Simultaenous effect of all-trans retinoic acid, 5-fluorouracil and cisplatin on KYSE-30, AGS cell lines

Abstract

Introduction: Recently, a side population of cancer cells has been identified in gastrointestinal tumors. Especially, CD44 positive cancer stem cells (CSC) isolated from human gastric and esophageal cancers may initiate tumor growth and targeting CD44 may eradicate the proliferating population of cancer cells. The drug resistance property of CD44 positive cells, however, is still unknown. Overall, studies suggest that tumor cells frequently develop resistance to cisplatin and 5-fluorouracil. All-trans retinoic acid (ATRA) has been reported to down-regulate CD44 and has differential role. Here, we investigated the additive effect of ATRA used in combination with cisplatin and 5-FU on esophageal (KYSE-30) gastric (AGS) cancer cell lines. Also, we compared the distribution of cell cycle in the $CD44^{\pm}$ cancer cells treated with combination of ATRA, cisplatin and 5-fluorouracil (5-FU).

Material & Methods: The cancer cell lines were treated with various concentrations of all-trans retinoic acid, cisplatin and 5-FU. Magnetic activated cell sorting was used to select CD44 positive cells. The *cytotoxicity* was examined using MTT, acridine orange/ethidium bromide (AO/EB) and in vitro clone formation assay. Their IC50 were compared with those of cisplatin. In order to examine the efficacy of ATRA alone or in combination with 5-FU and cisplatin on cell cycle status, we performed 4, 6-diamidino-2-phenylindole dihydrochloride (DAPi) assay and flow cytometry analysis.

Result: CD44 was positively expressed in ~5-6% of AGS and Kyse-30 cell lines. Pretreatment with ATRA increased the sensitivity to cisplatin and 5-FU in in-vitro. The AO/EB staining showed an increase in apoptotic cells. ATRA inhibited cell growth and induced cell cycle G_0/G_1 arrest. Consistent with the inhibition and G0/G1 arrest, ATRA and 5fu, in combination, induced increased the cell cycle arrest at G_1/S phase in Kyse-30 and AGS $CD44^+$ cells. While after pretreatment of $CD44^-$ KYSE-30 with ATRA, no remarkable changes were seen in cell cycle by cisplatin and 5-FU. Furthermore there was an indication that the combination of ATRA and cisplatin caused increased cell cycle arrest at G_2/M in AGS and Kyse-30 $CD44^+$ cells.

Conclusion: These result suggested that ATRA enhance the cytotoxic effect of CDDP and 5-FU on $CD44^+$ gastric, esophageal cancer cell lines and our data provide a rational for combined ATRA and 5fu and cisplatin therapies in gastrointestinal cancer.

Keywords: cancer stem cell, all-trans retinoic acid, cisplatin, 5-fluorouracil, CD44 marker, KYSE-30 and AGS cell lines