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Report

Down-regulation of telomerase activity by anticancer drugs in human ovarian cancer cells

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Maintenance of telomere length is crucial for survival of cells. Telomerase, an enzyme that is responsible for elongation of shortened telomeres, is active in human germ cells as well as most tumor tissues and experimentally immortalized cells. In contrast, most mature somatic cells in human tissues express undetectable or low telomerase activity, implying the existence of a stringent and negative regulatory mechanism. In this study we report the effects of anticancer drugs on telomerase activity in human cancer cells. In assaying for telomerase activity, we basically followed the original TRAP assay system, but with some modifications. A down-regulation of telomerase activity was found when cells of a human ovarian cancer cell line, A2780, were treated with *cis*-diamminedichloroplatinum(II) (CDDP; cisplatin). However, down-regulation of telomerase activity was not found in cells of a cisplatin-resistant cell line, A2780CP, treated with cisplatin. On the other hand, telomerase activity in both the cell lines A2780 and A2780CP was reduced when A2780 or A2780CP was treated with adriamycin, an anthracycline antibiotic having a broad spectrum of antineoplastic activity. The different effects on the telomerase activity of the two types of anticancer drugs may be due the distinct chemical functions of these drugs. The present results may indicate a positive relationship between anticancer effects and down-regulation of telomerase activity by anticancer drugs. [© 2002 Lippincott Williams & Wilkins.]

Key words: Anticancer drug, ovarian cancer cell, telomerase activity.

Introduction

The ends of vertebrate chromosomes are composed of specialized structures called telomeres which contain TTAGGG repeats. Telomeres are involved in stabilizing and protecting chromosomes. Normal somatic cells lose telomeric repeats with each cell division, which is known as the 'end replication problem'. As a factor to solve this problem, telomerase activity had been foreseen for sometime when Grider *et al.*[1] first reported in 1985 the detection of telomerase activity by biochemical methods in *Tetrahymena* extracts. Telomerase, a reverse transcriptase, contains an RNA component in it and can elongate shortened telomeric DNA. In humans, Morin *et al.* first reported telomerase activity in HeLa cells in 1989.[2]

Since Kim *et al.* reported the TRAP assay,[3] a variety of cells and tissues, including different kinds of cancer cells, have been assayed for telomerase activity with this new technique. Telomerase activity was detected in most cancer cells, but in only some types of normal somatic cells such as proliferating lymphocytes and stem cells in regenerating human tissues.[4] As reacquisition of telomerase activity in human adult tissues seems to be related to the emergence of immortal human cancer cells, this enzyme may offer a unique target for cancer therapies. *Cis*-diamminedichloroplatinum(II) (CDDP; cisplatin) is a frequently used and very effective chemotherapeutic drug in treating various human cancers of the brain, ovary, testicle, bladder, and head and neck.[5] Adriamycin (ADR) is an anthracycline antibiotic that is an important agent in chemotherapy of human cancers because of the broad spectrum of its antineoplastic activity.[6,7] In this study we have investigated the effects of these anticancer drugs on telomerase activity in human cancer cells as well as the different effects of the drugs on these cells.

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Materials and methods

Reagents

Cisplatin was purchased from Nihon Kayaku (Tokyo, Japan). ADR was a gift of Kyowa Hakko Kogyo (Tokyo, Japan). Other chemicals were of the highest purity among commercially available agents.

Cell cultures

A2780, a human ovarian cancer cell line derived from an untreated patient, and A2780CP, a cisplatin-resistant cell line, were kind gifts from Dr TC Hamilton. A2780CP cell line was derived from A2780 cells which had been treated with stepwise increasing concentrations of the drug up to 70 μM.[8] Cells of the two lines were maintained in RPMI 1640 medium supplemented with 6% fetal calf serum (Gibco, Grand Island, NY) and 2 mM glutamine at 37°C in an atmosphere of 95% air/5% CO_2. Then, cells were treated with or without anticancer drugs at low concentrations, showing no remarkable toxicity.

Assay for telomerase activity

In assaying for telomerase activity, we basically followed the original TRAP assay,[3] but with some modifications.[9] Samples of cell extracts were prepared as previously reported by Kim *et al.*[3] and were incubated at 23°C for 30 min to allow telomerase to synthesize extension products. Then, the samples were heated at 85°C for 10 min, mixed with Taq polymerase and subjected to 23 cycles of PCR of 94°C for 1 min, 62°C for 1 min, 60°C for 1 min and 74°C for 1 min, and then, further to the last two cycles for labeling PCR products with the ^{32}P-labeled primer, TS-3 (5′-GCCAATCCGTCGAGCAGAGTTAGGG-3′), 94°C for 2 min, 67°C for 1 min, 65°C for 1 min and 74°C for 5 min, and 94°C for 2 min, 67°C for 1 min, 65°C for 1 min and 74°C for 10 min.The PCR products were subjected to electrophoretic analysis. Telomerase activity was quantified after electrophoresis by phosphor imaging with BAS2000 (Fuji Film, Tokyo, Japan). To quantify the intensity of radioactivity of each band on each lane of a polyacrylamide gel, a rectangle of equivalent size and shape was measured, and we assessed only a single small telomerase-specific band instead of summing up all bands on each lane. Cell extracts were estimated telomerase-positive when a 6-bp ladder was clearly observed compared with a control lane of null cell extract (lysis buffer only). The telomerase activity was normalized by the same protein amounts in the cell extracts and was represented as percent of the control activity of non-treated cell extracts (0.8 μg protein).

Results and discussion

We used two human ovarian cancer cell lines, cisplatin-resistant A2780CP and its parental line A2780. We prepared cell extracts from cells treated with cisplatin or ADR to investigate the down-regulation of telomerase activity in cancer cells by these drugs. Figure 1 shows a representative result showing the time-dependent down-regulation of telomerase activity in A2780 cells treated with 20 μM of cisplatin for the indicated times. Telomerase activity was reduced to about 60% at 12 h, and was gradually decreased to 50% at 24 h and further later on. On the contrary, down-regulation of telomerase activity was not observed in the cisplatin-resistant A2780CP cells treated with 20 μM cisplatin for the indicated times. The telomerase activities of untreated A2780 cells and untreated A2780CP cells were of almost the same level. Cisplatin-resistant A2780CP cells had a 2-fold increase in glutathione (GSH) content compared to A2780 cells after cisplatin treatment.[8] Telomerase is known to be selectively inhibited by isothiazolone derivatives

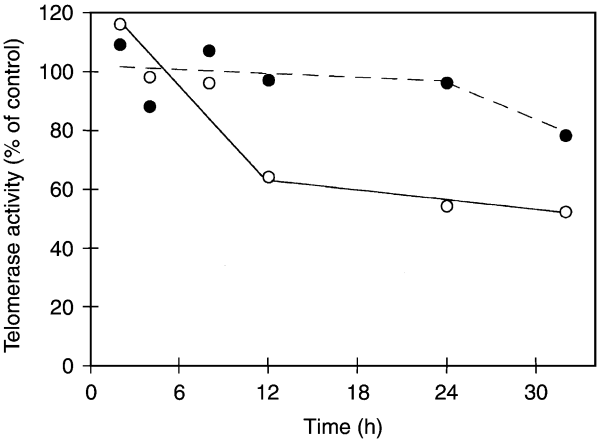

Figure 1. Time-dependent effects of cisplatin on telomerase activity of A2780 and A2780CP cells. Cells were treated with cisplatin (20 μM) for the indicated times. Telomerase activity is represented as percent of activity of the non-treated cells. (○) A2780 cells; (●) A2780CP cells. Representative result of two independent experiments.

which interact with cysteine in the enzyme's active center.[10] GSH may have protected telomerase activity from inhibitors due to competition at a cysteine residue, which may be one of the reasons for the lower reduction of telomerase activity in the cisplatin-resistant A2780CP cells. However, although there may be a relationship between the anticancer activity of cisplatin and the negative effect on telomerase activity by cisplatin, the mechanism by which cisplatin modulates enzyme activity is unknown.

Figure 2 shows the dose-dependent down-regulation of telomerase activity in A2780 cells and cisplatin-resistant A2780CP cells treated with different concentrations of cisplatin for 24 h. Although a remarkable reduction of telomerase activity was observed in the A2780 cells by cisplatin at 20 μM and higher concentrations (40–50% reduction), cisplatin-resistant A2780CP cells exhibited no down-regulation of telomerase activity at 50 μM or lower concentrations.

Figure 3 shows the time-dependent reduction of telomerase activity in A2780 cells and cisplatin-resistant A2780CP cells treated with 10 μM ADR for the indicated times. Almost similar down-regulations were observed in both the cells, which differed from the results with cisplatin. Reductions of about 20% at 12 h, 40–50% at 12 h and 70–80% at 24 h were observed.

Figure 4 shows almost the same dose-dependent decreases of telomerase activity in A2780 cells and cisplatin-resistant A2780CP cells treated with different concentrations of ADR for 24 h. In comparison to the cisplatin effect on the two cell lines, no difference in the down-regulation of enzyme activity by ADR treatment was found in the two cell lines, indicating that ADR might reduce the telomerase activity by a different mechanism from that of cisplatin. As A2780CP was established as a cisplatin-resistant cell line by exposure of A2780 cells to stepwise increasing concentrations of cisplatin, it may be reasonable that cisplatin did not show a reducing effect on telomerase activity of the cells. On the other hand, ADR exhibited strong inhibitory effects on telomerase

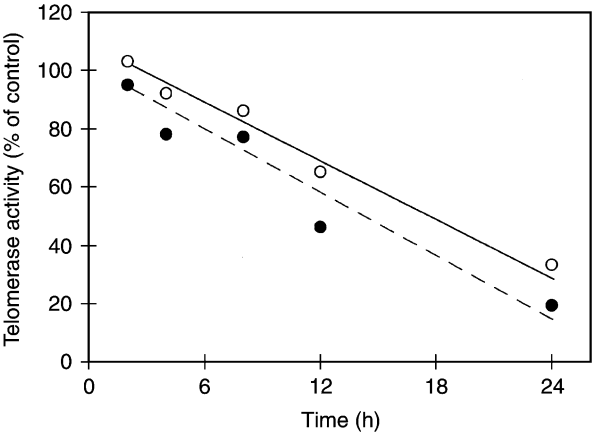

Figure 3. Time-dependent effects of ADR on telomerase activity of A2780 and A2780CP cells. Cells were treated with ADR (10 μM) for the indicated times. Telomerase activity is represented as percent of the activity of non-treated cells. (○) A2780 cells; (●) A2780CP cells. Representative result of the two independent experiments.

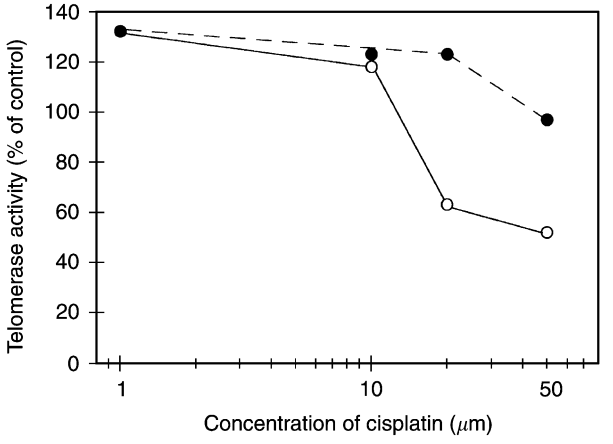

Figure 2. Dose-dependent effects of cisplatin on telomerase activity of A2780 and A2780CP cells. Cells were treated with cisplatin (1–100 μM) for 24 h. Telomerase activity is represented as percent of activity of the non-treated cells. (○) A2780 cells; (●) A2780CP cells. Representative result of the two independent experiments.

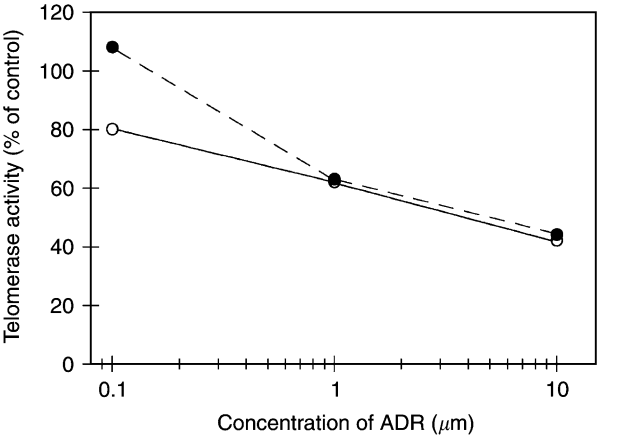

Figure 4. Dose-dependent effects of ADR on telomerase activity of A2780 and A2780CP cells. They were treated with ADR (0.1–100 μM) at 24 h. Telomerase activity was represented as percent of the activity of non-treated cells. (○) A2780 cells; (●) A2780CP cells. Representative result of the two independent experiments.

activities of both A2780 and A2780CP cells, which may be due to the difference in the chemical structure of ADR from that of cisplatin and due to the broad spectrum of antineoplastic activity of ADR.

To investigate whether cisplatin and ADR down-regulate other enzyme activities, lactic dehydrogenase (LDH) activities of the cell extracts prepared from the A2780 and A2780CP cells treated with the drugs were estimated. No down-regulation, but a slight enhancement (at high concentrations of the drugs), of LDH activity in the cell extracts was observed (data not shown), indicating that the down-regulation of telomerase activities in A2780 and A2780CP cells treated with these anticancer drugs probably does not reflect the non-specific inhibition of telomerase activity by those anticancer drugs.

It has been reported that telomerase activity is repressed during induced differentiation of tumor cells due to the reduced tumorigenic potential of the mature cells,[11,12] showing an inverse relationship between the degree of differentiation and telomerase activity. However, the factors responsible for the regulation of telomerase activity are not known. Further studies are required to elucidate the mechanism of down-regulation of telomerase activity by anticancer drugs.

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