Exploiting the p53 Pathway for the Diagnosis
and Therapy of Human Cancer

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After 26 years of research and the publication of 38,000 papers, our knowledge of the p53 human tumor suppressor protein (p53) is still incomplete, and internationally, we still have neither effective p53-based therapies nor diagnostics in approved clinical use except in the People’s Republic of China. The reasons for this are the finding that more than half of all human tumors have lost normal p53 gene function by virtue of mutation and the growing understanding of the critical function of p53 as a tumor suppressor (Greenblatt et al. 1994; Vogelstein et al. 2000). In this paper, I discuss the attempts that we and others have made to exploit our growing understanding of the p53 system for the diagnosis and therapy of human cancer. This has been an intense effort, and to date its success is very limited. However, the challenges faced in this task reflect those of translational medicine in general, and the intensity and focus of the p53 effort make it a particularly instructive case history. The considerations in this paper reflect not only on the scientific issues, but also on some of the organizational social and political points raised in Clifton Leaf’s article in Fortune magazine on why we are losing the war against cancer (Leaf 2004). If we calculate that the scientific funding required to produce the average paper is $100,000 (I suspect quite a conservative estimate), then academic p53 research has consumed a paper is $100,000 (I suspect quite a conservative estimate), then academic p53 research has consumed

Since its original description in 1979 (Lane and Crawford 1979), the p53 protein and the gene that encodes it have been the subject of intense study; over 38,000 papers are identified in Pub Med using p53 as the search term. The p53 gene encodes a 393-amino acid protein whose complete crystal structure has not yet been determined. However, we do have crystal (Cho et al. 1994) and NMR-based (Canadyllis et al. 2006) structural determinations of the core DNA-binding domain, the amino-terminal Mdm2-binding protein region (Kussie et al. 1996), and the oligomerization domain (Jeffrey et al. 1995; Miller et al. 1996). A large collection of monoclonal antibodies to p53 has been produced and epitope-mapped (Stephen et al. 1996). The p53 pathway in animal models dramatically regulates the cellular response to ionizing radiation and chemotherapeutic drugs. The ability to translate this knowledge to patient benefit is, however, still in its infancy. The many approaches to determining the status of the p53 pathway in human tumor biopsy samples and the attempts to develop p53-selective therapies are described. A great deal of our knowledge of the p53 system remains incomplete, and the issue of how to best conduct translational research in cancer is debated using the difficulties around the p53 system as an example. The need for a more unified and coordinated approach to critical technological developments and clinical trial protocols is discussed.

CERTAIN AND UNCERTAIN KNOWLEDGE ABOUT p53

This huge research effort has produced a great deal of “certain knowledge” about p53. I would define this as a core set of research findings that have been widely tested, reproduced, and accepted, and on which further confirmatory findings have been based. These findings usually lie in three areas: epidemiology, genetics, and structural and physical protein biochemistry. In all three of these broad areas, our scientific tools seem to be robust and reliable, subject to widely agreed upon and understood standards of proof. The uncertain knowledge about p53 refers to a vast literature of mostly cell-based transcription experiments that are widely discussed but are still to a large extent contradictory or unverified or which, although undoubtedly true in a given experimental context, have an unproven biological significance. Examples of such claims include reports that p53 is an exomuclease (Munnmenbrauer et al. 1996), or that p53 binds to cdk2 and tubulin (Maxwell et al. 1991), or closer to home, our own observation that p53 is modified by the Ned8 ubiquitin-like protein (Xirodimas et al. 2004). I discuss these issues in more detail below, but it should be recognized that observations in this uncertain category can sometimes cross over into the certain knowledge category by virtue of further rigorous experimentation usually involving genetic models. The problem is that so many are left to languish in the uncertain category.

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have been described in some vertebrate species (Moll and Slade 2004). These p53-like proteins share a particular biology with the DNA-binding domain of p53. It is disquieting that many basic questions about the quaternary structure of the p53 protein remain unresolved and the mechanisms of its regulation in vivo are not well understood. In particular, of the many posttranslational modifications of p53 that can be detected in “in vitro” systems, which ones are important in vivo is still unresolved. It is striking that as recently as last year we described that the human p53 gene could encode nine proteins, an observation missed by the field for over 20 years (Board et al. 2005).

The current central theory of p53 action is that it acts as a sequence-specific transcription factor to activate and repress a large number of genes that control cell growth and survival. The p53 response is very tightly controlled by multiple pathways and is induced in response to cellular stress. This large area of current research is the determination of the precise upstream signals to p53 and the mechanisms by which p53 exerts its tumor suppressor function. A large body of evidence strongly suggests that p53 function is critical in reducing cancer frequency in vertebrates, including man. The human evidence derives from two principal findings, first and most importantly from the studies of the Li-Fraumeni cancer family syndrome (Varley 2003). In the majority of families with this broad familial cancer syndrome, there is genetic linkage to the p53 locus, and the p53 gene is mutated to produce a protein that is nonfunctional in transcription activation function. The second supportive finding is the very high frequency of somatic mutations in p53 in human cancer (Hainaut et al. 1998). These mutations are often point mis-sense mutations that again result in proteins which have lost transcription activation function. How can one be certain that these mutations are not simply chance occurrences, but rather have significance for the properties of the tumor cell? One biological argument extends from the extensive work in mouse genetic model systems, and the other arises from the case of human cervical cancer, in which p53 mutations do not occur but p53 function appear to be inactivated by the activity of the viral HPV E6 protein that is invariably expressed in these cancers (Mantovani and Banks 2001). A key control pathway that regulates p53 activity is the function of two negative regulatory proteins, Mdm2 and Mdm4 (Bond et al. 2005a,b). These proteins bind to p53 and inactivate its function as a tumor suppressor. Mdm2 acts as an E3 ligase promoting the degradation of p53, and the inhibition of this activity appears to be a principal route by which p53 levels rise in response to stress signals and the p53 response is initiated. A small protein, p14 Arf, can bind to Mdm2 and block its inhibitory function, thus activating the p53 response. The promoter of the Arf gene is activated in many tumor cells, and the Arf protein accumulates in cells that have lost p53 function (Lowe and Sherr 2003).

**QUESTIONS RAISED BY MOUSE MODELS**

Mouse genetic models of the p53 pathway have proved to be of enormous value and establish certain key parameters of the p53 response. They also provide a critical link between biochemical observations and biological consequences. If I have come to any conclusions from being an active participant and interested observer of the p53 field since its inception, it is to emphasize the importance of these models. The key first finding was that mice which lacked p53 gene expression could mature to adulthood but spontaneously developed cancer at a greatly elevated rate (Donehower et al. 1992). The p53 knockout mouse has emerged as a key tool in the field, but many of the lessons from this model are still not fully absorbed by the community. Here I want to emphasize two of them; the first concerns the phenomenon of haplo-insufficiency and the second relates to issues around the p53 system and tissue culture. When Knudson (2001) developed his two-hit theory to explain the link between the somatic and inherited forms of retinoblastoma, he clarified in a remarkable way our understanding of the genetics of cancer. In this model, tumors occur more frequently in the inherited cases because only one allele of the Rb gene (the remaining wild-type allele) must be lost for the cell to lack all Rb function. Thus, the critical event is a loss of heterozygosity usually achieved by the deletion of a whole chromosome or chromosomal segment from the wild-type allele. In the p53 model, however, an additional complexity has emerged which is clearly seen in the mouse model. When tumors derived from p53 heterozygote mice are examined, loss of heterozygosity is found in only about half the cases examined (Wijnhoven et al. 2001). In the other half of tumors, the wild-type allele is retained. The same phenomena have been described for tumors isolated from Li-Fraumeni patients (Varley et al. 1997). One of the most important early observations made with the p53 knockout mice was their resistance to radiation-induced death. This observation had a critical impact, emphasizing the importance of the genetics of the host response to radiation rather than the precise dose of radiation as a key variable in radiobiology. Studies on tumors isolated from Li-Fraumeni patients (Varley et al. 1997) showed that most tumors isolated from Li-Fraumeni patients (Varley et al. 1997) showed cell-autonomous haplo-insufficiency (Clarke et al. 1993; Lowe et al. 1993). Thus, the biological response of cells having two copies of the p53 gene is readily differentiable from the response of those having a single copy of the gene. This finding of haplo-insufficiency has been extended in other mouse genetic studies to the key p53 regulators, p19Arf and Mdm2 (Eischen et al. 2004). The full impact of these observations has not, however, been broadly appreciated by the field. For example, many models of feedback loops of p53 regulation are discussed, since p53 can induce the synthesis of its negative regulators such as Mdm2, Hop1, and Pith2, but all of these models fail to appreciate that they are unable to cope with the difference in p53 levels brought about by haplo-insufficiency. The second key finding from the p53 knockout mice concerns the issue of genetic instability in tissue culture. Examination of normal tissues from these mice establishes that they are diploid, yet after even a very brief period in tissue culture, the cells of p53 knockout mice, but not p53 wild-type mice, become extraordinarily...
anaploid (Tsukada et al. 1993). Two explanations can be put forward to elucidate this remarkable observation. Either cell culture imposes an extraordinary level of stress on genetic stability compared to in vivo conditions, or in vivo mechanisms exist of great capacity to eliminate aneuploid cells when they arise. Either explanation carries important implications for most studies on p53-controlled genetic instability in cancer, but the issue has not been closely examined. More recently, mouse models have been used to challenge key biochemical observations made about p53 regulation. The carbonyl terminus of p53 contains seven lysine residues, and these are variably subject to modification by acetylation ubiquitination, SUMO, and Nedd8 conjugation. Alteration of these residues has profound effects on the function and regulation of p53 in the normal tissue culture model systems that are largely based on transient transfection. However, when a p53 gene in which six of these seven lysines had been replaced with arginine was used to replace the endogenous gene in a recently developed model, the mouse showed almost completely normal regulation of p53 function, including the critical property of tumor suppression (Krummel et al. 2005). More informative have been studies of the expression and activity of point mutant p53 proteins in the mouse. Since most somatic mutations that occur in human tumors and most Li-Fraumeni families have mutations of this type, these new models more accurately reflect the human situation than do the knockout mice. The new mice establish that the point mutant proteins can act as dominant negatives over wild-type p53 but can also have p53-independent oncogenic function. For example, mouse tumors arising in such mice are more prone to metatasis than are tumors arising in p53 knockout mice (Lang et al. 2004; Olive et al. 2004).

THE ROLE OF p53 IN RESPONSE TO RADIATION AND CHEMOTHERAPY

The clear and striking prediction of the discovery of the importance of the p53 response to ionizing radiation in the mouse models was that human tumors with mutant p53 may be resistant to radiation therapy. This concept was extended, largely through the work of Scott Lowe’s team, to other commonly used cytotoxic cancer therapies (Lowe 1995). The mouse results fitted the model well, and it became clear that the p53 response was very important to the therapeutic activity of many of the common anticancer drugs in these models. It has proved hard to extend these findings in a useful way to human cancers, and the reasons for this, which are discussed below, reflect many of the observations made by Leaf (2004).

DIAGNOSTIC USE OF THE p53 PATHWAY

Given that the animal studies described above implied that the presence or absence of p53 mutation may have a profound effect on the behavior and response to therapy of human tumors, it was expected that determining the p53 status of cancers would rapidly acquire major clinical significance (Wallace-Brodeur and Lowe 1999). A recent meta-analysis of such studies in colon cancer has, however, confirmed anecdotal understanding that this is not the case (Munro et al. 2005). Since p53 mutations are one of the commonest alterations found in human cancers, this can be seen as a massive failure of translational research, and it is well worth analyzing why this might be the case.

Lack of Suitable Samples

Human clinical material is much harder to study than mouse tissue. What is needed are large cohorts of fully consented patients with precisely diagnosed cancers and exact clinical histories. Tumor samples of whose tumors have been stored in suitable conditions to allow analysis. Although such collections are now being made in several centers, it is still a great challenge to obtain them. The service requirements of the surgery and pathology departments, combined with lack of standard operating procedures, result in great and confounding variability of sample type. This is often further troubled by issues of ownership. Clinicians involved in sample collection feel a sense of ownership, whereas science laboratories able to conduct the tests are staffed by scientists on short-term contracts who, after an initial exciting “claim,” do not want to be involved in tightly controlled repetitive analysis. These practical and cultural difficulties often conspire to block progress.

Lack of an Agreed Test Method

Broadly, three methods have been used to establish p53 status in human tumors. In the first, antibodies to p53 are used to stain tumor sections. In the second, the p53 gene or its mRNA is amplified from tumor material and examined by either direct sequencing or indirect methods of sequence or function analysis. Finally, in the third method, p53 function is deduced by the analysis of gene expression profile using microarrays. All of these methods have specific technical and theoretical problems, and typing of tumors by one method often contradicts typing by another. Recent in-depth analysis of the different methods, however, suggests that a useful and practical test can be developed.

WHY DO TUMORS STAIN WITH ANTI-p53 ANTIBODIES?

In the 1980s, it was first observed that the level of p53 protein was much higher in some tumor cell lines and some tumor tissue samples than in normal tissues. In the early 1990s, the development and wide distribution of monoclonal and polyclonal antibodies to p53 that worked on conventional paraffin sections motivated an enormous number of studies of p53 staining (Vojetsek et al. 1993). In some senses, the results were remarkable. In many cases, intense nuclear staining of p53 was seen to be confined to obviously neoplastic cells in the section, while the surrounding normal tissue failed to stain. Analysis of the large number of studies has revealed that not all tumors that have mutant p53 will stain, however. This is because some mutations create stop codons so that the com-
ple protein cannot be expressed. Staining can also be variable across sections in a way that seems not to reflect simple difficulties in processing. In general, but not always, point missense mutations give rise to tumors that stain strongly with p53 antibodies. The mechanism behind this staining is still not understood. In tissue culture systems, mutant p53 accumulates to high levels in every cell in the culture due to an extended half-life of the protein compared to that of wild-type p53. Initially, it was thought that the mutation itself directly stabilized the protein. However, the discovery of the Mdm2 protein as an E3 ligase for p53 suggested an alternate explanation. In this feedback model, Mdm2 transcription is regulated by p53, and so, when mutant p53 is present, the Mdm2 protein is not synthesized and so p53 is stable. Indeed, reintroduction of cDNAs encoding Mdm2 into human tumor cells will cause a reduction in mutant p53 levels (Midgley and Lane 1997). Although intellectually very satisfying, this model has also now been shown to be incomplete. Many human tumor cell lines that express high levels of p53 continue to express Mdm2. When mice are made that can only produce mutant p53, the protein is undetectable by immunohistochemistry in their normal tissues, whereas the tumors that these animals develop stain strongly with anti-p53 antibodies. This suggests an as-yet-undefined method by which the activity of Mdm2 is a...
and affect cancer incidence and aging reveal at least nine genotypes in the human population of varying sensitivity of p53 response.

THERAPEUTIC APPROACHES TO THE p53 PATHWAY

The discovery that mutation of the p53 gene was a frequent and common event in the majority of human cancers has led to great efforts to exploit this fact for therapy. One approach has been to ask how much the presence or absence of p53 function contributes to the response to existing anticancer drugs. As discussed above, these efforts have been of limited success, and it is still unclear whether this is because all such drugs have complex mechanisms of action that involve p53-dependent and -independent pathways or because of failure to accurately type the status of human tumors. Probably both factors are important. Thus, clinically few decisions are made on the basis of p53 status. One system where it has been used is in bladder cancer, where p53 status influences the clinical decision to surgically remove the bladder. Reports suggest that among the commonly used therapies, 5-FU works better in functionally p53 wild-type tumors, whereas the taxanes work best in functionally p53 mutant tumors. One of the most obvious routes to the use of p53 has been in the field of gene therapy. The delivery of the p53 gene using an adeno virus vector has been approved in China, and the treatment is in late-stage clinical trials in the U.S. The preclinical data are very encouraging and the clinical problem appears to be one of consistent delivery. In its approved use, the gene therapy is given as multiple injections along with radiotherapy in the treatment of head and neck cancer. Recent research suggests ways to engineer more active variants of p53 that may help these therapies (Liu et al. 2001). Another therapy based on a defective adenovirus reported to replicate only in cells that lack p53 function has also been adopted for full development in China after early trials in the U.S. The basis for the tumor-selective replication of this virus, however, turns out not to be due to p53, but rather to heat shock protein expression and events linked to nuclear export. In its approved use, the gene therapy is given as multiple injections along with radiotherapy in the treatment of head and neck cancer. Recent research suggests ways to engineer more active variants of p53 that may help these therapies (Liu et al. 2001). Another therapy based on a defective adenovirus reported to replicate only in cells that lack p53 function has also been adopted for full development in China after early trials in the U.S. The basis for the tumor-selective replication of this virus, however, turns out not to be due to p53, but rather to heat shock protein expression and events linked to nuclear export (O’Shea et al. 2005).

ATTEMPTs TO REACTIVATE MUTANT p53

The ready detection of high intracellular concentrations of mutant p53 proteins in human tumor cells and the finding that those cells retained susceptibility to wild-type p53 gene therapy have prompted the search for p53 reactivating small molecules. Initial work from our laboratory showed that wild-type p53 proteins produced in bacterial expression systems were inactive in DNA-binding assays. Their activity could be recovered, however, by modifications of the carboxy-terminal 30 amino acids of the protein ranging from simple deletion through to phosphorylation or the action of heat shock proteins (Hupp et al. 1992). We were further able to show that some mutant p53 proteins could also be induced to bind in a sequence-specific manner to DNA with similar activators (Hupp et al. 1993). Eventually, this led to the development of small peptide activators of p53 which have been shown to be effective in animal models. In an alternative approach, other groups have identified small molecules such as CP31398 (Foster et al. 1999), PRIMA-1 (Bykov et al. 2002), and MIRA-1 (Bykov et al. 2005) that can activate mutant p53 in cell-based assays. The mechanism of action of these small-molecular-weight compounds is still unclear. Work using macromolecular NMR, however, has shown how a small peptide able to bind the DNA-binding domain of mutant p53 proteins can help fold the protein into an active form (Wisdler et al. 2002). With the exception of CP31398, all of these compounds have emerged from the academic community rather than the pharmaceutical industry, and the concept of changing protein-folding patterns as a target for drug development is still seen as very challenging. The realization that mutant p53 proteins may show a gain of independent oncogenic function has, however, led to further support for these proposals, as loss of mutant p53 activity may be therapeutically significant.

ACTIVATING WILD-TYPE p53

Since half of all tumors retain the wild-type p53 gene sequence, it has been proposed that finding molecules able to activate the p53 response in tumor cells may be of therapeutic benefit. Two major criticisms have been raised against this proposal. First, many existing therapeutic drugs activate the p53 response and, second, treated tumors will gain resistance via p53 mutation, which is a common event. These criticisms have been countered by the successful development of p53 activating compounds that show efficacy in preclinical models (Vassiliev et al. 2004).

BLOCKING THE p53 Mdm2 INTERACTION

The most intensively studied route to the activation of p53 in tumor cells has been in the development of agents that block the p53 Mdm2 interaction. The first suggestions that such an approach may be viable emerged from an antibody microinjection experiment in which the anti-Mdm2 antibody 3G5 was found to cause the accumulation and activation of p53 in human tumor cell lines. In an intense study from my laboratory in collaboration with scientists from Ciba-Geigy (now Novartis), peptide libraries, phage display, and peptide display aptamers were used to validate this target (V. Bottger et al. 1996, 1999; A. Bottger et al. 1997b). The solution of the X-ray structure of a p53 peptide Mdm2 complex (Kaussie et al. 1996) confirmed the definition of the p53 Mdm2 interaction, and specific molecules that block the interaction were able to stabilize and activate p53 (A. Bottger et al. 1996, 1999; A. Bottger et al. 1997b). The solution of the X-ray structure of a p53 peptide Mdm2 complex (Kaussie et al. 1996) confirmed the definition of the p53 Mdm2 interaction, and specific molecules that block the interaction were able to stabilize and activate p53 (A. Bottger et al. 1996, 1999; A. Bottger et al. 1997b). The solution of the X-ray structure of a p53 peptide Mdm2 complex (Kaussie et al. 1996) confirmed the definition of the p53 Mdm2 interaction, and specific molecules that block the interaction were able to stabilize and activate p53 (A. Bottger et al. 1996, 1999; A. Bottger et al. 1997b). The solution of the X-ray structure of a p53 peptide Mdm2 complex (Kaussie et al. 1996) confirmed the definition of the p53 Mdm2 interaction, and specific molecules that block the interaction were able to stabilize and activate p53 (A. Bottger et al. 1996, 1999; A. Bottger et al. 1997b). The solution of the X-ray structure of a p53 peptide Mdm2 complex (Kaussie et al. 1996) confirmed the definition of the p53 Mdm2 interaction, and specific molecules that block the interaction were able to stabilize and activate p53 (A. Bottger et al. 1996, 1999; A. Bottger et al. 1997b). The solution of the X-ray structure of a p53 peptide Mdm2 complex (Kaussie et al. 1996) confirmed the definition of the p53 Mdm2 interaction, and specific molecules that block the interaction were able to stabilize and activate p53 (A. Bottger et al. 1996, 1999; A. Bottger et al. 1997b). The solution of the X-ray structure of a p53 peptide Mdm2 complex (Kaussie et al. 1996) confirmed the definition of the p53 Mdm2 interaction, and specific molecules that block the interaction were able to stabilize and activate p53 (A. Bottger et al. 1996, 1999; A. Bottger et al. 1997b). The solution of the X-ray structure of a p53 peptide Mdm2 complex (Kaussie et al. 1996) confirmed the definition of the p53 Mdm2 interaction, and specific molecules that block the interaction were able to stabilize and activate p53 (A. Bottger et al. 1996, 1999; A. Bottger et al. 1997b). The solution of the X-ray structure of a p53 peptide Mdm2 complex (Kaussie et al. 1996) confirmed the definition of the p53 Mdm2 interaction, and specific molecules that block the interaction were able to stabilize and activate p53 (A. Bottger et al. 1996, 1999; A. Bottger et al. 1997b). The solution of the X-ray structure of a p53 peptide Mdm2 complex (Kaussie et al. 1996) confirmed the definition of the p53 Mdm2 interaction, and specific molecules that block the interaction were able to stabilize and activate p53 (A. Bottger et al. 1996, 1999; A. Bottger et al. 1997b). The solution of the X-ray structure of a p53 peptide Mdm2 complex (Kaussie et al. 1996) confirmed the definition of the p53 Mdm2 interaction, and specific molecules that block the interaction were able to stabilize and activate p53 (A. Bottger et al. 1996, 1999; A. Bottger et al. 1997b). The solution of the X-ray structure of a p53 peptide Mdm2 complex (Kaussie et al. 1996) confirmed the definition of the p53 Mdm2 interaction, and specific molecules that block the interaction were able to stabilize and activate p53 (A. Bottger et al. 1996, 1999; A. Bottger et al. 1997b). The solution of the X-ray structure of a p53 peptide Mdm2 complex (Kaussie et al. 1996) confirmed the definition of the p53 Mdm2 interaction, and specific molecules that block the interaction were able to stabilize and activate p53 (A. Bottger et al. 1996, 1999; A. Bottger et al. 1997b).
PARADOXICAL p53 ACTIVATION BY INHIBITORS OF TRANSCRIPTIONAL ELONGATION

In surveys of molecules that can activate the p53 response, it was apparently paradoxical that agents which inhibit general transcription such as DRB or actinomycin D were very good activators of the p53 response (Berkson et al. 2005). In general, the response to DNA damage is to inhibit transcription, whereas in the case of the p53 pathway, DNA damage induces specific gene expression. These issues have become particularly pertinent as two inhibitors of kinases involved in transcriptional elongation have entered clinical trials as anticancer treatments. The two molecules, R-roscovitine (CYC202 or Seliciclib) and flavopiridol, were originally identified as cyclin-dependent kinase inhibitors that preferentially inhibited the cdk2/cyclin A and cdk2/cyclin E enzymes. Both compounds were found to induce apoptosis in cancer cells, with particular efficacy in B-cell tumors. The mechanism of action was eventually traced to a selective inhibition of transcriptional elongation via inhibition of RNAP II phosphorylation (probably by cdk9/cyclin T) which resulted in the loss of expression of the antiproliferative protein MeI (Guo et al. 2002; Alvi et al. 2005; MacCallum et al. 2005; Raje et al. 2005). At the same time, it was clearly established that R-roscovitine (CYC202 or Seliciclib) could induce p53 accumulation and a potent p53-dependent transcriptional response (Kotala et al. 2001). The p53 response, therefore, seems geared to work in the presence of a general shutdown of transcription, and a very recent paper suggests that this may reflect distinct requirements for RNAP II phosphorylation and a high degree of p53 dependence, suggesting that of all the effects of blocking nuclear export, the activation of the p53 response is the major apoptosis-inducing event (Lain et al. 1999; Smart et al. 1999). In comparative studies with the nutlin compounds, leptomycin B is almost as selective, but far more potent. A clear mechanistic distinction can be made, however, as leptomycin is able to activate p53 in HPV-transformed cells where p53 is controlled by the HPV E6 protein (Hietanen et al. 2000). As expected from its target, the nutlin compound is inactive in HPV-transformed cells. Our attempts to introduce leptomycin B into the clinic have met many of the obstacles described by Leaf (2004), despite the existence of dedicated support for such approaches within the UK. The major obstacles relate to the costs of toxicity testing and GMP manufacture for compounds that are in the public domain. Given the promising activity of the compound in the treatment of HPV-transformed cells and the desperate need for such an agent in the treatment of HPV-induced anogenital lesions, these delays are very frustrating.

CELL-BASED SCREENING FOR NOVEL ACTIVATORS OF THE p53 PATHWAY

The analysis of both R-roscovitine and leptomycin B as activators of the p53 response revealed two new ways that the p53 response might be controlled and underscored the value of “chemical-biology” approaches. We therefore set about establishing a simple cell-based screen for small-molecule activators of the p53 response (Berkson et al. 2005). The screen consists of a reporter cell line in which the endogenous wild-type p53 protein transcriptional activation is measured by a stably integrated β-galactosidase gene under the control of a minimal promoter and a repeated p53-binding site. Careful selection and maintenance of the line allows the screening of tens of thousands of compounds with low background and excellent signals from known inducers such as DNA damage and DRB. Screening of both the NC1 diversity set (Berkson et al. 2005) and, more recently, a large commercial library using limited robotics has been remarkably successful. Over 100 hits have been further characterized to eliminate generally toxic compounds or those that induce DNA damage, and a small subgroup of compounds is now entering further development. Excitingly, compounds that show nutlin-like characteristics have already been identified, underscoring the potential value of cell-based screens (S. Lain, pers. comm.).

CONCLUSIONS

The research on the p53 system represents a good case to study key questions about the structure of modern science. Has the way that we manage scientific research in the 21st century speeded or inhibited the expected translational benefit of the major discovery that over half of all human cancers suffer from a common genetic alteration? This question is at the heart of Clifton Leaf’s discussion. Why do so many people work on p53? Is it the appropriateness of the approach or the potential value of “chemical-biology” approaches? We therefore set about establishing a simple cell-based screen for small-molecule activators of the p53 pathway. The next such workshop will be in New York in 2006; the 2004 event was held in New Zealand. This meeting is unique in both its openness and its organization. The field is also distinguished by a high level of collegiality and the ready sharing of reagents and methods, even in the face of commercial pressures. Yet these very strengths also reinforce the investigator-led,
publication-driven model that Leaf describes. It is un-
doubtedly hard to see clearly when one is on the “inside” of such an environment, and retrospect is always the easy way to come to the right conclusions. I would finish then by making two observations. First, the field is not self-
critical enough. Despite all of the structures of peer re-
view, the pressure has not been sufficient to tilt the bal-
ance of research toward the more “certain” science. Anecdotally, the surprising and novel claim has a much higher chance of being published than a careful, precise, even dogged, study that can be relied on for perpetuity. Second, there comes a point when certain technological and organizational steps are needed to move a field for-
ward. These are not well supported by current funding
structures. It should be possible to design both a trial and a device to definitively answer whether p53 status is im-
portant in the treatment of human cancer. The device might be a lab on a chip that can be applied directly to
samples in the operating theater. Similarly, it should be possible to define all genes activated and repressed by
p53 and all p53-binding sites occupied in chromatin (Wei et
al. 2006). It should be possible to catalog all proteins
binding to p53 under physiological endogenous condi-
tions, and it should be possible to take p53 activating
compounds rapidly into clinical trial. If making these steps reduces the pool of investigator-led research for a
time, it would be a worthwhile pause. That time could be
lost, and organizational steps are needed to move a field
forward. We have started in Singapore, with central gov-
ernment funding to attempt to address these issues.

ACKNOWLEDGMENTS

The opinions are my own, the references are illustra-
tive rather than comprehensive. I thank the Cancer Re-
search UK and A*Star, Singapore, for support. I thank the
Cancer Research UK and the Natural Environment Fund-
ing to attempt to address these issues. I thank the Cancer Re-
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Cold Spring Harb Symp Quant Biol 2005 70: 489-497

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