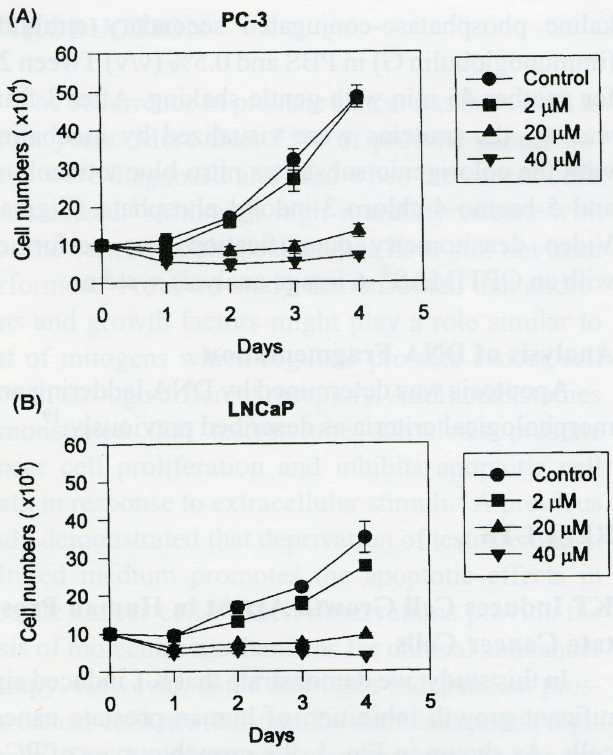

Fig. 1. Growth inhibition of human prostate cancer cells treated with KT. (A) PC-3, and (B) LNCaP cells were treated with various concentrations (2 to 40 M) of KT. The medium containing various doses of KT was renewed daily, and the total cell number was counted at the indicated time points. Results are the means ± s.d. of 3 independent experiments.

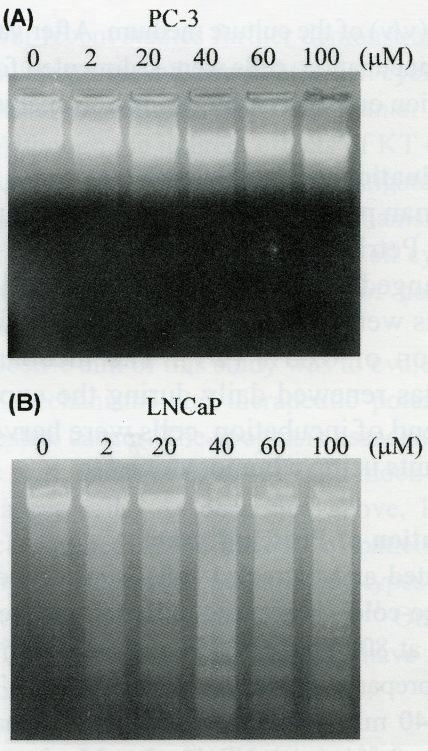

Fig. 2. DNA fragmentation analysis of human prostate cancer cells undergoing KT-induced apoptosis. (A) PC-3 cells and (B) LNCaP cells were treated with different doses (2-100 M) of KT, and DNA fragmentation was examined at 24 h after KT treatment. Cells in lane 1 received mock treatment as a control.

nificant Bax and Bad protein inductions were observed in both cells after exposure to KT (2-100 μM) (Fig. 3A, and B). As shown in Fig. 4B, caspase-3 was activated in LNCaP cells by 24 h after KT (40 μM) exposure. According to a previous report, the substrate of caspase-3 is PARP.[22] Western blot analysis revealed that the Mr 116,000 PARP molecule was degraded to a relatively stable Mr ~85,000 fragment (Fig. 3B) by 24 h after KT (60 μM) treatment. In order to evaluate the time-course changes of caspase 3 activation, immunoblotting analysis was performed (Fig. 4A, B). Significant caspase 3 activation and PARP protein degradation were observed at 12 h in response to KT in LNCaP cells. Cleavage of PARP was also observed at 18 h after KT treatment (Fig. 3B) at the time point that the DNA fragmentation (Fig. 2B) was observed when cells were treated with KT (40 μM).

As shown in Fig. 2B and 3B, fragmentation of DNA and activation of caspase 3 occurred simultaneously when LNCaP cells were treated with 40 μM of KT. These studies suggest that apoptosis was induced by KT in LNCaP cells through activation of a caspase 3 pathway. To further investigate whether the regulation of genes involved in apoptosis is induced in a time-dependent manner, 40 μM of KT was added to both p53 and LNCaP cells and the levels of Bax, caspase 3, and PARP activation were then determined (Fig. 4). Our data demonstrate that caspase 3 and PARP were drastically activated by 12 h after 40-μM KT treatment in LNCaP cells. However, in PC-3 cells, a significant increase in activated caspase 3 was only seen at 18 h after cells were exposed to 40-μM KT treatment (Fig. 4B). Such results are consistent with Fig. 2 which indicates that DNA laddering was more