as the controversy over the requirement for a defective p53 pathway to support the replication selectivity of ONYX-015—stimulated the development of numerous additional oncolytic virus platforms. The controversy surrounding the inactivated-p53 requirement for ONYX-015 replication has been somewhat resolved through studies demonstrating that a subset of tumor cells contain a wild-type p53 gene, yet are functionally p53 inactive, due to inactivation of the "upstream" $p14^{arf}$, resulting in the accumulation of mdm2, which binds p53 and targets it for degradation, much in the same way as does the adenovirus 55K protein (McCormick, 2000; Ries et al., 2000). While improved conditionally replicative adenoviruses like ONYX-411 and others (Li et al., 2001; Ramachandra et al., 2001) have been developed, the question still remains whether oncolytic agents developed from other virus genera will be more efficacious for local-regional or systemic applications. Table 1 summarizes some other viruses in development. The properties of these viruses in terms of the expression of their cognate receptors on various tumor cell types, reproductive cycle time, yield, cytotoxicity, and selectivity cover a broad spectrum. Importantly, the issue of virus-specific immune responses will need to be addressed in order to repeatedly administer any of these viruses intravenously. Another important issue related to some of these viruses may be their possible safety risk; in several instances, viruses are being used without genetic modification—such as deleting critical virus regulatory genes—suggesting the possibility of in vivo selection of virulent phenotypes. For these or any oncolytic viruses to eventually gain FDA approval and become the standard of care will require continued refinement and testing, both in preclinical models and in safety and efficacy trials in humans. Nevertheless, the complete makeover that ONYX-015 has received in the design and development of ONYX-411 would appear to be a step in the right direction toward oncolytic viruses becoming useful therapies for the treatment of selected cancers.

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Senescence: a companion in chemotherapy?

Mouse lymphoma model points to an unsuspected role of drug-induced "senescence."

For decades we have been attempting to understand the basis of drug resistance manifested by so many cancers. Even tumors never previously exposed to anticancer agents often already show intrinsic drug resistance, suggesting that gene mutations driving tumor development do not automatically confer resistance to anticancer agents. In two recent papers, Lowe and collaborators (Schmitt et al., 2002a, 2002b) convincingly show that in a lymphoid mouse tumor model, drug resistance can be conferred not only by lesions in the apoptotic pathway but also, surprisingly, by mutations in the senescence pathway. The realization that an intact senescence pathway can contribute to the success of chemotherapy may have profound consequences for the treatment of cancer patients.

Several mechanisms can contribute to drug resistance. Cytotoxic drugs, at the moment still the backbone for treatment of disseminated cancer, almost invariably damage DNA or interfere with DNA replication or chromosomal segregation. One way cells can prevent killing by anticancer drugs is to expel the drugs from the cell by transporters. Even low amounts of these transporters substantially decrease the sensitivity of cells to cytotoxic drugs (Allen et al., 2000, and references therein). However, this does not explain why tumors often are inherently resistant to anticancer agents. One has to assume that this tolerance arises as a side effect of the genetic alterations that drive the tumorigenic process. Indeed, mutations interfering with apoptosis can contribute both to tumor growth and confer resistance to chemotherapy in a mouse lymphoma model (Schmitt et al., 1999). However, clinical practice teaches that tumors with clear defects in the apoptotic machinery are not neces-

sarily more resistant to anticancer drugs, and therefore, skepticism about the contribution of mutations in the apoptotic pathways to chemotherapy resistance is justified (Borst et al., 2001; Brown and Wouters, 1999). This skepticism was also fostered by observations from clonogenic assays, which showed that defects in the apoptotic pathway did not necessarily reduce the number of surviving cell clones in response to treatment even though short-term cell survival was markedly reduced (Brown and Wouters, 1999; Schmitt and Lowe, 2002). Therefore, cells saved from early apoptosis would subsequently die anyway as a consequence of mitotic catastrophe. Finally, there is always the question whether drug resistance observed in a murine lymphoma model has any bearing on tolerance mechanisms in other cell types and, even more important, in other species. The two recent papers by Lowe's group (Schmitt et al., 2002a, 2002b) not only highlight the role of apoptosis in preventing Eμ-Myc-induced lymphomagenesis, they also reveal an unsuspected role of the senescence pathway in the resistance to the DNA methylating drug cyclophosphamide (CTX). An intact senescence pathway appears pivotal for the efficacy of CTX, and its disruption makes tumor cells highly refractory to the drug.

The Lowe group utilized the well-established Eμ-Myc tumor model to study response to chemotherapy (Schmitt et al., 1999). In this model, loss- or gain-of-function mutations that block apoptosis (p53 loss, Bcl2 overexpression) cause lymphoma acceleration. p53 deficiency shows the most potent synergy with the Eμ-Myc transgene. Bcl2 overexpression in Eμ-Myc;$p53^{-/-}$ cells confers virtually no additional advantage to the tumor cells, and such advantage is even fully absent when a construct encoding a dominant-negative caspase-9 is introduced, indicating that loss of p53 does it all. Interestingly, while tumors arising in Eμ-Myc;$p53^{+/-}$ compound mice invariably lose the remaining functional p53 allele, this does not occur when Bcl2 is simultaneously overexpressed, illustrating that blocking of the apoptotic pathway represents the primary driving force for loss of the remaining wt p53 allele in these lymphomas (Schmitt et al., 2002a). The Bcl2-overexpressing tumors nevertheless differ from the tumors with disrupted p53 in that the former remain pseudo-diploid, whereas the latter are aneuploid. However, this aneuploidy does not appear to add to the growth and metastatic potential of the lymphomas, arguing that the genetic instability seen in these Eμ-Myc;$p53^{-/-}$ tumors is not an important factor contributing to tumor progression.

When Eμ-Myc overexpression was combined with disruption of both the $p19^{Arf}$ and $p16^{Ink4a}$ genes, tumors developed after a short latency as previously reported (Schmitt et al., 1999). Inactivation of $p19^{Arf}$ but not $p16^{Ink4a}$ is responsible for the synergy with Eμ-Myc in accelerating lymphomagenesis (Eischen et al., 1999). This situation differs from that in other tumor models in which $p16^{Ink4a}$ loss does contribute to tumor development (Krimpenfort et al., 2001; Randle et al., 2001; Sharpless et al., 2001). $p19^{Arf}$ loss likely exerts its oncogenic effect in this model by preventing induction of p53 as a result of aberrant oncogene expression. It is worth noting that this tumor-suppressor activity of $p19^{Arf}$ is not seen in an epithelial tumor model where $p19^{Arf}$ loss fails to abrogate oncogene-induced p53-dependent apoptosis (Tolbert et al., 2002). Overexpression of Bcl2 in Eμ-Myc;$p19^{Arf-/-}$ lymphoma cells augments tumorigenesis and leads to comparable tumor characteristics with respect to latency and malignancy as observed for Eμ-Myc;$p53^{-/-}$ cells. A similar effect of Bcl2 is seen in Eμ-Myc;$p16^{Ink4a-/-}$;$p19^{Arf-/-}$ cells.

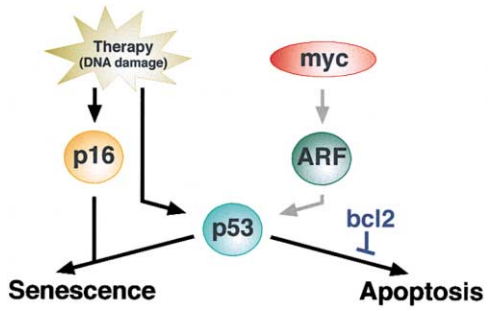

Figure 1. Cellular senescence and cancer therapy

Model of therapy-induced senescence controlled by $p16^{INK4a}$ and p53. Cyclophosphamide causes DNA damage, which activates p53 through a $p19^{ARF}$-independent mechanism. Block of p53-dependent and -independent apoptotic pathways by Bcl2 uncovers senescence as a drug-induced response program. Senescence is disrupted in the context of p53 or $p16^{Ink4a}$/$p19^{Arf}$ loss, whereas $p19^{Arf}$ deficiency alone is not sufficient to disable senescence. $p16^{INK4a}$, like p53, can be induced by DNA-damaging treatment and probably cooperates in a common arrest program (figure and legend from Schmitt et al., 2002b).

The surprise came when the various mutants were treated with CTX. As shown by elegant in vivo imaging, Eμ-Myc tumors rapidly regressed and the majority of the mice survived long term. By contrast, both Eμ-Myc;Bcl2 and Eμ-Myc;$p53^{-/-}$ tumors invariably progressed, albeit with distinct initial kinetics. As might have been predicted, Eμ-Myc;$p19^{Arf-/-}$ tumors responded equally well to CTX as the Eμ-Myc tumors. Most interestingly, however, Eμ-Myc;$p16^{Ink4a-/-}$;$p19^{Arf-/-}$ tumors appeared highly refractory to therapy and progressed rapidly, albeit somewhat slower than Eμ-Myc;$p53^{-/-}$ tumors. A retrovirally introduced Bcl2 gene had no impact on the overall survival of the p53 null group, whereas it accelerated tumor formation in the p16/p19 null group up to the level of the p53 null cohort. This indicates that $p16^{Ink4a}$, which itself does not influence tumor initiation and progression of B lymphomas, is a key factor in the response to CTX of this tumor. In accordance with this notion, $p16^{Ink4a}$ is upregulated in CTX-treated Eμ-Myc;Bcl2;$p19^{Arf-/-}$ tumors with the concomitant appearance of "markers of senescence." Utilizing a set of elegant genetic approaches (LOH of the common exon 2 of the $p19^{Arf}$/$p16^{Ink4a}$ locus; specific inactivation of $p16^{Ink4a}$ by antisense) Lowe and coworkers went on to show that $p16^{Ink4a}$ is responsible for this CTX resistance. Furthermore, $p16^{Ink4a}$ requires functional p53 for this in vivo senescence induction as was previously observed in mouse fibroblasts in culture.

These analyses show that, in this lymphoma model, at least two responses contribute to the cytotoxic therapy: the apoptotic pathway and the senescence pathway (Figure 1). The contribution of the latter is substantial and emphasizes the need to identify targets of p16 and/or p53 that impinge on the senescence response. These targets might well be known components of the cell cycle machinery, such as p21 and pRb, but their specific contribution to senescence, apoptosis, and mitotic catastrophe in response to different cytotoxic drugs

clearly needs to be further defined (Bunz et al., 1998; Wouters et al., 1999).

The current studies also provide a framework for why mutations only affecting the apoptotic pathway do not necessarily correlate with a poor prognosis. These mutations might have prevented the occurrence of other lesions, such as in p53, which are in fact more harmful because they could mitigate the efficacy of anticancer agents. The observations reported in these two papers (Schmitt et al., 2002a, 2002b) also argue that the genomic instability that is concurrent with p53 deficiency in this system does not significantly contribute to tumor initiation and progression but rather is an accidental side effect that nevertheless may have major consequences for the efficacy of subsequent treatment.

The obvious question that remains is how senescence plays out against apoptosis and mitotic catastrophe in the eradication of various tumors by anticancer agents. The CTX-induced senescence was most pronounced in the absence of apoptosis. Hence, the relative role of senescence in drug treatment sensitivity likely depends on the cell type, the genetic lesions that drove tumor development, and last but not least, the anticancer agent itself. Studies in other systems support this (Bunz, 1999). Therefore, we must understand the interplay between these lesions and their impact on the various resistance mechanisms. Only then can we offer patients—after appropriate molecular characterization of the tumor—the tailored treatment that takes into account tumor-specific resistance mechanisms similar to those described by Lowe and collaborators. Induction of senescence offers at least an appealing new option to include as a strategy for intervention.

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p53 leans on its siblings

Despite the common assumption that p53 by itself can induce apoptosis, results of a recent study implicate the homologous genes p63 and p73 in p53-mediated programmed cell death.

The p53 tumor-suppressor protein, first identified in 1979, acts as a major node in a complex network evolved to sense diverse cellular stresses including DNA damage and hyperproliferative signals (Ko and Prives, 1996). Once stabilized and activated by genotoxic stress, p53 can either activate or repress a wide array of different gene targets, which in turn can regulate cell cycle, cell death, DNA repair, angiogenesis, and other outcomes. p53 functions, including apoptosis, are thought to require its sequence-specific DNA binding and transcriptional activation activities, and a number of apoptosis-related genes are induced by p53 activation (Johnstone et al., 2002). Consequently, p53 is reported to be functionally inactivated in more than half of all human tumors, and murine models have confirmed p53's central role in tumorigenesis. Furthermore, the majority of tumor-associated p53 mutations occur in the core DNA binding domain and prevent interaction with target sequences. The cloning in the late 1990s of two p53-related genes, p63 and p73, caused a great excitement within the cancer biology community with the prospect of two additional tumor suppressors (Yang and McKeon, 2000). Initially the family resemblance was striking. p63 and p73 share with p53 an amino-terminal trans-