Karolinska Institutet

Institutionen för Onkologi-Patologi

Rescue of mutant p53 family members by the low molecular weight compound PRIMA-1^{MET}/ APR-246

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i lecture hall, CCK, R8:00

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ABSTRACT

The tumor suppressor p53, guardian of the genome, is induced and activated by cellular stress signals such as DNA damage, hypoxia and activation of oncogenes. p53 upregulates downstream target genes, that are involved in cell cycle arrest, senescence, apoptosis, etc. Mutations in p53 occur frequently (around 50%) in many human tumors. Tumors with p53 mutations often show increased resistance to chemotherapy, since many anti-cancer drugs induce p53-dependent apoptosis though DNA damage. Thus restoration of wild type function to mutant p53 appears as an attractive approach for novel cancer therapy.

The low molecular weight compounds PRIMA-1 and PRIMA-1^{MET} were previously identified in our laboratory. We have shown that both PRIMA-1 and PRIMA-1^{MET} (as denoted APR-246) are converted to methylene quinuclidinone (MQ), that binds covalently to the DNA binding domain of mutant p53, restores its wild type function and triggers massive apoptosis in cancer cells. However the exact molecular mechanism of the mutant p53-dependent apoptosis induced by these compounds was not elucidated.

In paper I, we demonstrate that PRIMA-1^{MET}/ APR-246 triggers the mitochondrial apoptosis pathway in mutant p53 expressing cells. We show that early activation of caspase 2, along with induction of wild type p53 target genes PUMA and Bax are crucial for triggering mitochondrial apoptosis pathway. In paper II, we show that STMA-1, as a Michael acceptor, inhibits cell proliferation and induces apoptosis in mutant p53- expressing tumor cells, but not human diploid fibroblasts. The effect of STIMA-1 is dependent on thiol modification.

p53 family members p63 and p73, particularly their DNA binding domains share high structure similarity to p53. That prompted us to test whether the mutant p53-reactivating compound PRIMA-1^{MET}/ APR-246 could also rescue mutant forms of p63 and p73. In paper III and paper IV we show that PRIMA-1^{MET}/ APR-246 enhances mutant p63 DNA binding and restores pro-apoptotic functions to mutant p63γ and p73β in tumor cells. Mutations in p63 in humans cause several hereditary developmental syndromes with impaired limb development and skin differentiation (such as the EEC syndrome). We found that treatment with PRIMA-1^{MET}/ APR-246 promotes differentiation of mutant p63 expressing keratinocytes isolated from patients with EEC syndrome.

In conclusion, PRIMA-1^{MET}/ APR-246 restores wild type function to mutant p53 family members presumably through interaction with homologous structures in their DNA binding domain. Our studies shed further light on the rescue mechanism of mutant p53 family members and raise possibilities for treatment of mutant p63 carrying development syndromes such as EEC in the future.

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