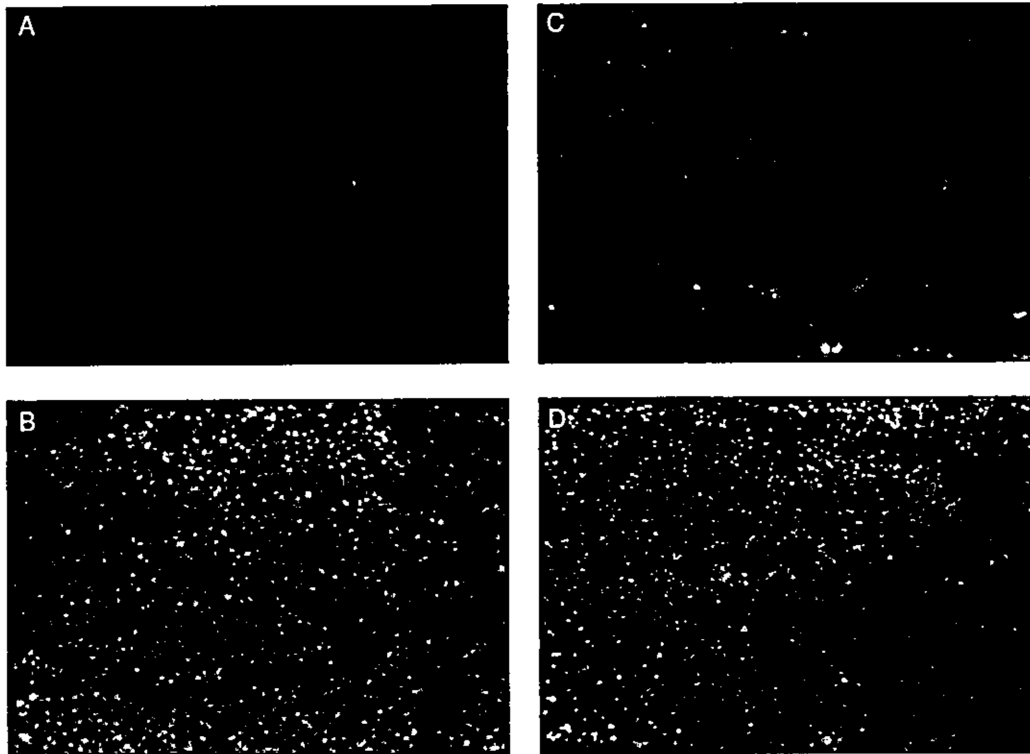

Figure 6. Detection of FITC-labeled ODN in tumors derived from HeLa cells in SCID mice. HVJ envelope vector containing unlabeled ODN (A, B) or FITC-ODN (C, D) was injected into tumors. FITC was detected in A and C. Hoechst 33 258 was used to counterstain the nucleus (B and D). The experiments were repeated three times and representative photos are shown

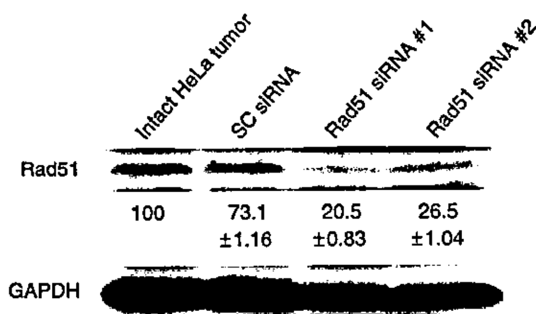

Figure 7. Rad51 transcript was detected by Western blot analysis after the delivery of either Rad51 siRNA or scrambled (SC) siRNA. The samples were isolated from two mice (#1 and #2) injected with the same Rad51 siRNA. This experiment was repeated twice and similar results were obtained. The percentage of Rad51 expression (mean ± standard deviation) below in each lane was calculated as described in Figure 2

the siRNA is gradually diluted after cell division. The use of lentivirus vector or retrovirus vector to insert siRNA expression DNA into the host chromosome has been proposed [38,39]. However, we believe that a combined treatment of synthetic siRNA and CDDP is sufficient for cancer treatment, because the cells that received Rad51 siRNA and CDDP in this study died in a few days. An important factor in the success of the combination treatment is the consecutive delivery of synthetic siRNA. Indeed, three injections of Rad51 siRNA into the tumor were more effective for tumor regression than two injections. The immunogenicity of the HVJ envelope vector is much less than that of native HVJ because of the inactivation of the viral genome. Consecutive injection is feasible with this vector system [28].

Rad51 siRNA enhanced the sensitivity to another anti-cancer drug, bleomycin, which can induce DNA double-strand breaks. The enhancement of bleomycin sensitivity by Rad51 siRNA was almost similar to that in a CDDP experiment (M. Ito and Y. Kaneda, unpublished data). It has been reported that Rad51 is also involved in the sensitivity of cancers to other anti-cancer drugs, such as etoposide (VP16) and imatinib mesylate (Gleevec) [40,41]. Since only Rad51 siRNA decreased cancer cell viability (Figure 4A), Rad51 siRNA can also enhance the sensitivity of cancer cells to other drugs which do not induce DNA double-strand breaks. This experiment is being performed in our laboratory. Furthermore, although Rad51 expression levels varied from cell line to cell line, all the cancer cells became very sensitive to CDDP in combination with Rad51 siRNA. The sensitivity of the cancer cell lines to CDDP did not appear to be related to the endogenous Rad51 protein level. These results suggest that the combination of CDDP with Rad51 siRNA will be generally applicable to various human cancers.

The enhancement of CDDP sensitivity by Rad51 siRNA was observed only in HeLa cells, not in NHDF. Similarly, apoptosis by Rad51 siRNA and CDDP increased in

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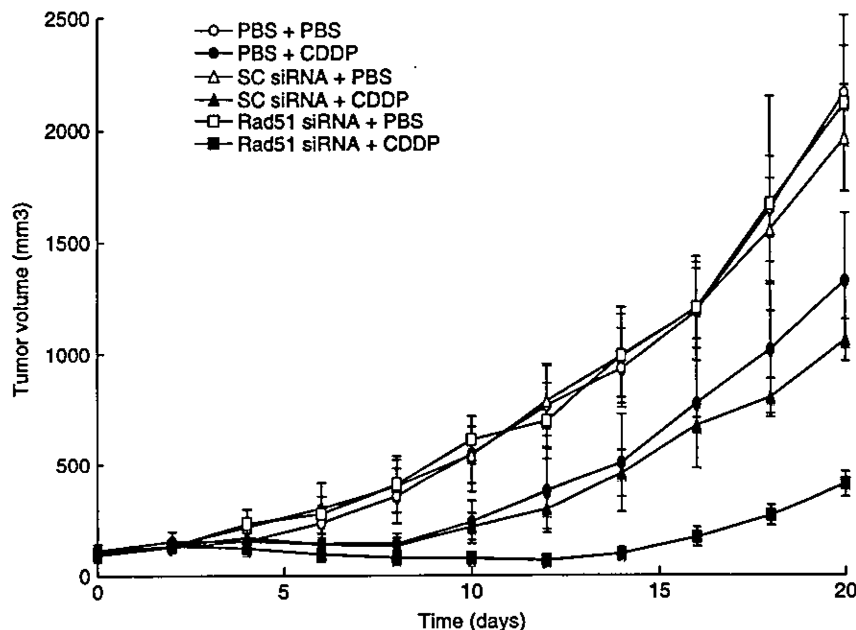

Figure 8. Tumor volume in SCID mice. Intraperitoneal injection of CDDP on day 2 transiently suppressed tumor growth *in vivo*, but tumors began to grow again 8 days after the treatment. To enhance the anti-tumor effect of CDDP, Rad51 siRNA or scrambled (SC) siRNA was injected on days 0, 2, and 4. In three groups, 200 µg of CDDP were injected into the abdominal cavity on day 2. In a negative control group, PBS was injected into both the tumor mass and peritoneal cavity. Each group contained five mice, and the representative result from three independent experiments is shown

HeLa cells, but not in NHDF. The discrepancy of CDDP sensitivity by Rad51 siRNA between NHDF and HeLa cells may be due to the difference of the CDDP uptake by the two cell lines. Indeed, the equitoxic dose of CDDP in NHDF and HeLa cells was 1.2 and 0.5 µg/ml, respectively, in our case (M. Ito and Y. Kaneda, unpublished data). Another possibility is that cell cycle difference between both cells may affect the sensitivity to CDDP in the presence of Rad51 siRNA. The precise mechanism of this different sensitivity to CDDP remains to be solved.

However, in human gene therapy, we should be very careful regarding the toxicity of Rad51 siRNA. As shown in Figure 5B, Rad51 siRNA alone induced apoptosis in both HeLa cells and NHDF, although the apoptotic cell ratio was much lower in the absence of CDDP. This may be consistent with the fact that Rad51 knockout mice are embryonic lethal [42]. To minimize the adverse effects to normal tissues, tumor-selective targeting is indispensable for cancer treatment. There are two ways to achieve selective targeting. One is the insertion of tumor-specific molecules to vectors, and another is the modification of vector size and charge. We have already reported that HVJ-cationic liposomes targeted tumor nodules in mouse peritoneum by intraperitoneal injection [43]. We are now constructing targeting vectors by modifying the HVJ envelope vector with polymers or tumor-specific single-chain antibodies.

When delivered by tumor-targeting vectors, siRNAs against genes resistant to cancer therapy hold great promise to become very effective anti-neoplastic therapeutics in combination with chemotherapy or radiotherapy.

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