0.025% (v/v) of the culture medium. After various periods of incubation, cells were sedimented for protein preparation or for DNA fragmentation analysis.

Determination of Cell Growth Curves

Human prostate cells (1×10^4) were plated in 35-mm Petri dishes. The next day, the medium was changed, and KT (0-40 μM) was added. Control cells were treated with DMSO in a final concentration of 0.05% (v/v). The incubation medium was renewed daily during the experiment. At the end of incubation, cells were harvested for cell counts using a hemocytometer.

Preparation of Protein Lysate

Treated and untreated cells were rinsed 3 times with ice-cold phosphate-buffered saline (PBS), pelleted at 800 x*g* for 5 min and lysed in 500 μL of freshly prepared extraction buffer (10 mM Tris-HCl, pH 7; 140 mM sodium chloride; 3 mM magnesium chloride; 0.5% (v/v) NP-40; 2 mM phenylmethylsulfonyl fluoride; 1% (w/v) aprotinin; and 5 mM dithiothreitol) for 20 min on ice. The extracts were cleared by centrifugation for 30 min at 10,000 x*g*.

SDS-polyacrylamide Gel Electrophoresis (PAGE) and Immunoblotting

Proteins were normalized to 50 g/lane resolved on a 12.5% (w/v) SDS-PAGE and blotted onto an immobilon P membrane with a semidry electroblotting apparatus (TE70; Hoefer Scientific Instruments, San Francisco, CA). The membrane was blocked overnight at room temperature with blocking reagent (20 mM Tris, pH 7.4; 125 mM NaCl; 0.2% (v/v) Tween 20; 4% (w/v) non-fat dry milk; and 0.1% (w/v) sodium azide). Antibodies used for immunoblot assays including polyclonal rabbit antisera specific for the human Bax protein (Ab-1, CAN Bioscience Co.) were raised against synthetic peptides corresponding to amino acids 150-165 of human p21-Bax. Mouse monoclonal antibodies included caspase 3, PARP, Bad, and GAPDH (Transduction Laboratories, Lexington, KY).

Filters were incubated for 1 h with the primary antibody, washed 3 times, and then incubated with an alkaline phosphatase-conjugated secondary antibody (immunoglobulin G) in PBS and 0.5% (v/v) Tween 20 for another 45 min with gentle shaking. After 3 final washes, the proteins were visualized by incubating with the colorigenic substrates nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate (Sigma.) Video densitometry quantification was performed with an OPTIMAS®-6 image analysis system.

Analysis of DNA Fragmentation

Apoptosis was determined by DNA laddering and morphological criteria as described previously.[19]

RESULTS

KT Induces Cell Growth Arrest in Human Prostate Cancer Cells

In this study, we demonstrate that KT induced significant growth inhibition of human prostate cancer cells. As shown in Fig. 1, the growth curves of PC-3 and LNCaP were significantly inhibited by KT (2-40 μM) treatment (Fig. 1A, B). As compared to PC-3 cells, KT-induced growth inhibition in LNCaP was more apparent. In order to determine whether KT has an apoptotic effect in human prostate cancer cells, cells were treated with KT (2-100 μM). The results showed that KT-treated cells exhibited morphological changes accompanied by progressive internucleosomal degradation of DNA to yield a ladder of DNA fragments (Fig. 2A, B). These results further confirm the occurrence of apoptotic prostate cancer cell death after KT exposure. As shown in Fig. 2B, after exposure of LNCaP cells to KT (2-100 μM), both the attached and detached cells were harvested at 24 h after drug treatment. In each case, nucleosomal DNA ladders typical of apoptosis were visualized on the agarose gel after staining with ethidium bromide (Fig. 2B). Interestingly, the DNA laddering effects were not observed in PC-3 cells treated by KT.

Bax Protein Induced by KT Treatment

The levels of Bax protein induction in PC-3 (Fig. 3A) and LNCaP (Fig. 3B) cells were investigated using Western blot analysis. The results show that sig-