From genetics to chemistry: tumor suppressor genes and drug discovery

An increasing understanding of how the protein products of tumor suppressor genes regulate the cell cycle offers new opportunities for the development of selective anti-cancer agents.

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Over the past few years, two major areas of cancer cell biology have converged: the study of tumor suppressor genes, which had its origin in the molecular genetics of human cancer and in DNA tumor virus biology, is now central to the study of the cell cycle [1,2]. The greater biological understanding that resulted in this convergence has major implications for drug discovery, specifically in the design of novel small molecules as new classes of selective anti-cancer agents.

The biological importance of tumor suppressors in the genesis of cancer has been fully appreciated for many years [3]. Several hereditary forms of human cancer, such as retinoblastoma and Li–Fraumeni syndrome, are caused by transmission of defective tumor suppressor genes (the *Rb* and *p53* genes, respectively). Equally important, DNA tumor viruses bring about oncogenic transformation by inactivating products of the same genes (Rb and p53 proteins). The recent experimental demonstration that restoration of tumor suppressor function to a tumor cell results in growth arrest or cell suicide (apoptosis) [2] confirms the importance of these genes in cancer biology, and makes it clear that therapeutic approaches that could restore tumor suppressor function would have tremendous clinical value.

The simplest way to achieve this therapeutic goal would be to re-introduce a wild-type version of the defective gene, using a viral vector or another means of gene delivery. From a practical perspective, this approach faces severe technical hurdles that currently restrict its value. It would be preferable to be able to use a small molecule drug to restore the function of a missing tumor suppressor protein. To do this, we need to understand precisely which cellular pathways are altered by the loss of tumor suppressor proteins so that we may intervene specifically in these pathways and restore them to a normal state. Alternatively, we may be able to take advantage of the abnormal physiology of a tumor cell in such a way as to provoke its death. Both approaches require the

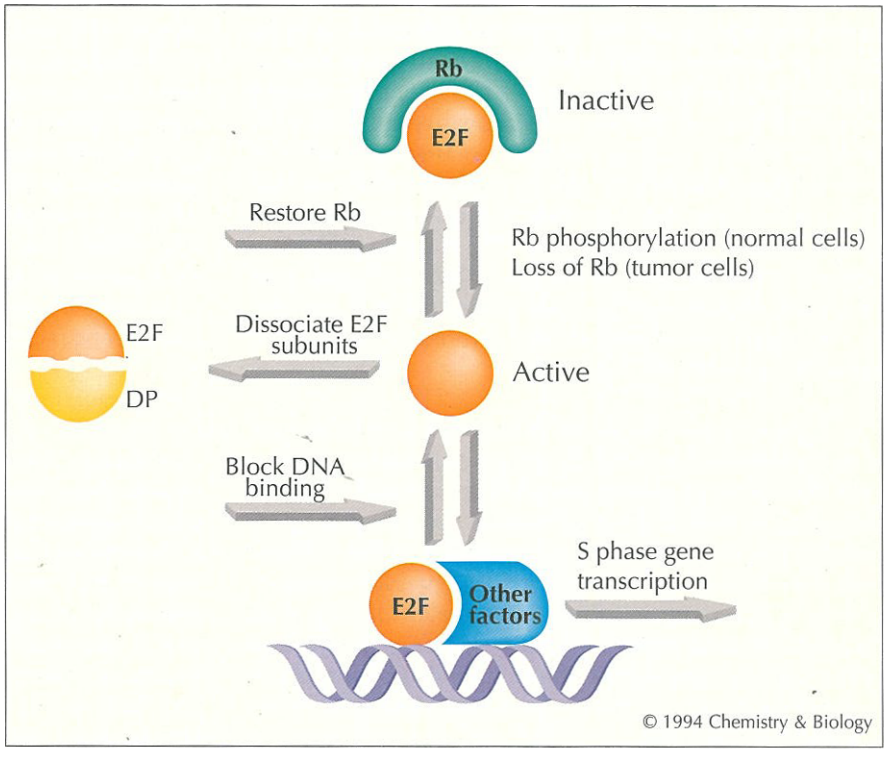

Fig. 1. Different approaches to inhibiting E2F activity. E2F is a transcription factor that activates the expression of a number of S phase genes. Normally E2F is negatively regulated by Rb, and thus loss of the Rb protein leads to E2F activation, which may contribute to uncontrolled cell growth. The figure shows a number of ways to block E2F function: restore Rb protein function, disrupt dimerization of the two subunits of E2F or prevent the interaction of E2F with its target DNA sequences.

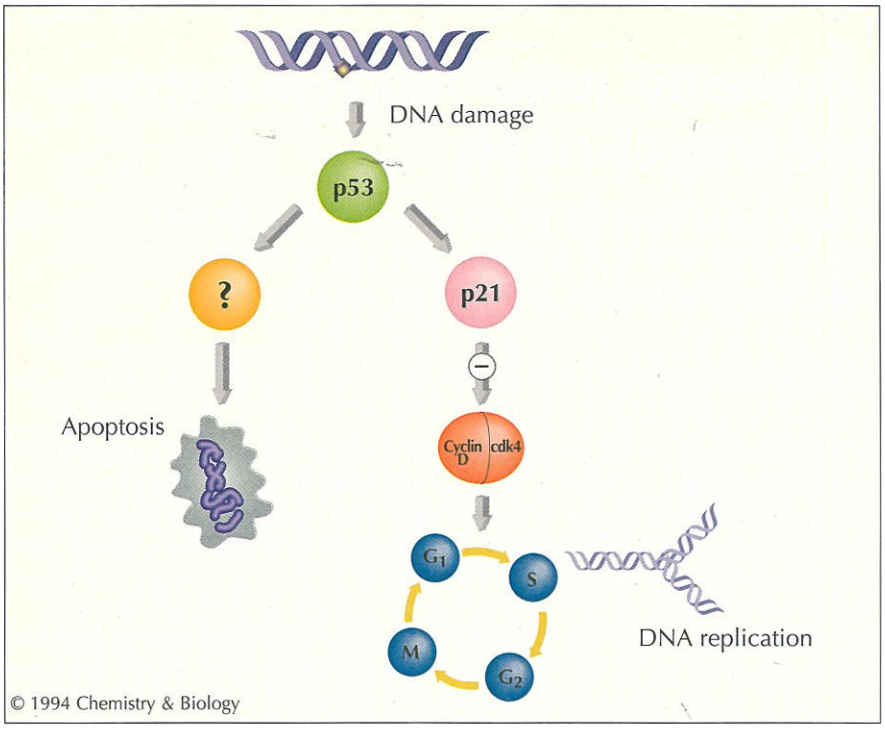

Fig. 2. p53 delays the cell cycle to allow an opportunity for DNA repair. Replicating cells normally respond to DNA damage by activating the *p53* tumor suppressor gene, which, in turn, activates transcription of *p21*. The p21 protein causes cell cycle arrest by inhibiting the cyclin-D/cdk-4 complex, which is required for the G1 to S transition. Arresting the cell cycle at this point allows the cell to repair the damage before DNA replication occurs in S phase. If DNA repair fails to happen in time, then the cell undergoes apoptosis and dies. In cells that lack p53, neither pathway is activated, and the cell therefore continues to divide, accumulating DNA mutations. One way to restore a part of the control normally exerted by p53 is to inhibit cyclin-D/cdk-4 directly, using specific cdk inhibitors.

identification of key targets for intervention, and the application of chemistry to develop and optimize small molecule inhibitors of these targets.

The Rb paradigm

The *Rb* gene was the first *bona fide* tumor suppressor identified in human cancers. Many human cancers contain mutations in *Rb* genes that result in loss of the Rb protein. Genetic analysis of these cancers reveals that loss of Rb plays a direct causal role in the development of cancer [3]. Restoration of Rb expression (by ectopic expression from gene vectors) causes growth arrest [4,5]. A major function of the Rb protein is to act as a negative regulator of the transcription factor family referred to as E2F. Loss of Rb leads to activation of E2F [6,7] which, in turn, may contribute to uncontrolled cell growth [8]. This leads to the attractive hypothesis that Rb is selectively lost in human tumors mainly because its loss leads to the activation of E2F. If the hypothesis is correct, it follows that inhibition of E2F in tumor cells by a small molecule drug could render the cells normal, by this criterion at least, so that treated cells resemble those that contain the Rb protein.

This paradigm is, of course, an over-simplification, and the actual effects that an anti-E2F drug would have on cancer cells remain to be determined. Efforts are under way to anticipate these effects using various experimental procedures. For example, it may be possible to make a 'dominant interfering' mutant of E2F that would interrupt normal E2F activity and thus mimic the effects of a small molecule. Mutants of this type are typically inactive versions of the authentic target (E2F in this case) that bind to regulators of the target to generate dead-end complexes, blocking the normal target's action. Other approaches might include production of genetically engineered animals in which E2F activity is either reduced or missing altogether, so that the effects of the putative drug can be anticipated.

An anti-E2F drug could, in principle, act at several levels (Fig. 1). E2F is a heterodimer of two subunits, an E2F component and a DP component, and it is therefore possible that E2F could be inactivated by dissociation of the subunits. Precedents exist for small molecules that disrupt protein:protein interactions; the estrogen antagonist ICI164384 inhibits dimerization of the androgen receptor [9], and considerable effort is being focused on small molecule inhibitors of a number of protein:protein interactions involved in signal transduction (for a discussion of inhibitors of SH2:phosphotyrosine interactions, see [10]). Alternatively, E2F inhibitors might prevent the interaction of E2F with its target DNA sequences, or prevent essential post-translational modifications to the E2F protein. Such modifications have yet to be discovered, however. Any one of these approaches presents a significant challenge to the chemist, but each of them seems to be technically feasible.

This paradigm as applied to the Rb protein, namely that the function of a tumor suppressor can be restored by elucidating down-stream events and thus identifying targets for drug intervention, can be applied to other tumor suppressor genes such as *p53*. This gene is mutated to a defective form in more than 50 % of human cancers. The mutant p53 protein fails to activate transcription of a number of genes. The product of one of these, referred to as p21 (also known as WAF-1 or sip1), is a protein that causes cell cycle arrest by inhibiting cyclin-dependent protein kinases [11–13]. Under conditions in which cell DNA is damaged (such as after exposure to radiation or

to DNA-damaging drugs), p53 induces the production of p21, and the cells stop dividing (Fig. 2). This gives the affected cell an opportunity to fix the DNA damage. If the damage cannot be fixed, cell suicide may occur [14]. In tumor cells that express the mutant p53 protein, the production of p21 protein cannot be induced. Thus the mutant cells can neither protect themselves from further damage, nor be induced to commit suicide when the correction mechanism has failed.

How could one intervene with drugs to correct these biological effects? The most direct approach would be to convert the mutant p53 protein to a wild-type conformation. Although the technical feasibility of this approach is unproven, recent evidence suggests that mutant proteins can indeed adopt a wild-type conformation *in vitro* under certain conditions of high salt and low temperature [15]. Thus, although one could hardly expose a cancer patient to such conditions, it may be possible to find some other means to force the mutant protein to function as if it were the wild-type one. Alternatively, one could attempt to find a method of turning on p21 expression that does not depend on wild-type p53, or to mimic the action of p21 in inhibiting the cyclin-D/cdk-4 (or cdk-6) complex. This latter approach is more familiar to the medicinal chemist, as the target could be an enzyme inhibitor rather than a molecule to disrupt protein:protein interactions. Crystal structures of cdk-like proteins have been solved [16], which could help with the identification of lead compounds using a number of different approaches to structure-based drug design. Structural information may also be important in the later stages of lead optimization. The ideal inhibitor would affect the cyclin-dependent kinase complex of a tumor cell without affecting similar complexes in normal cells. Whether sufficient differences between these complexes exist remains to be seen. However, the principle is clear: as more information accumulates regarding the critical pathways that p53 regulates, the probability of identifying targets for drug discovery and development increases.

The approach described above deals with only one of the actions of p53, the cell cycle arrest induced by DNA damage. A second aspect of p53 function may prove even more promising for therapeutic intervention: this is its role in promoting apoptosis. Tumor cells have suicidal tendencies (inappropriate expression of E2F may contribute to these tendencies); loss of p53 suppresses these tendencies and (presumably) allows sustained malignant growth. The pathways that p53 uses to regulate apoptosis are not yet known. However, a number of novel p53-binding proteins have been identified recently, using the yeast two-hybrid system, among other methods. Like the known signalling pathways (such as the Ras pathway, and the JAK/STAT pathway), the p53 pathway will presumably involve enzymes as well as specific interactions between proteins, and both of these types of components may become novel targets for drug intervention when their precise roles are known.

The improved understanding of signalling pathways has resulted in an explosion of new mechanistic targets. This improved understanding now needs to be effectively coupled with the major advances in drug development. Chief among these advances are the tremendous expansion in numbers of synthetic compounds available, made possible by combinatorial chemistry and structure-based drug design approaches. Together with the increasingly efficient screening made possible by robotics/automation procedures, these technologies promise to maximize the synergies between molecular biology and chemistry.

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