P63 and P73: Teammates or adversaries?

The status and interrelationship of p53 family members are critical elements in tumor progression. An intriguing paper in this issue of *Cancer Cell* (Rocco et al., 2006) reveals a new twist in the interactions between p63 and p73 following DNA damage, underscoring a role for p73 in the proapoptotic regulation of Puma, Noxa, and Bcl-2 in head and neck squamous cell carcinomas (HNSCC). These data define a pathway in which ΔNp63α promotes survival in squamous epithelial malignancy by repressing a p73-dependent proapoptotic transcriptional program, suggesting that p63 levels and p73 status may be key determinants of tumor response in patients with HNSCC.

At the cellular level, tumor progression usually involves blockage of normally regulated cell cycle control and apoptosis mediated by tumor suppressor genes. Inactivation of tumor suppressor genes or activation of protooncogenes can lead to a lack of proper control, especially under stress, leading to clonal outgrowth and tumor progression. These oncogenic events are evolving as important determinants in the response of human tumors to commonly used DNA damaging treatments. Head and neck squamous cell carcinomas (HNSCC) are malignancies derived from cells within the basal epithelia of the aerodigestive mucosa and are usually treated with platinum-based chemotherapy and radiation (Forastiere et al., 2001). P53 mutations are common in HNSCC, and the role of other p53 family members in this disease continues to be elucidated.

Several years ago, p73 and p63 joined the ranks of the p53 family. Both of these new members give rise to various protein isotypes predicting complex transcriptional activity. In addition to p63 and p73 isotypes capable of transactivating downstream target gene expression (TA isotypes), both genes also expressed dominant negative inhibitory isoforms (ΔN-isotypes). Amplification and overexpression of ΔNp63α was found to be the most common oncogenic event in primary HNSCC (Hibi et al., 2000). Specific amplification of p63 was just confirmed in over half of all squamous cell carcinomas of the lung, supporting its role in all common squamous cell carcinomas (Tonon et al., 2005). Moreover, ΔNp63 isoforms induced proliferation and growth of tumor cells in vitro and in vivo, and led to β-catenin-increased accumulation and signaling (Patturajan et al., 2002). We recently established that p63 is capable of regulating distinct sets of downstream target genes through a unique p63 *cis* element (Osada et al., 2005). One crucial target of ΔNp63α was found to be HSP-70, a stress response protein known to be a key determinant of cell death and cell transformation (Wu et al., 2003). Thus, ΔNp63α has been hypothesized to contribute to tumorigenesis through direct regulation of target genes, inhibition of the transactivation activity of p53 family members, or direct protein-protein interactions in key proliferative pathways.

What happens if ΔNp63α is removed from malignant cells overexpressing this oncogenic protein? After RNAi knockdown, no significant effect on cell viability was observed at early time points, but after 48 hr, a large fraction of cells underwent obvious death accompanied by cleavage of the poly(ADP)-ribosylating enzyme PARP-1. Furthermore, Rocco et al. show in the current study that inhibition of endogenous ΔNp63α by lentivirus RNAi in HNSCC cells induces the proapoptotic bcl-2 family members Puma and Noxa, suggesting that ΔNp63α is required for cell survival (Rocco et al., 2006). Induction of these genes following knockdown of ΔNp63α by RNAi is independent of p53, and instead requires transactivation of p73 isoforms. These data suggest that in most HNSCC tumors, TAp73 is functionally inactivated by high levels of ΔNp63α. In contrast, the rare HNSCC tumors with low ΔNp63α levels apparently bypass the requirement for p63-mediated p73 inhibition through upregulation of bcl-2 expression. They also found that bcl-2 expression rescues cells from death following loss of ΔNp63α and is inversely correlated with ΔNp63α levels in HNSCC cells.

ΔNp63α could conceivably inhibit the activity of p73 by a variety of mechanisms, including a direct association of both proteins or competitive interaction of p63 and p73 with similar *cis* elements in downstream gene target promoters (Rocco et al., 2006). Posttranslational modifications of p73 following DNA damage might also contribute to p73 activation and its promoter selectivity (Strano et al., 2005). This fact might explain differences in proapoptotic genes induced by p73 upon DNA damage versus those they find induced following inhibiton of p63 with RNAi (Strano et al., 2005). Consistent with this report, a prior study found that overexpression of p73 in the absence of DNA damage leads to induction of endogenous Puma mRNA and protein (Melino et al., 2004). The new findings support the notion that endogenous ΔNp63α suppresses the proapoptotic activity of p73 both through its direct association with p73 and through direct repression of p73-dependent transcription.

From the work presented here, it is becoming clear that in addition to the known role of p53, both p63 (ΔNp63α) and p73 are critical mediators of cell death following chemotherapy in HNSCC. ΔNp63α was shown to be dramatically downregulated following DNA damage, and p63 levels correlated with patient response to cisplatin-based treatment (Zangen et al., 2005). This report suggests that p73-mediated cell death following DNA damage may represent the cumulative effect of increased p73 levels in addition to decreased p63-mediated transcriptional inhibition. This new discovery may also shed light on the connection between accumulation of p73 and the loss of ΔNp63α levels necessary for the apoptotic program activation in HNSCC. The data further explain the lack of p73 or PUMA mutations in HNSCC, since p73 is inactivated by ΔNp63α overexpression in most of these tumors.

Head and neck cancers are commonly treated with a combination of DNA damaging agents, including radiation and/or chemotherapy. Although p53 status and response to chemotherapy may be linked, many studies published over the years have failed to show a direct connection between p53 mutational status and favorable patient response to chemotherapy. The new work presented here may in part explain previously observed prognostic correlations involving p63 and bcl-2 (Massion et al., 2003; Zangen et al., 2005; Andrews et al., 2004). For head and neck cancers expressing ΔNp63α, downregulation of ΔNp63α and subsequent activation of p73 may be an important mechanism for a favorable response of patients to treatment (Massion et al., 2003; Zangen et al.,

2005). Thus, tumors that have circumvented the requirement of p63-mediated survival may exhibit resistance to common cancer treatments (Rocco et al., 2006).

In contrast, upregulation of bcl-2 through alternative mechanisms may identify tumors that are resistant to the proapoptotic effect of these treatment modalities regardless of p53 family member status. Specific bcl-2 inhibitors show promise as cancer therapeutics in lung and other cancers. Other agents hold promise for increasing wild-type p53 levels, thus abrogating the p63 survival function. It would be interesting to explore whether novel biologic treatments that abrogate EGFR signaling in combination with DNA damaging agents can also overcome this resistance. A recent study showed that the addition of an EGFR antibody to local radiation therapy and platinum treatment markedly improved patient survival. Understanding the status of all p53 family members in HNSCC is crucial for developing more individualized combinations with standard DNA damaging agents and newer molecular therapies.

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Is Cyclin D1-CDK4 kinase a bona fide cancer target?

Previous studies have demonstrated that mice lacking Cyclin D1 were refractory to mammary tumor development induced by the c-*neu*/*erbB-2* oncogene, the rodent ortholog of the HER-2 receptor frequently overexpressed in human breast carcinomas. Two new studies in this issue of *Cancer Cell* provide additional evidence on this issue. Knockin mice expressing a mutant form of Cyclin D1 that binds to Cdk4/6 but cannot activate their catalytic activity are resistant to c-*neu*/*erbB-2* tumorigenesis in spite of undergoing normal epithelial cell expansion during pregnancy. Moreover, knockdown of Cdk4 in mammary tumor cells abrogates tumor formation. These observations provide new compelling evidence that inhibition of Cyclin D1-Cdk4/6 kinases might be beneficial for cancer therapy.

In 2001, a seminal paper by Sicinski and coworkers (Yu et al., 2001) reported that mice lacking Cyclin D1 were refractory to tumorigenesis induced by MMTV-driven Ha-*ras* and c-*neu*/*erbB-2* oncogenes. c-*neu*/*erbB-2* is the rodent ortholog of the human *HER-2* receptor gene frequently overexpressed in human breast carcinomas. Indeed, HER-2 is one of the few oncogenes already targeted in the clinic by means of specific monoclonal antibodies (reviewed in Hynes and Lane, 2005).

Earlier studies by Sicinski et al. (1995), then in the Weinberg laboratory, and by Fantl et al. (1995) in the Dickson laboratory had described that ablation of *cyclin D1* in the germline prevented the breast epithelial compartment of adult female mice to undergo the massive proliferative changes associated with pregnancy despite normal levels of ovarian steroid hormones. In their 2001 study, Yu et al. (2001) concluded that c-*neu*/*erbB-2* (and the Ha-*ras* oncogene) induced tumorigenesis by activating the *cyclin D1* promoter. Hence, ablation of Cyclin D1 expression prevented oncogenic signaling (Figure 1). In contrast, other oncogenes, such as c-*myc* and *wnt*-1, that could activate expression of other effectors, such as the related *cyclin D2*, efficiently induced mammary tumors in these Cyclin D1-defective mice (Yu et al., 2001). Intriguingly, c-*neu*/*erbB-2* and Ha-*ras* oncogenes induced other types of tumors (mainly salivary gland tumors) and efficiently transformed *cyclin D1* null fibroblasts in culture (Yu et al., 2001), indicating that the selective role of Cyclin D1 in mediating c-*neu*/*erbB-2* and Ha-*ras* oncogenesis is unique to mammary epithelial cells (Figure 1).

Cyclin D1 is not an obvious druggable target. Yet, one of the main biological activities of Cyclin D1 involves activation of its partners Cdk4 and Cdk6, two kinases whose catalytic activity is absolutely dependent upon binding of any of the D-type Cyclins (reviewed in Malumbres