



**Karolinska
Institutet**

Institutionen för Onkologi-Patologi

Pharmacological targeting of mutant p53 family members

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i seminarierummet R8:00, Cancercentrum Karolinska, Karolinska Universitetssjukhuset, Solna.

Torsdagen den 12 maj 2011, kl 9.30

av

Nina Rökaeus

Huvudhandledare:

Professor Klas G. Wiman
Karolinska Institutet
Institutionen för Onkologi-Patologi

Fakultetsopponent:

Professor Urban Gullberg
Lunds universitet
Institutionen för laboratoriemedicin

Betygsnämnd:

Professor Karin Öllinger
Linköpings Universitet
Institutionen för klinisk och experimentell medicin

Docent Leonard Girnita
Karolinska Institutet
Institutionen för Onkologi-Patologi

Professor Bengt Fadeel
Karolinska Institutet
Institutet för miljömedicin

Stockholm 2011

ABSTRACT

The tumor suppressor p53 serves as a guardian of the genome and functions mainly as a transcription factor. In response to various stress signals p53 binds to specific DNA sequence motifs and regulates transcription of a large group of target genes involved in cellular processes such as cell cycle arrest, senescence and apoptosis. Inactivation of p53 is critical for the formation of most tumors. Around half of all human cancers carry mutations in the *p53* gene (*TP53*) and mutant p53-harboring tumors often show increased resistance to conventional chemotherapy. Therefore, pharmacological restoration of wild type function to mutant p53 is a promising strategy for novel cancer therapy. We have identified a low molecular weight compound, STIMA-1, that selectively targets tumor cells in a mutant p53-dependent manner. STIMA-1 contains a reactive double bond that can potentially participate in Michael addition reactions and may restore the tumor suppressive function to mutant p53 by affecting its redox status.

Several other small molecules that reactivate mutant p53 have been identified in our group. PRIMA-1 and its more potent analog PRIMA-1^{MET} (also denoted APR-246) both induce p53 target genes and mutant p53-dependent apoptosis in human tumor cells. PRIMA-1 and PRIMA-1^{MET} are under physiological conditions converted to MQ that binds covalently to the p53 core domain and this modification *per se* is sufficient to endow mutant p53 with pro-apoptotic properties. To further explore the effects of PRIMA-1 and its analogs on tumor cells we analyzed the subcellular distribution pattern of several proteins upon drug treatment. We found that PRIMA-1 and PRIMA-1^{MET}, but not PRIMA-Dead (a PRIMA-1 analog that is unable to induce apoptosis) induced nucleolar accumulation of mutant p53. In addition, PRIMA-1^{MET} induced the levels of heat shock protein (Hsp) 70 and a redistribution of the PML nuclear body-associated proteins CBP, PML, Hsp70, and the Epstein-Barr virus encoded protein EBNA-5 to nucleoli. Our results suggest that relocation of mutant p53 and/or PML nuclear body-associated proteins to nucleoli may play a role in PRIMA-1^{MET}-induced apoptosis.

Since p53 and its family members p63 and p73 share high sequence and structural homology, we examined if PRIMA-1^{MET} also affects mutant p63 and p73. We found that PRIMA-1^{MET} restores wild type activity to some mutant forms of p63 and p73. PRIMA-1^{MET} enhanced mutant p63 DNA binding, and induction of target gene expression and apoptosis in human tumor cells in a mutant p63/p73 dependent manner. PRIMA-1^{MET} also induced a redistribution of mutant p63 to PML nuclear bodies and to nucleoli. Our data indicate that PRIMA-1^{MET} exerts its effects through a common mechanism for all three p53 family members, presumably involving homologous structural domains in the three proteins.

A better understanding of the exact molecular mechanisms of p53-targeting compounds is highly relevant for further drug optimization and the design of novel compounds with improved target selectivity and potency. The effect of PRIMA-1^{MET} on mutant p63 also raises the possibility of pharmacological rescue of p63 mutants in human developmental disorders caused by mutations in p63.