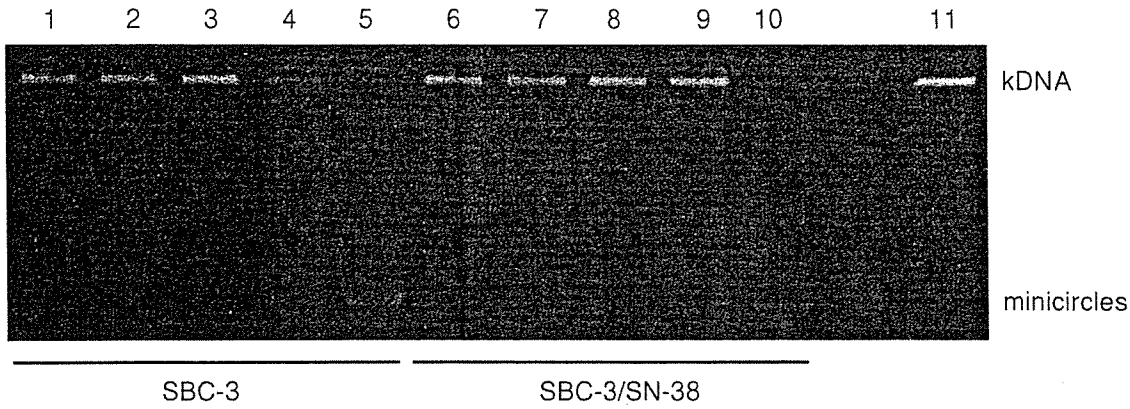

Figure 3. *DNA topo II activity determined by decatenation assay of kDNA showing a decreased topo II activity in SBC-3/SN-38. The amount of nuclear extracts was 0.00025 μg for lanes 1 and 6, 0.0005 μg for lanes 2 and 7, 0.001 μg for lanes 3 and 8, 0.002 μg for lanes 4 and 9, 0.004 μg for lanes 5 and 10, and none for lane 11 as a negative control. Lanes 1-5: SBC-3; lanes 6-10: SBC-3/SN-38.*

the SBC-3/SN-38 cells was considered to be half that of the SBC-3 cells. Topo II activity was determined by a kDNA decatenation assay (Figure 3). The formation of minicircles increased and kDNA disappeared in the presence of over 0.002 μg of the SBC-3 (lanes 4-5) and over 0.004 μg of the SBC-3/SN-38 (lane 10). This indicates that the topo II activity of the resistant cells is half that of the parent cells.

Discussion

We established an SN-38-resistant SCLC cell line *ex vivo* derived from SBC-3 cells. Several sublines resistant to a topo I inhibitor, such as camptothecin-resistant leukemia cell lines (14,15), a camptothecin-resistant Chinese hamster ovary cell line (16), a camptothecin-resistant non-small cell lung cancer, colon cancer and gastric cancer cell lines (17,18), an irinotecan-resistant non-small cell lung cancer cell line (19), an SN-38-resistant SCLC cell line (20) and a topotecan-resistant ovarian cancer cell line (21), have been reported. Although there is a slight difference in the cross-resistance pattern among these sublines, they are generally non-cross-resistant or collaterally sensitive to topo II inhibitors and non-cross-resistant to platinum, alkylating agents, antimicrotubule agents or methotrexate. On the contrary, the SBC-3/SN-38 cells were resistant to these anticancer agents. In addition, the relative resistance values of bleomycin and 5-fluorouracil were 0.81-fold and 1.2-fold, respectively. Bleomycin has not been examined in topo I inhibitors-resistant sublines to our best knowledge. A CPT-11-resistant non-small cell lung cancer cell line was cross-resistant to 5-fluorouracil (20), but an SN-38-resistant SCLC cell line was not (21).

Several mechanisms of resistance to topo I inhibitors have been reported (22). P-glycoprotein, which contributes to reduced accumulation of adriamycin, etoposide or antimicrotubule agents in the cells, is not overexpressed in the topo I inhibitor-resistant sublines as confirmed in our study. Another transporter, breast cancer resistance protein (BCRP), is responsible for the enhanced efflux of SN-38 (22). Another SN-38-resistant SCLC cell line (23) overexpressed BCRP, which has been confirmed in the SBC-3/SN-38 cells (24). A decrease in topo I activity and/or content also contributes to the resistance. In this study, we demonstrated that topo I activity in the SBC-3/SN38 cells was approximately half of the parent cell line. However, the 73-fold resistance value of SN-38 could not be explained by the reduced activity alone. On the other hand, topo II activity was elevated in the topo I inhibitor-resistant sublines (25, 26). In the present study, the decline of topo II activity in SBC-3/SN-38 was demonstrated and is responsible, in part, for the development of resistance to adriamycin (3.4-fold) and etoposide (5.5-fold). Regarding the drug detoxification system, Goto *et al.* (27) reported that irinotecan induced an increase in intracellular GST-π level. GST-π level was elevated in the cisplatin-resistant subline (SBC-3/CDDP) (28), adriamycin-resistant subline (SBC-3/ADM100) (29) and etoposide-resistant subline (SBC-3/ETP) (30), compared to that of the parent cell line. However, it was not elevated in the SBC-3/SN-38 cells. In addition, the GSH level was lower than the detection level in the SBC-3/SN-38, although it was elevated in the SBC-3/CDDP (29) and SBC-3/ADM100 (30). Accordingly, GST-π and GSH were not responsible for the resistance to platinum, alkylating agents and anthracyclines in the SBC-3/SN-38 cells.

Other mechanisms of resistance to topo I inhibitors, such as cellular localization of topo I, stabilization of DNA-topo I complexes, ubiquitin/26S proteasome-dependent degradation of topo I, DNA repair activity and regulation of NF-κB, *etc.*, have also been reported (reviewed in Ref No. 22). Further studies are needed to

clarify the cross-resistance pattern in the SBC-3/SN-38 cells. However, the resistant subline described here would be useful in the screening of anticancer agents showing sensitivity to irinotecan-resistant SCLC. Jensen *et al.* (31) reported that the different cytotoxicity patterns for a panel of acquired drug-resistant cells could enable the selection of non-cross-resistant drugs. The drugs that are cytotoxic to both SBC-3/SN-38 and SBC-3/CDDP cells might be effective in refractory SCLC patients previously treated with irinotecan and cisplatin. The SBC-3/CDDP cells were significantly more sensitive than the parent cells to 5-fluorouracil (29) and were equally sensitive to bleomycin (unpublished data).

There were no sets of adriamycin-, etoposide-, cisplatin- and SN-38- (or irinotecan)-resistant cell lines derived from the same parent cell line. Adriamycin-resistant SBC-3/ADM, SBC-3/ADM100, etoposide-resistant SBC-3/ETP and cisplatin-resistant SBC-3/CDDP cells were established in our laboratory and now SN-38-resistant SBC-3/SN-38 cells are presented here. Using these resistant cell lines, the drug-resistant mechanisms induced by each drug can be compared and reported (24).

In conclusion, the irinotecan-resistant cell line selected by continuous exposure of SBC-3 cells to SN-38 will be useful to elucidate the mechanism of irinotecan resistance and to explore new drugs for irinotecan-resistant SCLC.

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