Stability of mutant p53 proteins

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Identification of Mdm2 (Hdm2) and JNK as proteins that target degradation of wild-type (wt) p53 prompted us to examine their effect on mutant p53, which exhibits a prolonged half-life (Figure). Of five mutant p53 proteins studied for association with the targeting molecules, two no longer bound to Mdm2 and JNK. Three mutant forms, which exhibit high levels of p53 expression, showed lower affinity for association with Mdm2 and JNK in concordance with greater affinity to $p14^{ARF}$, which is among the molecules stabilizing p53. Monitoring mutant p53 stability *in vitro* confirmed that, while either JNK or Mdm2 no longer affects certain forms of mutant p53, JNK and Mdm2 target others for degradation, albeit at lower efficiency when compared with wt p53 protein. Expression of wt p53 in tumor cells by gene transfection revealed a short half-life, suggesting that the targeting molecules are functional. Forced expression of mutant p53 in p53 null cells confirmed pattern of association with JNK and Mdm2 and prolonged half-life, as found in the tumor cells. Overexpression of Mdm2 in either tumor (which do express endogenous and functional Mdm2) or in p53 null cells decreased the stability of mutant p53, suggesting that, despite its expression, Mdm2 and JNK are insufficient (amount and/-or affinity) for targeting mutant p53 degradation. Based on both *in vitro* and in vivo analyses, we conclude that the prolonged half-life of mutant p53 depends on the nature of the gene mutation, which either alters association with targeting molecules, ratio between p53 and targeting and/or stabilizing molecules, or their targeting efficacy.

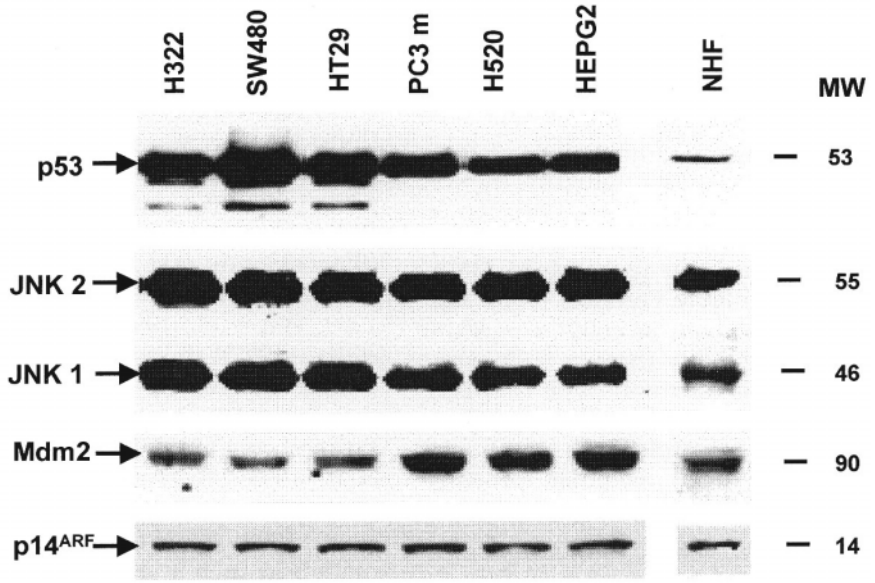

Figure. Expression levels of mutant and wild-type p53 proteins in human tumor cell lines and normal human fibroblast. Whole cell extracts (125 μg each) of tumor cell lines and normal fibroblast were separated on SDS-PAGE and transferred to nitrocellulose membranes that were probed with the monoclonal antibodies against p53 (DO1), JNK (333), Mdm2/Hdm2 (2A10) and p14ARF (Labvision), respectively. Molecular weight of the protein of interest is indicated on the right to the panel. Origin of cell lines (mutant codon and type of mutation); H322, bronchioalveolar carcinoma (248^{G-T}); H520, squamous cell carcinoma of the lung (146^{G-A}); SW480 and HT29, colon adenocarcinomas (273^{G-A} and 309^{G-T}, both); PC3m, prostate carcinoma (282^{C-T}); HEPG2, hepatocellular carcinoma (wild-type); NHF, normal human fibroblast GM00038A.