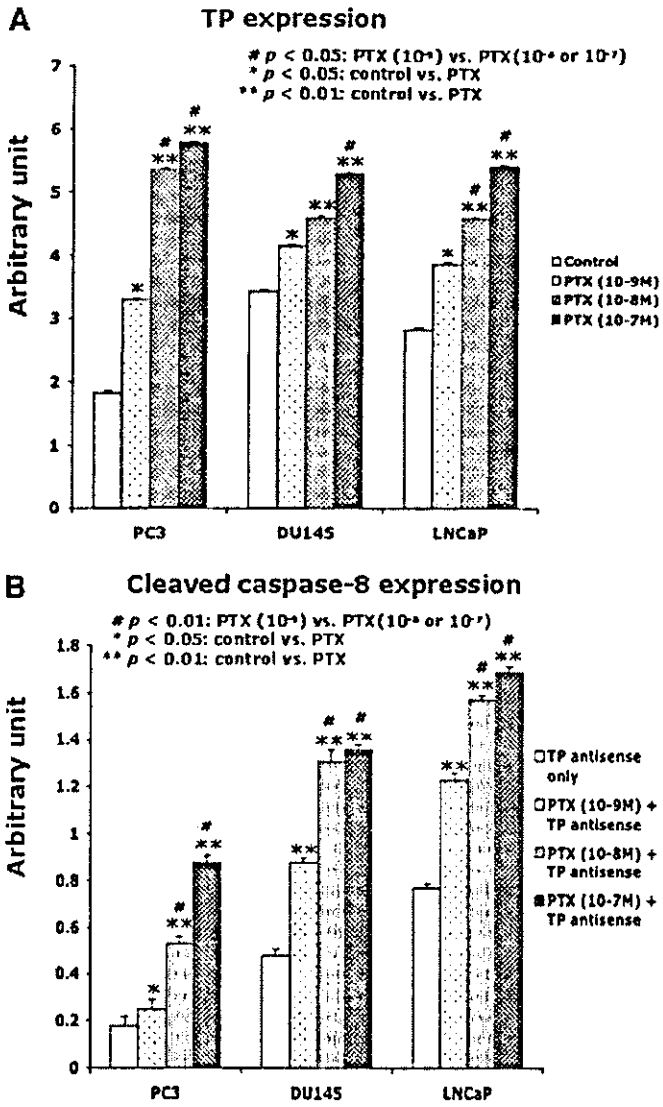

Fig. 6. TP expression after treatment with PTX (*A*), and cleaved caspase-8 expression after combined treatment with PTX and TP antisense (*B*) in PC cell lines are shown.

accelerating PTX-mediated apoptosis. As shown in Fig. 5, in PC-3, DU145, and LNCaP cell lines, PTX exposure clearly increased TP expression. The cell viability of PTX-treated PC cells with inhibition of TP translation by TP antisense transfection was significantly diminished in a time-dependent and dose-dependent manner compared with the PTX treatment alone (Fig. 3). Likewise, the apoptotic index was significantly increased in PC cells with combined treatment of PTX and TP antisense in comparison with those PC cells treated with PTX alone. However, the treatment with TP antisense alone did not make any influence on the cell viability and apoptotic index. These findings suggest that inhibition of PTX-induced TP up-regulation could confer more proapoptotic effect on PTX-treated PC cells.

Next, to verify the mechanism underlying PTX-induced apoptosis in relation to simultaneous TP expression, we focused on the molecular pathway involved in the apoptotic process. Two major apoptotic pathways are known in mammalian cells. One is the Fas-induced caspase-8 activation pathway, and the other is the mitochondrial pathway. Although these two apoptotic pathways operate independently, they converge at the level of caspase-3 activation (17). PTX-induced apoptosis has also been implicated in caspase-8 activation in breast and colon cancer cell lines (18, 19). TP has been reported to inhibit Fas-induced caspase-8 cleavage followed by the release of cytochrome *c*, the activation of caspase-3, and the apoptosis (3). However, no reports have shown a positive link between apoptotic pathways involved in PTX-induced TP expression and caspase-8 activation in PC cells. In breast and colon cancer cell lines, PTX induces proapoptotic effect on cancer cells through caspase-8 activation in addition to the activation of mitochondrial membrane potential (18, 19). However, as shown in Fig. 5, PTX-induced apoptosis in PC cell lines seems to be independent of caspase-8 activation. Thus, the mechanism underlying the antitumor effect of PTX on cancer cells appears to be potentially varied among cancer cells of different origins. On the basis of the present finding of dose-dependent TP induction by PTX treatment in PC cell lines as well as the previous report of potential inhibitory effect of TP on caspase-8 activation (3), we hypothesized that blockade of inhibitory effect of TP on caspase-8 activation could enhance the PTX-induced apoptosis in PC cells. In all three PC cell lines, after complete blockade of TP translation by TP antisense transfection, proapoptotic events such as cleaved form of caspase-3 and PARP were enhanced in a PTX dose-dependent manner. In addition, this exaggeration of apoptosis also ran parallel with the caspase-8 cleavage in a PTX dose-dependent manner. On the other hand, in all of three PC cell lines TP blockade itself did not confer any effects on the acceleration of apoptosis despite caspase-8 activation. These results suggest that cross-talk between caspase-3 activation through mitochondrial membrane potential and direct effect of caspase-8 activation pathway on cytochrome *c* release can modulate proapoptotic effect of PTX as a chemotherapeutic agent on PC cells. In turn, we can expect more antitumor apoptotic effect of PTX on PC cells with an inhibition of "adverse" effect of PTX-induced TP overexpression.

To our knowledge, this study is the first report to investigate the induction of TP expression by PTX and to present the possibility that overexpressed TP might be related to the potential decrease in caspase-8 cleavage in PC cell lines. Our results support the hypothesis that TP could be a new molecular target for enhancing the potency of PTX-mediated apoptosis in PC cells. Therefore, it is necessary to perform the clinical trial after treatment with a combination of TP antisense and PTX in the future.

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