Institutionen för Medicin, Huddinge

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Mechanistic Studies of APR-246 in Leukemia

AKADEMISK AVHANDLING

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ABSTRACT

PRIMA-1 and its analog APR-246 are novel drugs that restore the active conformation of mutated and unfolded p53 protein and induce apoptosis and cell death in various tumors in pre-clinical models. We first aimed to explore the effects of APR-246 alone and in combination with other drugs in acute myeloid leukemia (AML) in vitro. APR-246 induced dose-dependent apoptosis and increased active caspase-3 and p53 protein levels as well as the Bax/Bcl-2 ratio independently of *TP53* mutational status. AML patient cells with *TP53* mutations and complex karyotype were more resistant to conventional chemotherapeutic drugs but retained their sensitivity to APR-246. Pronounced synergism was found when combining APR-246 with DNR in AML patient cells and pre-incubation with APR-246 induced more synergistic effects compared to other treatment schedules in the AML cell line KMB3.

As APR-246 was shown to induce expression of genes protective of oxidative stress in global gene expression profiling, we furthermore aimed to study the effects of APR-246 on the redox status of AML cells. We confirmed that APR-246 increased ROS formation and depleted cells from glutathione. *HO-1*; a gene protecting from oxidative stress, was one of the most up-regulated genes in response to APR-246. Both *HO-1* and its transcriptional regulator *NFE2L2* (Nrf2) were up-regulated as detected by q-RT-PCR. APR-246 treatment induced Nrf2 activation by translocation of the Nrf2 protein from the cytosol to the nucleus. Transient knockdown of Nrf2 in KMB3 cells obliterated APR-246-induced upregulation of *HO-1* and increased its antitumoral effects. The PI3K inhibitor wortmannin and the mTOR inhibitor rapamycin; both up-stream regulators of Nrf2, inhibited APR-246-induced nuclear translocation of Nrf2 and induced synergism with APR-246.

A phase I first-in-man study including 22 patients with hematologic malignancies and prostate cancer was conducted. Dose escalations from 2 mg/kg to 90 mg/kg revealed a maximum tolerated dose (MTD) of 60 mg/kg and a half-life of 4-5 hours. The most common adverse effects were fatigue, dizziness, headache, and confusion. Dose limiting toxicities (DLTs) were increased ALT/AST (n=1), dizziness, confusion, and sensory disturbances (n=2). Tumor cells showed cell cycle arrest, increased apoptosis, and up-regulation of p53 target genes.

We finally showed that miR-34b/c is epigenetically silenced by DNA methylation in chronic lymphocytic leukemia (CLL). As being down-stream regulators of p53, miR-34b/c expression levels were induced by PRIMA-1 as well as by doxorubicin and decitabine. Over-expression of miR-34b/c in CLL cells increased apoptosis which suggest a tumor suppressor function for these microRNAs.

In conclusion, AML cells are sensitive to APR-246 in vitro irrespectively of *TP53* mutational status. The substance induces oxidative stress and activates the Nrf2/HO-1 protective pathway. In a first-in-man study, APR-246 was shown to be to have a favorable pharmacokinetic profile and to induce p53-dependent biologic effects *in vivo*. Either in combination with conventional chemotherapeutic drugs or PI3K inhibitors, synergistic antileukemic effects can be obtained which holds a promise for further combination studies *in vivo.*

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