es any additive toxicity from F16, consistent with a requirement for mitochondrial accumulation of the compound. Dose-response measurements at low concentrations of F16 (100 nM) should demonstrate concordance between the direct mitochondrial effects of F16 (uncoupling, increased O_2 consumption) and cell toxicity. Structure-activity relationships may demonstrate that cytotoxicity of a series of related compounds is correlated with relative mitochondrial uptake. Despite strong correlative evidence, nonmitochondrial sites of action can emerge to complicate conclusions about mechanism. MKT-077, a rhodacyanine DLC dye entered into Phase I clinical trials, has since been reported to have direct effects on additional targets, including telomerase inhibition, crosslinking of F actin, and reactivation of wild-type p53 via binding the hsp70 family member, mot-2 (mortalin) (Maruta et al., 1999; Naasani et al., 1999; Wadhwa et al., 2000).

The central importance of mitochondria in apoptotic cell death pathways offers up several molecular targets in mitochondria that are highly expressed in cancer cells. Foremost among these are the Bcl-2-related survival proteins, Bcl-2 and Bcl-x_L. There are now several examples of small molecules that bind to the hydrophobic groove of these proteins and either displace proapoptotic family members (Bax, Bak) from a heterodimeric complex or alter membrane topology of the survival proteins (Degterev et al., 2001; Tzung et al., 2001). Although the report by Fantin et al. (2002) does not examine the effect of Bcl-2 expression on F16 cytotoxicity, a separate group compared the ability of Bcl-2 to rescue L929 cells treated with neutral hydrophilic, anionic lipophilic, and cationic lipophilic photosensitizers (Klein et al., 2001). Overexpression of Bcl-2 sensitized L929 cells to the cationic drug, Victoria Blue BO, even in the dark, associated with increased mitochondrial uptake. Since the proposed functions of the Bcl-2 antiapoptotic proteins include regulation of mitochondrial membrane permeability, compounds with selective accumulation in mitochondria, including F16, may represent another approach to eradicate cancer cells expressing high levels of Bcl-2/Bcl-x_L and resistant to standard chemotherapy.

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Functions of p53 suppress critical consequences of damage and repair in the initiation of cancer

A pivotal study reveals a long-sought-after mechanism for gene amplification and provides important implications for oncogenesis.

One of the most frequent and mystifying types of abnormalities observed in human carcinomas is the amplification of large genomic regions associated with complex chromosomal rearrangements (complicons). These abnormalities are believed to be the functional basis for tumor progression and drug resistance both in vivo and in vitro (Kuehl and Bergsagel, 2002; Federspiel et al., 1984). In a recent publication by Zhu et al., the molecular dissection of these complicated structures in the experimental generation of lymphomas has illuminated a molecular mechanism of gene amplification and several factors that can modulate this process. As the result of a long series of fundamental observations, culminating in a recent elegant study (Zhu et al., 2002), the Alt laboratory has identified several of the major determinants that generate the intermediates in this process and has been able to propose a mechanism. They show that, in a setting where V(D)J recombination is initiated, deficiencies in both p53 function and nonhomologous end-joining abilities conspire to generate complex genomic rearrangements. In this system, the chromosomal rearrangement is initiated by RAG (recombination-activating genes)-mediated recombination that catalyzes a

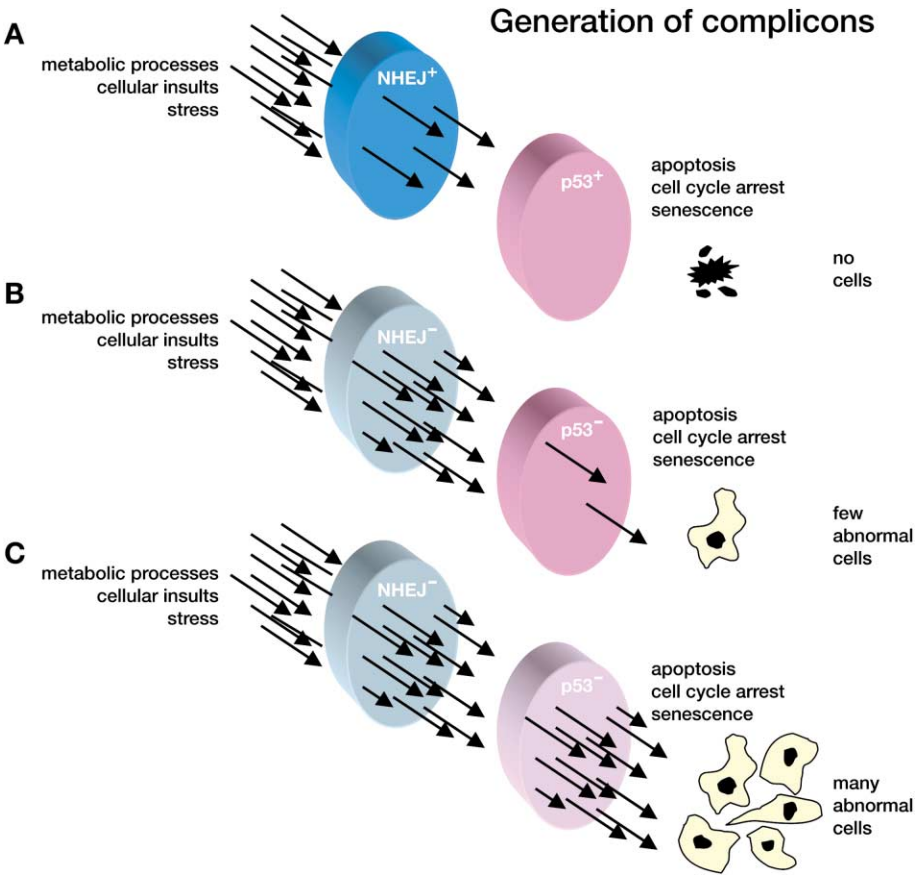

Figure 1. Generation of complicons

In a properly functioning cell (**A**), the majority of continually produced DNA damage is repaired. The few lesions that avoid repair activate functions of p53 that cause these cells to be cleared from the population. Inadequate removal of damaged DNA can overwhelm the second line of defense (p53), and rare cells with rearrangements survive (**B**). Finally, in cells that have lost both NHEJ and p53 functions (**C**), abnormal cells are readily produced and are no longer cleared from the population. These cells continue to proliferate and acquire the genomic changes that allow for malignant outgrowth.

DNA double-strand break. The inability to repair this break (NHEJ dysfunction) and the inability to eliminate the damage-containing cell (p53 dysfunction) allows the cleaved chromosomes to associate into dicentric intermediates that, through subsequent bridge-fusion-breakage events in the surviving cell, generates the amplified and rearranged units. The chromosomal intermediates in this process have been documented using contemporary cytogenetic advances and provide support for the predicted mechanism. Although V(D)J recombination is a specialized process and the only site-directed recombination process known in vertebrates, it holds lessons for a much broader appreciation of the factors that influence cancer initiation.

The astounding generation of multiple copies of genomic DNA (i.e., gene amplification) in tumor cells was first described in the 1970s. Early chromosome analysis by Biedler identified unique and unusual chromosomal abnormalities (double-minute chromosomes and homogeneously staining regions) that were associated with drug resistance in human tumor cells (Biedler and Spengler, 1976). Rigorous, groundbreaking studies by Alt and coworkers in the Schimke laboratory identified the molecular basis of drug resistance and the accompanying chromosomal abnormalities as amplification of genomic sequences (Alt et al., 1978). Over the last 25 years, many laboratory groups have sought understanding of this process for several reasons. The first and most direct reason is because the process of gene amplification can circumvent drug efficacy during chemotherapy. A molecular understanding of the process should allow us to prevent or reduce the generation of drug resistance. This is true for standard chemotherapies that have been used for decades, as well as the new "targeted" drugs that have been designed to abrogate the function of specific mutated enzymes. STI-571 is a prime example of this, since amplification of the rearranged BCR-ABL locus contributes to drug resistance and their eventual therapeutic failure (Gorre et al., 2001).

However, while elucidation of the generation of amplified loci and complicons is important in its own right, it also holds critical significance in the greater context of tumorigenesis. Thus, a second and equally important reason to study these processes is to gain understanding of how a normal, nontransformed cell loses genomic integrity. It has long been appreciated that normal somatic mammalian cells rarely, if ever, amplify genomic sequences (Tlsty, 1990; Wright et al., 1990), yet this event occurs readily in transformed cells. The acquisition of drug resistance (via gene amplification) and tumorigenic progression in transformed cells share several critical traits that suggest that understanding the former will contribute to controlling the latter. Both drug resistance and tumorigenesis are multistep processes whose products are believed to emerge from step-wise selection, both depend on functional consequences of identical chromosomal abnormalities, and both demonstrate dynamic instability and heterogeneity in their phenotype. Finally, both drug resistance and tumorigenesis are modulated by the same agents, and both can be enhanced by viruses and exposure to carcinogenic agents. The postulated mechanism of complicon formation in Zhu et al. provides the potential to integrate these diverse observations into a unified vision.

The initiating event in complicon formation is a DNA double-strand break. As indicated in Figure 1, there are many processes that occur within the cell as a consequence of normal metabolism that can generate this initiating event. Additionally, there is a plethora of exogenous agents (insults and stresses) that also generate DNA lesions and subsequent DNA breaks. Hence, in addition to carcinogen exposure, physiological processes as diverse as inflammation,

aging, and hypoxia can all potentially generate a multitude of initiating events. Each cell deals with the consequences of these processes every day.

The first line of defense against these assaults is the prevention or removal of the DNA double-strand breaks. As demonstrated by the Zhu study, the RAG-induced breaks invoke the action of a series of repair enzymes (Ku70, Ku80, DNA-PKC) that have a dual function in site-directed recombination and repair of double-strand breaks. These same proteins have more recently been reported to play a critical role in the capping of mammalian telomeric structures (Bailey et al., 1999; Goytisolo et al., 2001). Thus, critically short or uncapped telomeres rely on the same reparative proteins as the radiation-induced breaks and the free radical-induced scissions. Deficiencies in these protein functions probably contribute to the generation of complicon intermediates by failure to repair recombinogenic chromosome ends. When these proteins are inactivated, chromosomal rearrangements are generated, but in the presence of functional p53, these cells are cleared from the population (Figure 1B).

It is through its function in surveillance of genomic integrity and chromosomal structure that the p53 protein acts as a second line of defense against the initiating breaks. In the Zhu study, p53 function is removed via the knockout of p53 genes, but in other tumors, a variety of mechanisms exist to accomplish the same purposes. These functions can be permanently eliminated by a variety of means that include (but are not confined to) mutation of the gene, expression of viral oncoproteins that bind and inactivate the protein, or altered p53 metabolism leading to increased proteolysis or sequestration in an improper cellular compartment. In addition to these more standard inactivation mechanisms, p53 can be transiently inactivated by alterations in epithelial cell adhesion (Nigro et al., 1997) or Sirα2-mediated negative control of p53 function during stress (Luo et al., 2001). Without proper p53 function, cells with DNA lesions fail to arrest cell cycle progression, fail to senesce, and fail to activate apoptotic pathways. Other collaborators of p53, such as p63 and p73, are instrumental in executing the apoptotic response (Flores et al., 2002). These cells, deficient in p53 function and gravid with the products of improperly repaired intermediates, now have the ability to generate a productive set of genomic rearrangements that can provide selective advantage and oncogenic potential to their progeny (Figure 1C).

In the model system described by Zhu et al., three specific events were necessary to generate amplified DNA sequences with accompanying rearrangements. These specific events identify three general classes of alterations that can contribute to the formation of these structures in epithelial-derived tumors that comprise the majority of malignancies in humans. Other processes endogenous or exogenous to the cell (i.e., spontaneous hydrolysis of DNA or radiation-induced breaks) can also generate DNA strand breaks similar to those catalyzed by RAG. Deficiencies in Ku70/80 function can be mimicked by mutations in most of the other proteins that contribute to the formation of the complex protein machinery that repairs DNA lesions and maintains genomic integrity. Finally, abrogation of specific p53 functions can occur through mutation of the gene itself or alterations in its critical downstream effectors. These new insights into the events that generate amplified and rearranged DNA sequences provide a mechanistic appreciation of cancer initiation and progression independent of the RAG model system.

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