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Understanding the role of p53 in cancer

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Our main goal is to elucidate the biochemical and biological processes that underlie the activity of the p53 tumor suppressor protein. Within this frame we are also interested in the *mdm2* gene – an oncogene whose protein product serves as the main negative regulator of p53. Furthermore, both p53 and Mdm2 are components of an intricate signaling network, which surveys genome stability and serves as a major protective mechanism against cancer (Fig. 1A). Our studies are therefore aiming to identify the other key nodes in this network and to elucidate the molecular mechanisms that govern its functionality.

The p53 gene is mutated in about half of all human tumors. p53 is a transcription factor whose activity gives rise to a variety of cellular outcomes, most notably cell cycle arrest and apoptosis, eliminating cancer-prone cells from the replicative pool. Usually, p53 protein is present within a cell in minute amounts. The Mdm2 oncoprotein binds to p53, represses its transcriptional activity, and promotes its ubiquitination and rapid degradation. Recent data indicate the Mdm2 can promote either monoubiquitination or polyubiquitination of p53, with different outcomes (Fig. 1B). We are investigating the consequences of each type of ubiquitination.

Upon DNA damage, p53 is phosphorylated. We found that following treatment of cells with ionizing radiation, Mdm2 also undergoes rapid phosphorylation by the ATM kinase. The phosphorylated Mdm2 is less able to promote p53 degradation. Thus, in response to DNA damage, ATM promotes p53 stability and activity by mediating the simultaneous phosphorylation of both partners of the Mdm2-p53 autoregulatory loop. Recently, we identified an additional modification of Mdm2: acetylation. This modification occurs in the domain of Mdm2 that is involved in p53 ubiquitination. Acetylated Mdm2 becomes defective in promoting p53 degradation, suggesting a novel mechanism for regulating Mdm2 function and p53 levels.

Activated oncogenes also affect p53 activity. Interestingly, we found that deregulated excess beta-catenin induces accumulation of active p53, via induction of another tumor suppressor – ARF. As a consequence, excess beta-catenin triggers a p53-dependent growth inhibitory response. Aberrant accumulation of beta-catenin occurs in many human tumors, including colorectal cancer. We propose that aberrant activation of beta-catenin may alert p53 and increase its anti-proliferative activity. This would provide a safeguard against

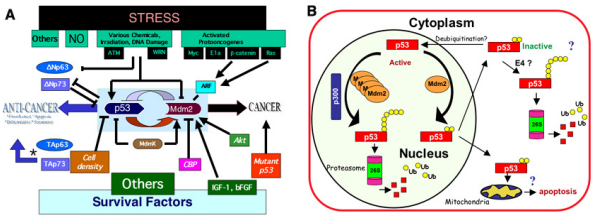

Fig. 1 *Regulatory processes in the p53 network.*
A. The p53 network. See text for details. Arrows indicate positive inputs; horizontal bars indicate inhibitory inputs. Two p53-related proteins, p63 and p73, exist in transactivation competent (TA) and N-terminally deleted forms.
B. Outcomes of Mdm2-mediated p53 ubiquitination. p53 acts primarily in the nucleus. Low levels of Mdm2 are proposed to promote p53 monoubiquitination and nuclear export. Cytoplasmic p53 may eventually be degraded, probably after further polyubiquitination by an E4-like enzyme, or deubiquitinated and recycled into the nucleus. High levels of Mdm2 are proposed to promote p53 polyubiquitination and degradation by nuclear proteasomes, perhaps through combined activity of Mdm2 with nuclear E4-like proteins (e.g. p300). While nuclear export of p53 might mainly spell inactivation, cytoplasmic p53 may also translocate to mitochondria to cause cytochrome C release and apoptosis.

oncogenesis, imposing a selective pressure for mutational inactivation of either ARF or p53 itself.

The ARF protein is also likely to be subject to extensive regulation. To explore the roles and regulation of ARF, we purified ARF-binding proteins. One of those proteins turned out to be B23 (NPM/nucleophosmin). B23 is a nucleolar protein. Notably, ARF is also typically found in the nucleolus, and nucleolar localization is critical for its ability to inactivate Mdm2 and induce p53. We found that B23 can affect the localization of ARF within the nucleus (Fig. 2), suggesting that ARF-B23 binding may be important for recruitment of ARF into the nucleolus. Morover, ARF-B23 binding is tightly regulated in a cell-cycle dependent manner, offering an interesting explanation for the ability of growth factors to override ARF-mediated growth inhibition.

Additional proteins also regulate p53 activity. Within this group, members of the p53 family – p63 and p73- play an important role. We found that naturally-occurring truncated forms of p63, generally believed to be effective inhibitors of p53, can actually promote the activation of apoptosis-related gene promoters by p53, while repressing promoters of genes involved in growth arrest and other p53 activities. This suggests a new level of interplay between p53 and its family members, which may be relevant for cancer development.

Recently, we found that Mdm2 can directly interact with chromatin histones, and promote the monoubiquitination of histone H2B. This may lead to transcriptional repression. Since Mdm2 is recruited to p53 target genes via p53-Mdm2 interactions, this finding offers a novel mechanism whereby Mdm2 can suppress the transcriptional activity of p53.

The p53-Mdm2 module (Fig 1A) is an integration hub for a variety of incoming signals. Mdm2 is regulated by many proteins, including ARF, the Akt kinase, bFGF and IGF-1, and the Mdm2-related protein MdmX. We are presently trying to assess the relative significance of each component.

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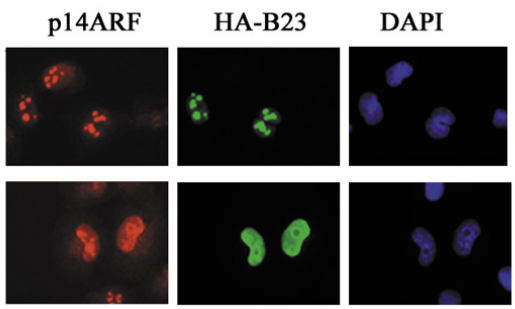

Fig. 2 *B23 affects the nucleolar localization of ARF.*
H1299 cells were transfected with HA-tagged B23 expression plasmid. After 24 hours, cells were fixed and immunostained for endogenous ARF (left panel, red) and HA-B23 (middle, green) antibodies. Nuclei were visualized by DAPI (right panel). Upper row: low B23 expression, normal nucleolar retention of ARF. Lower row: high B23 overexpression causes B23 spillover into the nucleoplasm, and relocates some of the endogenous ARF out of the nucleolus.

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