Development of antitumor cytosine nucleosides

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2'-Deoxy-2'-methylidene cytidine (DMDC) and 2'-C-cyano-2'-deoxy-1-β-D-arabinofuranosylcytosine (CNDAC) are novel antitumor cytosine nucleosides with unique mechanisms of action similar in part to those of gemcitabine (dFdC) which has been shown to be an effective therapy against solid tumors. These antimetabolites are incorporated into the cells and phosphorylated by enzymes including deoxycytidine (dCyd) kinase (EC 2. 7. 1. 74). We studied mutational events in dCyd kinase mRNA expression, focusing on aberrant dCyd kinase mRNA which has been frequently observed in established cell lines resistant to antitumor dCyd nucleoside analogues such as Ara-C, dFdC and CNDAC. We reported aberrant dCyd kinase mRNA expression identified as splicing mutants including deletion of the fifth exon, the third exon or the forth exon. The various mutations in the dCyd kinase gene may be caused by acquisition of resistance against several antitumor cytosine nucleosides and dCyd kinase may also be the most important molecule for the activation of the antitumor cytosine nucleoside and their acquisition of resistance.

1-(3-C-ethynyl-β-D-ribo-pentofuralnosyl)-cytosine (ECyd) is a new cytidine analogue showing significant cytotoxicity and antitumor activity in preclinical therapeutic models. The results of several studies have shown that ECyd acts by interfering with RNA synthesis. We examined the effects of dosage schedule on antitumor activity *in vitro* and *in vivo* to determine the optimal administration schedule for ECyd. Furthermore, when the metabolism of ECyd in tumor cells was examined, it was found that ECyd were retained at high concentration for prolonged periods. To elucidate more detail mechanisms, we have established resistant cells to ECyd. ECyd sensitivity of cells was well correlated with the intracellular accumulation of ECTP, which may be affected by both the cellular membrane transport mechanism and uridine/cytidine kinase (UCK; EC 2. 7. 1. 48) activity. UCK is thought to be a rate-limiting enzyme in the pyrimidine salvage pathway for DNA biosynthesis in growing cells. We isolated cDNA encoding the enzyme from human fibrosarcoma cells, then determined its nucleotide sequence by the 5'-RACE method followed by confirmation employing the human genome DNA library. As a result, UCK has two isoforms (UCK1 and UCK2). We investigated the relation between expression of UCK1 and UCK2 at both the mRNA and protein levels and UCK activity in a panel of 10 human cancer cell lines. Expression of UCK2 appeared to be correlated with cellular sensitivity to ECyd, and it may contribute to the tumor-selective cytotoxicity of ECyd.

Moreover, we investigated the antitumor activity of other antitumor nucleoside, 1-2-(deoxy-2-fluoro-4-thio-β-D-arabinofuranosyl)cytosine (4'-thio-FAC) in peritoneal dissemination models of gastrointestinal cancers. Oral administration of 4'-thio-FAC showed a marked effect on the development of ascites and on the survival of nude mice implanted with the highly metastatic MKN-45-P and HCT-15-P. 4'-Thio-FAC is a promising therapeutic agent for peritoneal dissemination of gastrointestinal cancers.