high-grade chondrosarcoma.

REFERENCES


