p53 mutations and resistance to chemotherapy: A stab in the back for p73

p73 is essential for apoptosis induced by many cytotoxic agents, but this function can be blocked by a particular category of p53 mutations that have consequently acquired a gain of function.

"Et tu quoque, mi fili!"
(And thou too, my son)
-Julius Caesar

The history of p53 is a chaotic voyage from the world of oncogenes to the world of tumor suppressor genes, while retaining a certain degree of individuality. Apart from artifactual problems related to involuntary cloning of mutant p53, this ambiguity is also due to our propensity to overcategorize in order to satisfy our Cartesian and oversimplistic view of science. Two articles published in this issue of *Cancer Cell* suggest that, once again, we need to revise our ideas concerning, not wild-type p53, which for the moment retains its tumor suppressor gene status, but mutant p53, which must now be considered to be oncogenes actively participating in the tumor phenotype (Bergamaschi et al., 2003; Irwin et al., 2003).

The idea that some p53 mutations can actively participate in cellular transformation was already postulated in 1990, and several arguments are in favor of such a model (Eliyahu et al., 1990; Lane and Benchimol, 1990). First of all, the mode of "inactivation" of wild-type p53: unlike most other tumor suppressor genes that are inactivated by frameshift or nonsense mutations leading to disappearance or aberrant synthesis of the gene product, almost 90% of p53 gene mutations are missense mutations leading to the synthesis of a stable protein, lacking its specific DNA binding function and accumulating in the nucleus of tumor cells (Soussi and Béroud, 2001). This particular selection for accumulation of p53 mutations in tumor cells can have two consequences: (1) a dominant negative role by heterooligomerization with wild-type p53 expressed by the second allele, or (2) a specific gain of function of mutant p53. Many studies have tried to distinguish between these two hypotheses, with no clear-cut conclusions. This task is further complicated by the fact that not all p53 mutations appear to be equivalent and present a marked heterogeneity of structure or loss of function. Transfection of various p53 mutations into cells devoid of endogenous p53 leads to an increase in their carcinogenicity, which varies according to the type of mutation (Dittmer et al., 1993; Halevy et al., 1990). This research into the oncogenic potential of certain p53 mutations is not purely theoretical, but has obvious clinical implications, as it could explain the marked disparity of the results of studies trying to demonstrate a relationship between the presence of a p53 gene mutation and various clinical parameters, such as survival or response to treatment. In breast cancer patients, the response to adriamycin is very strongly correlated with the presence of a mutation specifically localized in the loop domains L2 or L3 of the p53 protein (Aas et al., 1996). In vitro, the expression of p53 mutations in position 175 (R175H) specifically induces resistance of cells to etoposides compared to other p53 mutations (Blandino et al., 1999).

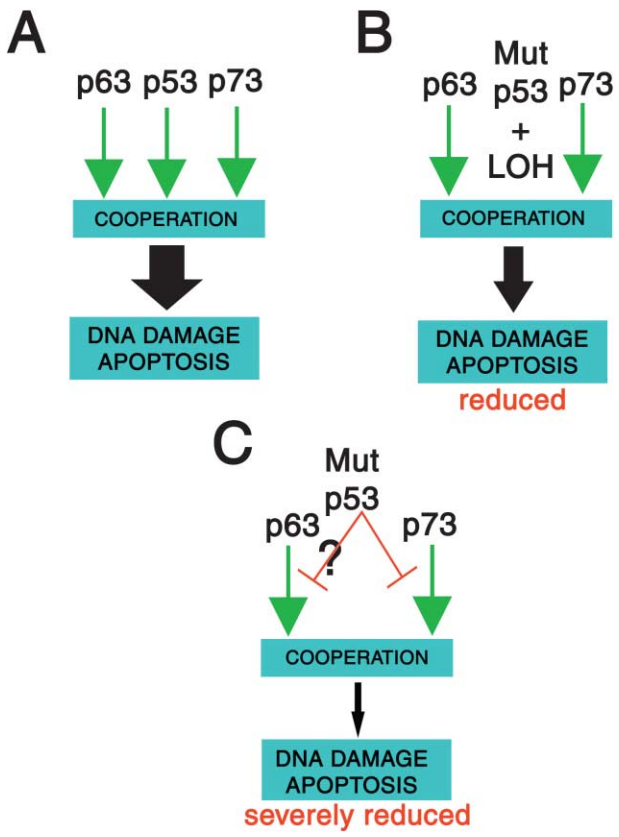

Figure 1. Cooperation of p53, p63, and p73 in response to DNA damage

In a normal cell, the signaling pathways leading to DNA damage apoptosis is controlled by a cooperation of p53, p63 and p73. How these 3 proteins cooperate is still unknown. In tumor cells, alteration of p53 can lead to at least 3 types of mutant p53: (**A**) inactive mutants with no dominant negative activity on p53; (**B**) inactive mutants with a dominant negative activity by interaction with wild-type p53; and mutants with a gain of function via inactivation of p73-dependent apoptosis and perhaps p63 as well (**C**).

The studies described by Crook and Kaelin in this issue of *Cancer Cell* show that this activity of resistance to anticancer agents involves inactivation of the apoptotic function of p73 protein by mutant p53 (Bergamaschi et al., 2003; Irwin et al., 2003). The two homologous genes of p53, p63 and p73, discovered 6 years ago, express many isoforms due to alternating use of transcription promoters and alternative splicing. Long isoforms (TA-p73 or TA-p63) are able to transactivate the same target genes as p53 and induce apoptosis, while short forms (ΔN-p63 or ΔNp73) have an opposite activity via dominant negative mechanisms (Melino et al., 2002). p63 and p73 are able to cooperate with p53 to induce apoptosis, suggesting the existence of a complex network of interactions between the products of these three genes (Flores et al., 2002). The mechanism by which p53 and its two siblings

cooperate for apoptosis after DNA damage is largely unknown, but two models have been provided (Urist and Prives, 2002). In the "dynamic exchange" model, p53 and p63 and/or p73 reside in a large transcriptional complex that sequesters the 3 proteins close to the promoter element. At any time, one of the 3 proteins may bind to DNA to activate transcription (Figure 1). It is possible that this large complex is stabilized by p63, p73, or both. The "dual site stabilization" model is based on the observation that several genes contain more than one p53 response element. Engagement of these two response elements by p53 and p73 and/or p63 could be required for the most efficient transcription.

Irwin et al. show that many genotoxic agents can induce accumulation of p73 protein in cell lines of tumor cells (Irwin et al., 2003). Inactivation of p73 by a dominant negative mutation or si-RNA leads to resistance of cells to apoptosis induced by genotoxic agents. Similarly, $p73^{-/-}$ murine embryonic fibroblasts immortalized by SV40 antigen T and Haras are more resistant to apoptosis induced by these genotoxic agents than the same cells expressing wild-type p73. This chemoresistance induced by inactivation of p73 is independent of the p53 gene status. Similarly, Crook's team shows that adriamycin, cisplatin, taxol, and etoposide induce accumulation of p73 protein and induction of AIP gene transcription (apoptosis induced protein), thereby confirming that p73 is an important component in the cell response to cytotoxic agents (Bergamaschi et al., 2003).

As the p73 gene is only rarely genetically or epigenetically altered in human tumors, is it possible to consider another indirect mechanism leading to p73 inactivation? This is a fundamental question, as p73 inactivation could explain certain mechanisms of resistance to chemotherapy. The answer to this question can be found in studies of the protein interactions between the various members of the p53 family. Although it has now been fairly clearly established that wild-type p53 cannot form stable heteroligomers with p73 or p63, this is not the case for mutant p53. A strong interaction involving the DNA binding domain of the two partners has been characterized between certain p53 mutations and p73 or p63 (DiComo et al., 1999; Marin et al., 2000; Strano et al., 2002). This interaction also leads to inactivation of the transactivational functions of p73 and p63. Various types of p53 mutations can interact with p73, depending on the author and experimental conditions, but recent studies show that a change of conformation of the central domain of mutant p53 is the essential component in this interaction with p73 (Bensaad et al., 2003). On the other hand, in a joint study, Kaelin and Crook showed that p53 polymorphism in codon 72 (Arg or Pro) had an important influence on this interaction, as it is only detected in mutant p53 with Arg polymorphism (Marin et al., 2000).

In this issue of *Cancer Cell*, Bergamaschi et al. used isogenic cell lines expressing a great diversity of p53 mutations either in the Arg form or the Pro form and show that only those mutants expressing the Arg form are resistant to cytotoxic agents (Bergamaschi et al., 2003). Furthermore, analysis of a homogeneous population of patients with head and neck cancer presenting identical p53 mutations to those studied in vitro demonstrate that the majority of patients expressing mutant p53 associated with Arg polymorphism have a poor response to chemotherapy and a shorter survival. This is the first large-scale study combining both basic research and clinical data in order to more accurately evaluate the role of the p53 signaling pathway in human tumors.

These two studies published in *Cancer Cell* will have important repercussions for both basic research and clinical practice. First of all, if the integrity of the p73 signaling pathway is important in the cellular and tumor response to genotoxic agents, it is possible that p73 inactivation may be mediated by pathways other than p53 mutations. This hypothesis is not easy to verify because, unfortunately, it would be difficult to develop a test evaluating the functional status of p73 in tumors in view of the mechanisms that inactivate p73. Only large-scale studies combining observation of the molecular status of tumors (expression profiles or mutational profiles) and functional studies would be able to characterize these signaling pathways. The second consequence of these studies is that mutant p53 can once again be considered to be oncogenes with a well characterized gain of function activity. Mutant p53 must be considered to be a family of extremely heterogeneous proteins, both structurally and functionally, which will one day need to be individually characterized (Bullock and Fersht, 2001). The p53 gene mutation database currently contains 3,200 variants for 15,000 listed tumors (Soussi and Béroud, 2001). It would be perfectly feasible to perform systematic structural and functional analysis linked with clinical data on the 50 variants most frequently identified in human cancers (corresponding to 7,300 tumors, i.e., almost 50% of the database), in order to establish the profile of each mutation.

Finally, the most promising aspect of the studies described here concerns the therapeutic potential, as the use of si-RNA specifically directed against mutant p53 should restore chemosensitivity of the tumor. It has already been demonstrated that wild-type p53 and mutant p53 can be distinguished by si-RNA, suggesting that it would therefore be possible to specifically target tumor cells (Martinez et al., 2002). The mechanism of action of p53 mutations, as demonstrated by these studies, could well be its Achilles' heel. It is up to us to take advantage of this opportunity!

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Cdk2 dethroned as master of S phase entry

The prevailing view of cdk2 as a critical regulator of cell cycle progression and optimal therapeutic target in cancer cells is now challenged by the observation that tumor cells deficient in cdk2 protein and kinase activity are not impaired in proliferation.

Deregulation of cell cycle control mechanisms is an obligatory step in tumorigenesis. Myriad individual genetic events lead to circumvention of checkpoints that restrain the activity of cyclin/cyclin-dependent kinase (cdk) complexes that are responsible for managing cell cycle transitions. Indeed, it is now widely accepted that alteration of some component of the retinoblastoma protein (pRb) pathway, the core of which is depicted in Figure 1, occurs in virtually all human tumors. In some tumor cells, the RB gene is a direct target of inactivating mutations, but most often pRb is inactivated consequent to inappropriate activation of the cyclin D/cdk4(6) complex. This in turn can be achieved through overexpression or mutation of one of the subunits, or through loss of the negative regulator $p16^{INK4a}$ (Sherr and McCormick, 2002).

One of the most significant consequences of pRb inactivation is activation of cyclin E/cdk2 subunits, often as a result of increased cyclin E expression. Cyclin E/cdk2 complexes can themselves participate in maintained inactivation of pRb in tumor cells that express this protein, but also appear to have several other critical roles in cell cycle progression (Bartek and Lukas, 2001). As shown in Figure 1, cyclin E/cdk2 complexes are thought to play critical roles in centrosome duplication (Hinchcliffe and Sluder, 2002), replication origin firing (Krude et al., 1997; Takisawa et al., 2000), and histone protein expression (Ma et al. 2000; Zhao et al., 2000). Consistent with such crucial roles for cyclin E/cdk2 downstream of pRb, many tumor cells are exquisitely sensitive to inactivation of cyclin E/cdk2 whether or not they express pRb. This conclusion has been drawn from a multitude of experiments demonstrating antiproliferative effects of overexpression of $p27^{KIP1}$, a protein inhibitor of cdk2, or of dominant-negative cdk2 subunits. Further, injection of antibodies against cdk2 activators cyclin E and cyclin A blocks proliferation, as does treatment of many different cells with cdk2 inhibitors (Tetsu and McCormick, 2003; Knockaert et al., 2002).

The view that pRb pathway inactivation has cyclin E/cdk2 activation as its ultimate proliferative consequence is supported by observations of mice engineered to express cyclin E in place of cyclin D1. Animals lacking cyclin D1 have profound proliferative defects in a subset of tissues, and fail to activate cdk2 in those tissues. These phenotypes are significantly suppressed in a knockin animal that expresses cyclin E from the cyclin D1 locus (Geng et al., 1999), suggesting that loss of cyclin D-mediated inactivation of pRb is inconsequential if cyclin E synthesis is no longer dependent on pRb inactivation. Indeed, excess cyclin E/cdk2 subunits can also overcome ectopic expression of a nonphosphorylatable, and thus constitutively active, pRb (Bartek and Lukas, 2001). This tumorigenic role of cyclin E/cdk2 may be most clearly manifest in human breast cancer cells, where reduced $p27^{KIP1}$ expression or cyclin E overexpression correlates well with aggressiveness of the tumor (Catzavelos et al., 1997; Porter et al., 1997; Keyomarsi et al., 2002). All together, these studies suggest that cyclin E/cdk2 regulation is targeted directly and indirectly by multiple, collaborative mutational events in a wide variety of tumor cells and thus chemical inhibition of cdk2 might provide an insurmountable obstacle to continued tumor cell proliferation.

This view of cell cycle control in cancer is now challenged by work from Tetsu and McCormick reported in the March issue of *Cancer Cell*. Using primarily cell lines derived from colon cancers, Tetsu and McCormick have shown that direct chemical inhibition of cdk4(6) or indirect reduction of D cyclins and cdk4 by MEK inhibitors blocks proliferation, but multiple modes of cdk2 inhibition are without effect. For example, colon cancer cell lines proliferate without regard to $p27^{KIP1}$ or dncdk2 overexpression, but these same reagents do cause arrest in other cell lines previously shown to respond to cdk2 inhibition. Thus, colon cancer cells in general appear to evade the effects of cdk2 inhibition seen in other cancer cell types. The authors suggest that one reason for this is the ability of deregulated cdk4 to fully inactivate pRb as a consequence of eroded phosphorylation site specificity in tumor cells. Consistent with this, Tetsu and McCormick show that one form of dncdk4 can block proliferation of