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Review

A miracle if restored, p53

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The tumour suppressor function of p53 is due to its highly regulated activity as a transcriptional factor. This very important player is a frequent target of mutation in majority of human cancers. p53 activation is a valid anticancer approach that can result in development of new cancer treatments. New discoveries are emerging rapidly about the functional control of p53 that attempts to exploit the system for developing better therapeutics and diagnostics. This article is an attempt to explore the recent advancements in p53 biology for its restoration in human tumors. We have highlighted some of the potential molecules targeting the p53 pathway for tumor suppression and it is hoped that they will become effective and powerful weapons against tumors/cancers.

Key words: p53, tumour suppressor function, human tumors, cancers.

INTRODUCTION

p53 is a well known and well studied transcription factor critical for tumor suppression, apoptosis, cellular senescence (Johnson et al., 2008; Bohlig et al., 2010) and is also capable of inducing transcription activation of a variety of genes (Vousden and Prives, 2005; Knights et al., 2006). The p53 tumor suppressor gene is a frequent target of mutation in human cancer (Vogelstein et al., 2000). The current model for p53 function as a tumour suppressor is that the protein acts as a highly regulated transcription factor. Normally, p53 is a very unstable protein and is present only in minute concentrations in the cell. Even these small amounts of p53 are not fully active as a transcription factor, because the negative regulator protein, Mdm2, binds them. When cells are exposed to a wide variety of aberrant growth signals, the p53 protein is activated and stabilized and triggers the expression of downstream genes such as p21 that trigger cell cycle arrest, or Puma that triggers apoptosis (Lane, 2004). Genetic and biochemical studies have demonstrated a rich variety of genes and pathways that act to control p53 function and thresholds. These must act in concert to precisely control the activity of p53. These pathways are nearly always disrupted in cancer cells that need to avoid the full p53 response if they are to grow and survive (Venkatachalam et al., 1998). p53 activation remains a valid anti-cancer approach that will likely result in development of new cancer treatments in the near future (Gudkov and Komarova, 2007). Recent studies have shown that restoration of p53 leads universally to a rapid regression of established *in situ* tumors (Shangary and Wang, 2008).

The use of p53 in gene therapy has already been approved and several small molecules that can activate the p53 pathway without non-specific toxicity are under development (Lane, 2004). This article is an attempt to explore the recent advancements in p53 biology for its restoration in human tumors. We have highlighted some of the potential molecules targeting the p53 pathway for tumor suppression and it is hoped that they will become effective and powerful weapons against tumors/cancers.

IMPLICATIONS OF THE p53 PATHWAY IN CANCER TREATMENT

Cancer research has reached an exciting phase of its

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evolution, as we now have an increasingly detailed molecular description of the genomic abnormalities and the biochemical pathways that drive the malignant progression of cancers. Investigation of the p53 tumour suppressor pathway, in particular, has become a key focus of current cancer research (Woods and Lane, 2003). The p53 pathway has been shown to mediate cellular stress responses; p53 can initiate DNA repair, cell-cycle arrest, senescence and, importantly, apoptosis. These responses have been implicated in an individual's ability to suppress tumour formation and to respond to many types of cancer therapy. Studies of the genetics of p53 pathway components, in particular p53 itself and its negative regulator MDM2, in cancer cells has proven useful in the development of targeted therapies. Furthermore, inherited single nucleotide polymorphisms in p53 pathway genes could serve a similar purpose (Vazquez et al., 2008). Cancer treatment with implications of the p53 pathway has been in focus for the last few years. A number of available treatments activate the p53 response in tumour and normal tissues.

As compared to human material data which is less clear cut, animal models have been observed with improved effects of p53 wild-type tumors responding better to chemotherapeutic drugs. It shows the genetic complexity of cancers in human and the possibility that a number of 'wild-type' p53 tumors may have some other defects in the pathway. Discoveries and development of cancer therapies based on p53 pathway understanding are perhaps more encouraging (Lane, 2004; Edelman and Nemunaitis, 2003).

p53 IN GENE THERAPY

In China, Wt p53 delivery through adenoviral expression vector has already been approved for treating head and neck cancer as part of a radiotherapy-gene therapy combination and as similar in clinical trial at their late stages in the USA (Edelman and Nemunaitis, 2003). It is not possible to deliver the virus to all tumour cells but immune phenomena may possibly be acting for the effectiveness of these therapies. More active variants designing of p53 is possible through mutation that either blocks the negative regulator Mdm2 binding or that helps to stabilize the core structure of the p53 protein. In model systems, these approaches clearly provide better performance but in human trials more research is needed to test the safety related features of the wild-type p53 virus (Bischoff et al., 1996). Mutant status is a better candidate for p53 therapy. However, this treatment is also considered effective for those who have inactivated wt-p53, which is a common condition in tumors. Human wt-p53 gene has multiple anti-tumor effects and the loss of p53 is thought to be responsible for the lack of apoptotic signals in tumor cells and thus for their uncontrolled proliferation and recurrence (Kouraklis, 1999). In lung cancer, the incidence of p53 mutation was reported as 50 to 60%. In addition, recent clinical studies have demonstrated that most human cancers have an abnormality in some of the molecules associated with the p53 pathway (Huang et al., 2007). Many studies proved that p53 gene transfer as mediated by retroviral or adenoviral expression vectors increased drug and radiation sensitivity or directly induced apoptosis (Nguyen et al., 1996; Nielsen, 1997); and the combination of p53 gene transduction with radiation or chemotherapy has resulted in local tumor control that is superior to either therapy alone (Gjerset, 1995; Sandig et al., 1997).

Although, restoring p53 function alone is sufficient to treat certain human cancers, tumors may be able to quickly generate resistance by finding other ways to disrupt the p53 pathway (Kastan, 2007). It is proposed that p53 gene therapy combining BAI, as a promising anti-tumor method, could have better efficacy in lung cancer (Guan et al., 2007). It has been noted previously that osteosarcomas with p53 mutations show resistance to chemotherapy and that their prognosis is poor (Park et al., 2001). Hence, studies of p53 gene therapy for osteosarcoma have been implemented. Many such studies have used viral vectors, and growth inhibition *in vitro* has been reported to be approximately 60% (Son et al., 2001). Transferrin-liposome-mediated p53 gene therapy is effective against growth inhibition of osteosarcoma with p53 mutations. The increase in the efficacy of p53 gene transfer and the subsequent increase in p53 expression seemed to have an effect on enhancement of HOSM-1 growth inhibition. Cells are directed to cell cycle termination or apoptosis by increased p53 expression, and the transcriptional activity of the bax gene is directly associated with the p53-dependent apoptosis pathway (Nakase et al., 2005).

The introduction of exogenous wild-type p53 enhanced bax expression in HOSM-1 cells, and induction of apoptosis by transferrin-liposome-p53 treatment occurred through a bax-dependent pathway (Im et al., 2001).

RESCUING MUTANT P53

To rescue the mutant p53, a number of strategies have been proposed. Some of the promising early leads have shown better activities in animal models but the exact action mechanism is still disputed. In some cases, the DNA binding function of the mutant p53 proteins could be activated by peptides produced from the C-terminus of p53 up to some extent (Hupp et al., 1995); but still needs more research work to achieve the complete picture. However, in mice, it has been shown recently that such peptides when binds to a cell transport sequence derived from D-amino acids can cure an aggressive intraperitoneal tumour completely (Snyder et al., 2004). Researchers have identified certain residue peptides that bound to a defined site on the tumor suppressor p53 and

stabilized it against denaturation. These molecules have been tested for their activity as chaperones and can rescue the tumor-suppressing function of oncogenic mutants of p53 in living cells. Human tumor cells when treated with the peptide Fl-CDB3 (fluorescent derivative of CDB3), it induced a substantial up-regulation of wild-type p53 protein and representative mutants. The mutants, His-273 and His-175 p53, adopted the active conformation, with a dramatic decrease in the fraction of denatured protein. In all cases, there was p53-dependent induction of expression of the p53 target genes mdm2, gadd45 and p21, accompanied by p53-dependent partial restoration of apoptosis.

Fl-CDB3 sensitized cancer cells that carried wild-type p53 to p53-dependent γ-radiation-induced apoptosis. It did bind to and rescue p53 in cells and so can serve as a lead for the development of novel drugs for anticancer therapy (Issaeva et al., 2003). Adaptation to deleterious mutations by a compensatory mutation at a different site of the genome is a common occurrence in evolution (Poon et al., 2005). The analysis of second site suppressor mutations is therefore a powerful tool for studying functional interactions within and among proteins (Wray et al., 1999; Sujatha et al., 2001).

In the case of p53, studies on second-site suppressor mutations are particularly interesting, because they provide important clues as to whether activity can be restored to common cancer mutants, which has far-reaching consequences for the development of therapeutic anti-cancer strategies. In yeast and mammalian, second-site suppressor mutations restoring of many oncogenic mutants have been identified by using genetic approaches (Brachmann et al., 1998; Baroni et al., 2004).

The structure of p53 core domain quadruple mutant provides the framework for understanding the molecular basis of stabilizing mutations in p53. The fact that the overall structural features of wild type are conserved makes this superstable mutant a suitable substitute for wild type in biochemical and biophysical studies where the intrinsic thermodynamic instability of the wild type protein causes experimental difficulties (Nikolova et al., 1998).

The scientists are focusing on the mutations N268D and N239Y to use them as suppressors for a number of oncogenic mutations. Quadruple mutant M133L/V203A/N239Y/N268D provides strong evidence about their mode of action that is, to locally stabilize some of the regions on protein. Stabilization of some major sites on the p53 can compensate for the loss of structurally important interactions like the salt bridge between Arg-249 and Glu-171. During the course of evolution of p53, both its thermodynamic stability and conformational flexibility have been carefully balanced out. It is therefore predicted that, to carry out various functions *in vivo,* p53 has to maintain a certain degree of plasticity in the L3 region (Joerger et al., 2004).

DIFFERENTIAL RESPONSES TO p53 ACTIVATION

Activation of p53 through therapeutic strategies has to take advantages from the ability of p53 to modulate or its differential responses. It is hoped that cancer stresses like activation of oncogenes, hypoxia etc. would lead to differential and heightened sensitization to undergo apoptosis of the concerned cells only. The control over deregulation of transcriptional factor E2F1 is a good example of the mechanism that underlies the difference between normal and cancer cells in human. In cooperation with p53, E2F1 can induce apoptosis both in independent and dependent fashion (Stanelle and Putzer, 2006). E2F1 also induce the expression of ASPP1, ASSP2 and p73 which can act as helpers of p53 to induce expression of the apoptotic target genes. It is clear that, increased E2F1 activity in tumour cells is vital for its apoptotic response to p53 by virtue of the p53 cofactors expression in apoptosis. In practice, the usefulness of the differential between normal and cancer cells in response to p53 is still to be explored. In normal tissues, inducible loss of MDM2 followed by the activation of p53 can induce apoptosis in some of the normal tissue types. It is therefore suggested that, tumour to normal cells differential might not be as tight as we have hoped (Marine et al., 2006). MDM2 inhibitors are also hoped to be responsible for the inactivation of p53.

Studies have been proved of nutlin-3, which is one among such inhibitors is efficient in decreasing tumorigenesis and is also not toxic (Vassilev et al., 2004). Due to its antitumor activity, Nutlin-3 is also an effective *in vivo* in xenograft models of human cancer with wild-type p53 (Sarek et al., 2007). Their discovery provided the base for designing and development of small molecule MDM2 inhibitors (Vassilev, 2007).

USING INTERACTION OF MDM2-P53 FOR CANCER THERAPY

MDM2 negatively regulates the p53-mediated transactivation (Momand et al., 1992). Genetic studies provide the MDM2 physiologic relevance as a p53 critical inhibitor and showed that the deletion of the p53 gene can lead to rescue MDM2 null mice embryonic lethality (Montes et al., 1995). Reactivation of p53 through targeting MDM2-p53 interaction by small molecules has emerged as an effective strategy for cancer therapeutics. High-resolution crystal structures of the NH_2-terminal domains of MDM2 complexed with short peptides of p53 showed that MDM2 has well-defined hydrophobic pockets on its surface and p53 has four key hydrophobic residues which are responsible for the interaction of MDM2-p53. Hydrophobic residues of p53 are Leu22, Leu26, Phe19 and Trp23. This interaction has provided the basis for designing inhibitors of MDM2-p53 interaction which are nonpeptide, drug-like small molecules to reactivate p53 (Shangary and Wang, 2008). They induce

the accumulation of p53 and its pathway activation by blocking the intracellular interaction of MDM2-p53 both in tumor and normal cells (Vassilev et al., 2004).

PHARMACOLOGICAL RESTORATION OF P53 TUMOR SUPPRESSOR FUNCTION

Any dominant inhibitor which can suppress p53 has always been considered as a potential drug target with the hope that its suppression would lead to p53 functional restoration. This approach is supported by a group of researchers because of the p53 anti-tumor effect activated either by genetic recombination (Xue et al., 2007; Ventura et al., 2007) or through small molecules targeting its interaction with inhibitory proteins or restore the mutant p53 functionality (Vassilev, 2005). In this regard, an interesting window of opportunity was opened by the discovery of a novel NF-κB-dependent mechanism frequently involved in p53 suppression in tumors (Bonizzi and Karin, 2004), NF-κB is constitutively active in the vast majority of cancers (Lin and Karin, 2003). Interestingly, among pharmacological agents capable of simultaneous inhibition of NF-κB and activation of p53, we identified quinacrine (Gurova et al., 2005), a drug historically used for treatment of malaria. The advantages of NF-κB inhibition as an approach to p53 activation include: i) its specificity for cancer cells; and ii) its potential usefulness even against cancers with mutant p53.

Although, many small-molecules of HDM2 inhibitors have shown potent *in vitro* activity, only a limited number of compounds have displayed acceptable pharmacokinetic properties for *in vivo* evaluation. To date, the most studied chemotypes have been *cis*-imidazolines (for example, Nutlins), benzodiazepines (BDPs) and spirooxindoles. The *cis*-imidazolines were the first reported potent, selective small-molecule inhibitors of the p53–MDM2 interaction, and continue to show therapeutic potential (Sharmila and Player, 2008). Another notable agent is PRIMA-1 discovered in a screen for compounds that could suppress the growth of tumor cells in a mutant p53- dependent manner (Bykov et al., 2002). PRIMA-1 also results in an accumulation of p53 with wild type conformation and transactivation function. It can convert previously unfolded p53 mutant protein into wild type active form. The biochemical targets and mechanisms of action are not yet fully understood. Administration of PRIMA-1 in SCID mice resulted in antitumor activity against human mutant p53-carrying tumor xenografts. Several p53-activating compounds have been identified in protein and cell based screening assays (Wiman, 2006). Most of these compounds, however, do not directly interact with p53 but function via different mechanisms, some of which are still a matter of debate. Rational structure based drug design that directly targets p53 is still in its infancy. The underlying principle for a direct pharmacological rescue of p53 cancer mutants follows simple thermodynamic considerations. The low kinetic stability of p53, and in particular of destabilized mutants, further requires that such a compound acts immediately upon biosynthesis of the protein and acts as a chemical chaperone.

Studies on the CDB3 peptide provided a proof of principle that pharmacological rescue of p53 mutants by small molecule drugs is a feasible strategy. CDB3, a 9-residue peptide derived from one of the p53-binding loops of 53BP2, and its fluorescein-labelled form FLCDB3 bind to the p53 core domain, albeit with a strong electrostatic component, and raise the melting temperature of the wild-type and conformationally destabilized mutants (Friedler et al., 2002). It partly overlaps with the DNA-binding region but, interestingly, differs from that of the corresponding loop in the p53–53BP2 complex. CDB3 not only raises the melting temperature of p53 and its mutants but also increases the half-life of kinetically unstable mutants *in vitro* (Friedler et al., 2003) and was found to induce upregulation of wild-type p53 and oncogenic mutants in human cell lines (Friedler et al., 2002; Issaeva et al., 2003). The availability of high-resolution structural data on p53 mutants, combined with data on the energetic and functional consequences of mutation, has opened novel avenues for the design of small molecule drugs to reactivate p53 mutants. Since p53 mutants have distinct structural and functional characteristics, different rescue strategies can be applied, which can also include drugs that specifically target the folding state of a particular mutant. The ideal candidates for generic small molecule drugs are mutants, such as the b-sandwich mutants V143A and F270L, that are destabilized while retaining the structural features of the wild-type in important functional regions (Baroni et al., 2004).

The hotspot mutant Y220C falls into a category of mutants that is a particularly an attractive target for structure-based drug design. Its temperature-sensitive behavior makes it a good candidate for a generic small molecule drug, and the mutation-induced crevice could also be specifically targeted. This crevice has its deepest point at the mutation site, Cys-220, thus providing a binding pocket for a small molecule drug with a moiety that selectively targets mutant Y220C (Joerger et al., 2006). Such a drug would allow specific targeting of the mutant without binding to the wild-type protein in a heterozygous scenario. The frequency of this mutation in human cancer is high enough to make the development of a mutant-specific drug an attractive proposition.

RESTORATION OF P53 PATHWAYS BY USING SYNTHETIC SMALL INHIBITING RNAS

After the discovery of small inhibiting RNAs (siRNAs) as gene suppressors in mammals (Elbashir et al., 2001A; Caplen et al., 2001) opens new horizons for the analysis of gene targeted therapies and gene functions (Elbashir

et al., 2001B), siRNAs have been used for various purposes (Novina et al., 2002) and are able to distinguish between point mutant mRNA targets (Elbashir et al., 2001A). It is therefore estimated that, siRNAs can be used to eliminate oncogenic mutant proteins (Martinez et al., 2002) as they are regarded as powerful tools for the inhibiting mutated genes in cancer cells. To analyze transformed cells and delineate the functions of mutated oncogenic proteins, synthetic siRNAs can be used. In p53 model, this property is of immense interest because mutant p53 is considered to have some gain-of-function oncogenic activity which may be caused by the inactivation of other members of this family (Di Como et al., 1999), in addition to its interaction with cofactors that regulate the WT protein's function (Joers et al., 1998). They can provide a tool for cancer prevention in certain cases for example, in families with Li–Fraumeni syndrome having only one copy of mutated p53, genome surveillance by WT p53 can be restored by blocking mutant p53.

It will decrease the risk of causing cancer and also the development of multiple malignancies (Aas et al., 1996). The use of chemically synthesized synthetic siRNAs necessitates the need to address the variety of both technical and ethical problems which can be raised by using expression vectors. It should also be made possible to use synthetic siRNAs simultaneously in combination against various targets than siRNAs derived from plasmids. These therapies are hoped to be valuable in preventing oncogenic mutations and adaptations of cancerous cells to treatment (Martinez et al., 2002).

SELECTIVE ELIMINATION OF CELLS DEVOID OF P53 ACTIVITY

Virus dependent lysis of cancerous cells can lead to elimination of cells devoid of p53 activity. There are a number of viruses which inhibit p53 activity and thus can efficiently control cell processes. Human adenoviruses have the ability to activate the quiescent cells to enter the S phase, necessary for viral genome replication. These are due to the viral E1A, which blocks the pRB suppressor and induces p53-dependent apoptosis. E1B-55kDa simultaneously inhibits p53 and acts together with E4-ORF6 product to accelerate proteolytic degradation of p53. It is therefore estimated that dl1520 adenovirus mutant (Almazov et al., 2007; Barker et al., 1987) having a partial deletion from E1B is not replicated in normal cells but does reproduce and cause death in cancer cells having a functional defected p53 (Bischoff et al., 1996). The mutant is unable to reproduce in primary cells; but reproduces well in cancer cells devoid of p53 and thus causing their death (Habib et al., 2002). There are evidences that Onyx-015 well reproduces in cancer cells with the wild-type p53 (Rothmann et al., 1998).

Cancerous cells are usually poorly differentiated and produce a large amount of surface associated coxsackie and adenovirus receptor (CAR) (Hutchin et al., 2000); their infection with these viruses is therefore more efficient, which explains selective oncolysis (Dix et al., 2001). Regardless of the state of p53, cancer cells with normal p53 are sensitive to Onyx-015 because their p53-dependent mechanisms are defective (McCormick, 2000a). A defect in p14ARF leads to a loss of p53 function (McCormick, 2000B). p53 cannot be induced by Onyx-015 in p14ARF-deficient cells and, consequently, is efficiently reproduced. The selective effect of Onyx-015 can therefore be related to the fact that cancerous cells sustain the nuclear export of adenoviral RNA essential for adenovirus expression (O'Shea et al., 2004). Experiments with cell cultures and preclinical trials have shown that selectivity and safety of Onyx-015, allowing a phase III clinical studies in patients with head-and-neck cancer. The virus in this case selectively replicates in cancer cells and cause their death (Nemunaitis et al., 2000). Onyx-015 is even more effective in combined standard chemotherapy with 5-fluorouracil (5FU), cisplatin (Nemunaitis et al., 2000), or doxorubicin and taxol (Portella et al., 2002).

Owing to selective infection of cancer cells, recombinant adenoviruses with an E1B deletion can be used to simultaneously deliver the gene for uracil phosphoribosyltransferase, which helps to overcome 5FU resistance. It has been proved highly effective in pancreatic cancer (Sunamura et al., 2004).

FUTURE PERSPECTIVES

New discoveries about the function and control of p53 are still in progress and it is hoped to develop better therapeutics and diagnostics by exploiting this system (Hupp et al., 2000; Lane and Hupp, 2003). Modern research strategies through manipulating the p53 pathway are emerging rapidly and one can predict the extensive clinical use of this protein in the near future for the human benefit worldwide (Lane, 2004; Khan et al., 2011).

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