

(NDEA) decreased DNA fragmentation and the alkylation of DNA in both liver and esophageal mucosa.⁷ By contrast, it also was found that DTDS increased the toxicity and carcinogenicity of *N*-nitrosomethylbenzylamine.^{8,9} It seems that DTDS itself might be toxic and even carcinogenic when fed to rats in combination with amine.¹⁰ PDTC was the better analog and was found to be less toxic than DDTC and DTDS. The dithiocarbamate derived from amino acids is more hydrophilic and stable. PDTC still retained the anticarcinogenic effect against NDEA-induced rat live tumors and inhibited cytochrome p-450-dependent oxygenase. DDTC and PDTC have been tested for their capability to increase the therapeutic effects in neoplasma and extend the life span of leukemic mice.¹¹ Prolinethiuramdisulfide (PTDS) is the oxidized form of PDTC. The metabolite, PTDS, is more hydrophilic than PDTC. PTDS was found in urine of rats treated with PDTC, but it is not excreted via bile or blood.¹²

In this study, we examined the effects of PDTC and PTDS on colon carcinoma HT29 cell proliferation and found that its effect on proliferation is exerted through mechanisms independent of cell differentiation.

MATERIALS AND METHODS

Chemicals

Prolinethiuramdisulfide (PTDS) and prolinedithiocarbamate (PDTC) were synthesized by the procedure described previously.¹³ DMSO was used to dissolve the oil-soluble compounds before their addition to the cultures. Control cultures received DMSO only.

Cell Culture

The human colon adenocarcinoma cell line, HT29, was obtained from Veterans General Hospital (Taipei, Taiwan, ROC). The cells were grown in Dulbecco modified Eagle medium (DMEM) supplemented with 10% heat-inactivated and dialyzed fetal bovine serum (FBS) (Gibco, Gaithersburg, MD, USA), 100 units/mL penicillin, and 100 mg/mL strep-

tomycin. HT29 cells were grown as a monolayer and incubated at 37 °C, in 5% CO₂ and 90% relative humidity. Cell morphology was recorded daily.

Growth Assay

HT29 cells were treated with PDTC, PTDS, or DMSO only. At different time points, quadruplicates of control and treated cells were removed by trypsinization and counted. For the thymidine incorporation assay, cells were seeded into 24-well plates for 18 h, and then treated with PDTC, PTDS, or DMSO for 24 h, followed by the determination of [³H]-thymidine (1 µCi/mL) incorporation into DNA during the last 4 h of treatment. For clonogenic capacity analysis, cells were seeded in triplicate at a density of 1000 cells per well and grown for 14 days in the absence or presence of drug. The resulting colonies were fixed, stained with 0.25% methylene blue in 50% methanol/water, and counted. Colonies composed of at least 50 cells were scored. The cloning efficiency was determined by dividing the number of colonies by the number of cells plated. All experiments were performed on at least 2 separate occasions and in triplicate samples.

Alkaline Phosphatase Activity

Alkaline phosphatase activity was determined using 4-nitrophenyl phosphate (Boehringer Mannheim) as a substrate. A solution of 10 mM 4-nitrophenyl phosphate in alkaline buffer (50 mM NaCO₃, pH 10, 50 mM MgCl₂) was added to 20 µg aliquots of protein extracts. Hydrolysis of 4-nitrophenyl phosphate was measured as absorbance at 405 nm using the Dynatech MR-7000 reader (Dynatech Labs, Chantilly, VA, USA).

Western Blotting

Protein extraction and Western blot analysis were done as described.¹⁴ Total proteins (for alkaline phosphatase and α -tubulin) containing 50 µg of protein were analyzed on blots and incubated overnight at 0 °C with 10% bovine serum albumin in phosphate-buffered saline and then incubated with an α -tubulin monoclonal antibody (Zymogen) or anti-alkaline phosphatase polyclonal antibody (Dako, Carpinteria, CA, USA). Alkaline phosphatase or α -tubulin pro-