Retinoic Acid Induced G1 Arrest in Hepatocarcinoma Cell Lines

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Background Retinoids (RA), a group of vitamin A derivatives, have been known to regulate growth and differentiation of epithelial cells and to involve in carcinogenesis. Therefore, it has been examined its chemotherapeutic and chemopreventive activity in various types of cancers. Treatment of various types of cancer cells with RA resulted in growth inhibition and apoptosis. Biological actions of RA are mediated through nuclear receptors, including the retinoic acid receptors (RARs) and retinoid X receptors (RXRs) families. Among them, RARβ has been suggested to play an important role in the biological functions of RA. RARβ expression is suggested to associate with the cellular sensitivity to retinoid in cancer cells. It has been shown that RA treatment caused cell cycle arrest at G1 phase through enhanced expression of cyclin dependent kinase inhibitor p21 in leukemia cells and lung cancer cell lines. Therefore, we examined whether all-trans retinoic acid (atRA) caused to arrest a cell cycle, resulting in a growth inhibition in Korean hepatoma cell lines. We investigated the expression of proteins related to a cell cycle.

Methods Korean hepatoma cell lines (SNU354, SNU449) were purchased from Korea Cell Line Bank and HepG2 from ATCC. Cell lines were maintained by RPMI medium containing 10% fetal bovine serum. Cells were treated with 10μM atRA as an indicated time period. Percent growth inhibition was calculated by cell number from atRA treated cells compared to that from control cells. After atRA treatment, cells were harvested with ice-cold PBS and were lysed with 200μl of ice-cold RIPA buffer containing protease inhibitors. Total cell lysates were resolved on 8–12% SDS-PAGE gel and transferred to PVDF membrane. Blots were reacted with desired antibodies. Cell cycle arrest was analyzed by FACScan after atRA treatment.

Results Treatment of hepatoma cells with atRA resulted in cell growth inhibition and the sensitivity of cells to atRA seemed to be related to induction of RARβ expression. atRA-treated hepatoma cells showed cell cycle arrest at G1 phase starting from 3h treatment in atRA-sensitive SNU354 but it was delayed in SNU449 cell line. HepG2 cells also showed G1 arrest after 48 h atRA treatment. Increased expression of both p21\textsuperscript{Waf1/Cip1} and p27 proteins were observed in atRA-treated SNU354 cells. Since expression of p53 was not changed by atRA treatment in both cell lines, induction of p21 and p27 could independently occur in atRA treated SNU3545 cells. Expression of p27 was not increased in SNU449 cells after atRA treatment.

Conclusion Based on our results, we concluded that retinoic acid treatment induced cyclin dependent kinase inhibitors p21 and p27, resulting in cell cycle arrest at G1 phase in Korean hepatoma cell lines.