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Review paper

Does p53 status influence tumor response to anticancer therapies?

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Abnormalities in the tumor suppressor gene p53 have been identified in over 60% of human cancers. Since it plays such a pivotal role in cell growth regulation and apoptosis, the status of the p53 gene has been proposed as one of the major determinants of a tumor's response to anticancer therapies. In this review we examine the relationship between functional p53 and sensitivity/resistance to both chemotherapy and radiotherapy, and discuss the potential use of some of the current gene therapy approaches to restore functional p53 to tumors as a means of modulating the effects of radiation and chemotherapy. [© 2000 Lippincott Williams & Wilkins.]

Key words: Chemotherapy, clinical studies, gene therapy, p53, radiation therapy, resistance.

Introduction

The development of somatic gene therapy has created the potential to restore wild-type function of critical tumor suppressor genes. One intensely pursued target of gene therapy for the treatment of cancer is the tumor suppressor p53 (reviewed in 1), the most widely mutated gene in human cancer. Abnormalities in p53 may impact the efficacy of standard anticancer therapies, such as radiation and chemotherapy. Below, we will briefly examine the role of p53 in human cancer, specifically its ability to mediate responsiveness to cytotoxic anticancer therapies, and examine some of the current approaches toward developing effective p53-based gene therapies.

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p53

The tumor suppressor gene p53 has a diverse range of functions including regulation of cell cycle checkpoints, apoptosis, senescence, DNA repair, maintenance of genomic integrity and control of angiogenesis, which together make p53 a gene critical for the inhibition of tumorigenesis (reviewed in 2–4). However, mutations in p53 abolish its activity and have been implicated in more than 60% of human cancers (reviewed in 5) (Figure 1). p53 is a critical regulator of the cellular stress response, primarily through the transcriptional regulation of genes involved in cell cycle control, DNA repair and apoptosis (reviewed in 6). p53 can be activated in response to a number of cellular stressors including hypoxia, the depletion of nucleotide pools and, most notably, DNA damage. The ATM gene is particularly important in p53 activation after DNA damage (reviewed in 7). p53 has been implicated in the generation of both G_1/S and G_2/M cell cycle arrest, as well as a mitotic spindle checkpoint. p53 can lead to a G_1 cell cycle arrest primarily through the induction of the cyclin dependent kinase inhibitor $p21^{waf1/cip1}$ and proliferating cellular nuclear antigen (PCNA), and may contribute to G_2 arrest through the induction of proteins such as p21, GADD45 and 14-3-3-δ (reviewed in 8). In addition to mediating cell cycle arrest in response to DNA damage, p53 can induce apoptosis. There are a number of p53 inducible genes whose expression may play a critical role in apoptosis including, Bax, Bcl-x_L, IGF-BP, Fas/Apo1, TRAIL, TRID and TRUNDD (reviewed in 9). Additionally, it has been suggested that p53 plays a role in DNA damage repair through the induction of GADD45, which has been implicated in nucleotide excision repair, and p21, which has been demonstrated to directly interact with the mismatch repair enzymes MLH1 and MSH2. Additionally, the DNA binding domain of p53 exhibits 3′–5′ exonu-

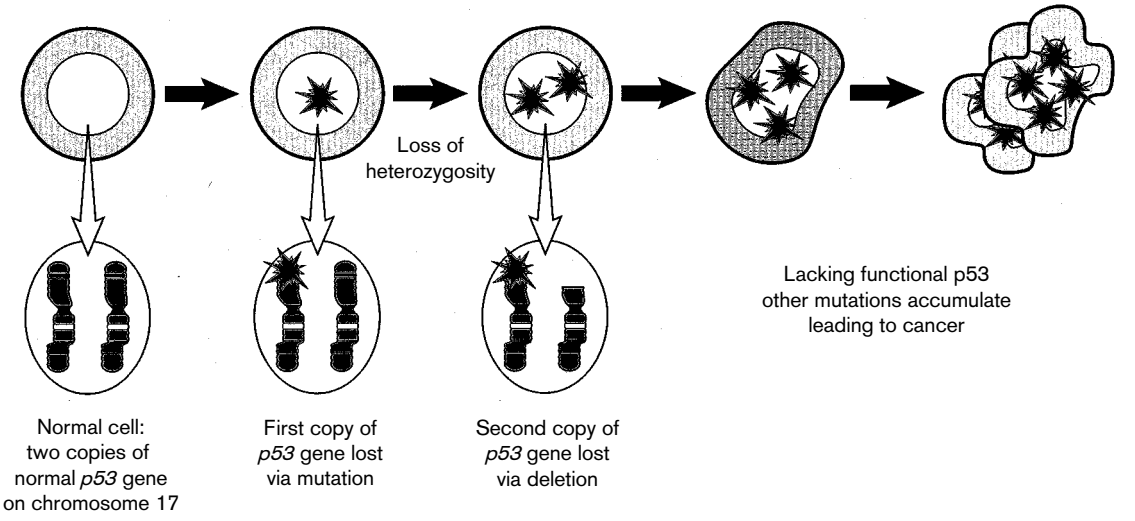

Figure 1. p53: guardian of the genome.

clease activity, thus p53 may play a proofreading function during DNA replication and excise DNA damage mismatches (reviewed in 10).

Given the integral role that p53 plays in cellular growth arrest and apoptosis, a growing body of literature recognizes a role for p53 in mediating the cytotoxic effects of anticancer drugs and ionizing radiation. In a seminal series of papers, Lowe *et al.*[11] demonstrated for the first time a direct role for p53 in the modulation of sensitivity to cytotoxic anticancer therapies. Using embryonic fibroblasts from p53 knockout mice they showed that p53 (−/−) cells were resistant to treatment with ionizing radiation and various cytotoxic drugs both *in vitro* and *in vivo*. This lack of responsiveness correlated directly with an inability of cells to undergo apoptosis in response to DNA damage. Thus, the authors demonstrated a direct link between p53 status, apoptosis and sensitivity to cytotoxic anticancer therapies. Similar results were demonstrated by Clarke *et al.*[12] Subsequently, innumerable studies have been conducted in cancer cell lines and tumors of differing origins to determine if this observed relationship between p53 status and responsiveness to anticancer therapies is a general phenomenon or specific for particular cell types or therapies.

Loss of functional p53 and response to chemotherapy

It has been observed that, in general, cancers that contain wild-type (wt) p53 are sensitive to cytotoxic drugs, while tumors that contain mutant (mt) p53 are not. However, a number of studies have been conducted to specifically establish this relationship. One study aimed at broadly assessing the nature of the relationship between p53 and responsiveness to cytotoxic drugs was conducted by O'Connor *et al.*[13] In this study the authors examined 60 human cancer cell lines, with varying p53 status, for sensitivity to 123 'standard' anticancer drugs, in order to determine if p53 status plays a role in responsiveness to cytotoxic drugs. They found that cells with wtp53 were more susceptible to growth inhibition by the majority of drugs examined. However, they found that sensitivity to antimitotic drugs was independent of p53 status.

The relationship between p53 status and sensitivity to anticancer drugs has been extensively studied in breast and ovarian cancers. The majority of studies in these cancers support the idea that mutations or alterations in p53 can lead to decreased sensitivity or resistance to cytotoxic anticancer drugs. Studies by Clahsen *et al*,[14] Koechli *et al.*[15] and Itaya *et al.*[16] found that immunohistochemically p53(+) breast tumors showed lowered sensitivity to a variety of anticancer drugs including 5-fluorouracil (5-FU), doxorubicin, cyclophosphamide, methotrexate and mitomycin C. It should be noted that, conventionally, p53(+) tumors denote tumors with p53 mutations and/or the presence of a stabilized p53. [In normal cells, the expression of p53 is kept low due to a short half-life. Mutations in, or activation of, p53 stabilize the protein and allow for immunohistochemical (IHC) detection]. Additionally, studies by Elledge *et al.*[17] and Faille *et al.*[18] observed a similar trend in breast tumors although these data were not statistically significant. In contrast, a number of studies have found no correlation between p53 status and chemosensitivity

in breast cancer. A study by Fan *et al.*[19] found that loss of functional p53, through the use of a dominant negative transgene or human papilloma virus E6, sensitized MCF-7 breast cancer cells to cisplatin. The authors proposed that this sensitization resulted from a loss of nucleotide excision repair capacity. Additionally, they demonstrated that p53 status had no effect on treatment with other drugs, such as adriamycin and etoposide.

Platinum-based therapies are a mainstay in the treatment of ovarian cancer, thus many studies have assessed the relationship between p53 status and sensitivity to cisplatin. Righetti *et al.*[20] found a significant relationship between p53 mutations and resistance to cisplatin in advanced ovarian cancer. Consistent with this, Calvert *et al.*[21] found that p53 mutation status was a potent predictor of responsiveness to platinum-based therapies in advanced ovarian cancer. A similar relationship was observed by Marx *et al.*[22] and Buttita *et al.*[23] A study by van der Zee *et al.*[24] showed no significant relationship between p53 status and responsiveness to chemotherapy in ovarian cancers.

There is evidence to support the relationship between p53 mutations and decreased drug sensitivity in other types of cancer as well, including colorectal and male germ cell tumors. 5-FU is the standard chemotherapy for patients with colorectal cancer. A number of studies have shown that colorectal tumors containing p53 mutations showed little or no response to treatment with 5-FU. Interestingly, Bunz *et al.*[25] demonstrated that targeted deletion of p53 in colorectal cancer cell lines resulted in resistance to 5-FU both *in vitro* and in an *in vivo* xenograft model. However, these cells showed an increased sensitivity to adriamycin and radiation-induced apoptosis *in vitro*. Additionally, Fan *et al.*[19] showed an increase in sensitivity to cisplatin in RKO colon cancer cells which were engineered to be deficient for p53.

In contrast to colorectal cancers, which often have p53 mutations, male germ cell tumors rarely contain mutations in p53. It has been suggested that this may account for their superb sensitivity to cisplatin. Houldsworth *et al.*[26] have identified a population of cisplatin-resistant tumors and found them to contain mutations in p53. However, there are studies, such as that by Burger *et al.*[27] which find no significant correlation between p53 mutations in germ cell tumors and sensitivity to cisplatin. Tables 1 and 2 contain a representative sampling of studies examining the relationship between p53 status and responsiveness to cytotoxic anticancer drugs *in vitro* and *in vivo*, respectively.

There is evidence that p53 may be a molecular determinant of chemoresponsiveness in other types of cancer as well including gastric, esophageal and non-small cell lung cancers, head and neck squamous cell carcinomas, and melanomas (see Tables 1 and 2). Finally, it should be noted that p53 has been shown to repress the transcription of the multidrug resistance genes, MDR1 and MRP. Accordingly, inactivation of p53 has been shown to lead to increased MDR1 expression and subsequently to a multidrug-resistant phenotype.

The influence of p53 status on cellular radiation resistance

Numerous *in vitro* and *in vivo* studies indicate that loss of p53 function results in increased post-irradiation clonogenic cell survival, which correlates with an abrogated G_1 checkpoint and changes in apoptosis.[28] Such studies cross a broad spectrum of cell types derived from sources as diverse as rat and mouse cells[11,12,28–31] to normal human fibroblasts[32,33] and tumor cell lines.[13] An example is the report of McIlwrath *et al.* where a significant correlation was found between p53 status, as represented by the presence of a G_1 arrest, and radiation killing in 12 human tumor cell lines displaying a wide range of radioresponsiveness.[34] However, it is important to note that the relationship between p53 status and radiation resistance is not entirely clear and may not completely or directly correlate in all cell types. This is exemplified in studies of the involvement of p53 in radiation-induced apoptosis in glioblastoma cells, where different studies have found the radiobiological response of these cells to be either p53 dependent[35] or independent.[36,37] Table 3 is a representative sampling of *in vitro* studies examining the correlation between p53 status and the radiation response of cell lines.

Of more significance, however, is whether p53 status affects the response of tumors to therapeutic radiation in the clinical setting. Results from some clinical studies attempting to determine if a correlation exists between p53 status and radioresponsiveness are given in Table 4. A number of recent reports, in various tumor types, have indicated that alterations in p53 correlate with an increase in survival post-irradiation.[38] For instance, p53 status has been related to prognosis and response to adjuvant radiation therapy in breast cancer.[39,40] The results of these, and other recent studies, including those discussed above, regarding the relationship between functional p53 and response to chemotherapy, indicate a striking

Table 1. Correlation between chemosensitivity and p53 status in *in vitro* studies

Cell type	Cell line	p53 status	Drug(s)	Response	Comments	Reference
Multiple including breast lung colon kidney ovary CNS leukemia melanoma prostate	60 various cell lines that comprise the NIH anticancer drug screen	wt mt	123 standard anticancer agents	Sensitive Resistant	Response to antimiotic drugs differed—appeared to be p53 independent	13
Breast	MCF-7 MCF-7 transfectants	wt mt	Cisplatin, ADR, MMS, VP-16	Sensitive to all drugs Increased sensitivity to cisplatin	wt and transfectants showed similar sensitivity to ADR, MMS and VP-16	19
Colon	RKO RKO transfectants	wt mt		Sensitive to all drugs Increased sensitivity to cisplatin		
Human foreskin fibroblasts	Primary human fibroblasts Primary cell transfectants	wt mt	Cisplatin, carboplatin, nitrogen, mustard, melphalan, taxol	Sensitive to all drugs Increased sensitivity to all drugs		90
Mouse embryonic fibroblasts	Primary mouse fibroblasts	p53 (+/+) p53 (+/−) p53 (−/−)		Sensitive to cisplatin Sensitive to cisplatin Increased sensitivity to cisplatin	Mouse fibroblasts treated only with cisplatin	
Human colon cancer cell lines	Cell lines + homologous recombination	p53 (+/+) p53 (+/−) p53 (−/−)	ADR, 5-FU	Sensitive to 5-FU Intermediate sensitivity to 5-FU Decreased sensitivity to 5-FU	p53-deficient cells sensitized to ADR	25
Gastric and esophageal	SK-GT-1 (gastric) SK-GT-2 (gastric) SK-GT-4 (esophageal) SK-GT-5 (gastric) NU-GC-4 (gastric) MKN-45 (gastric) MKN-74 (gastric)	Null mt mt mt wt wt wt	5-FU, mitomycin C, cisplatin	Resistant Resistant Resistant Resistant Sensitive Sensitive Sensitive	Results correspond for each drug	91
Testicular germ cell	NT2 2101 EP S2 NCCIT	wt wt wt mt	Cisplatin	Sensitive Resistant Sensitive Sensitive		27

ADR = adriamycin; MMS = methylmethanesulfonate; VP-16 = etoposide.

Table 2. Correlation between p53 status and response to chemotherapy in clinical studies

Tumor type	No. of patients	p53 analysis	Drug(s)	Tumor response	Comments	Reference
Breast	441	IHC	5-FU, ADR, cyclophosphamide	Decreased sensitivity		14
Breast	35	IHC	5-FU + radiation	Decreased sensitivity		40
Breast	40	ELISA	Cisplatin, MTX, 5-FU	Decreased sensitivity	*In vitro* sensitivity assay	15
Breast	11	IHC	Mitomycin C, 5-FU, ADR, cisplatin	Decreased sensitivity		16
Breast	261	IHC	Cisplatin, MTX, 5-FU	Decreased sensitivity	Non-statistically significant trend	17
Breast	39	IHC, SSCP, seq.	ADR, cyclophosphamide	Decreased sensitivity	Non-statistically significant trend	18
Breast	167	IHC	5-FU, ADR, cisplatin	No correlation		92
Breast	90	IHC	Mitoxantrone, TAM, MTX, Mitomycin C	No correlation		93
Breast	347	IHC	Cisplatin, MTX, 5-FU	No correlation		94
Breast	139	IHC	Cisplatin, MTX, 5-FU	Increased sensitivity		95
Ovarian	33	IHC, SSCP, seq.	Cisplatin	Increased sensitivity	Only associated with missense mutations	20
Ovarian	46	IHC, SSCP, seq.	Carboplatin	Decreased sensitivity		21
Ovarian	187	IHC	Cisplatin, cyclophosphamide	Decreased sensitivity		22
Ovarian	33	IHC, SSCP	Cisplatin	Decreased sensitivity		23
Ovarian	70	IHC	Cisplatin, ADR, cyclophosphamide	No correlation		24
Colorectal	17	SSCP, seq.	5-FU	Decreased sensitivity		96
Colorectal	39	SSCP	5-FU, camptothecin	Decreased sensitivity		97
Colon	11	IHC	Mitomycin C, 5-FU, ADR, cisplatin	Decreased sensitivity		16
Esophageal	56	IHC	5-FU, cisplatin, +/− radiation	Decreased sensitivity		98
Esophageal	9	IHC	Mitomycin C, 5-FU, ADR, cisplatin	Decreased sensitivity		16
Gastric	30	IHC	Cisplatin, 5-FU, ADR	Decreased sensitivity		99
Gastric	30	IHC	5-FU	No correlation		100
Male germ cell	28	SSCP	Cisplatin	Resistant		26
Osteosarcoma	32	IHC, LOH	MTX, cisplatin	No resistance	Resistance associated w/LOH	101
Stomach	11	IHC	Mitomycin C, 5-FU, ADR, cisplatin	Decreased sensitivity		16
Liver	13	IHC	Mitomycin C, 5-FU, ADR, cisplatin	Decreased sensitivity		16

ADR = adriamycin; MMS = methylmethanesulfonate; VP-16 = etoposide; IHC = immunohistochemistry; SSCP = single-stranded conformational polymorphism; LOH = loss of heterozygosity.

Table 3. Correlation between radiation sensitivity and p53 status in *in vitro* studies

	p53 status	Radiation response	Reference
Lymphoma/lymphoblastoma			
17 various cell lines	wt	Sensitive	121
	Null	Increased resistance	
Breast			
MCF-7	wt	Sensitive	102
MCF-7/ADR	mt	Increased resistance	
Thyroid			
various	wt	Sensitive	103
tumor derived	mt	Increased resistance	
primary cell lines	Null		
Ovarian			
A2780	Assumed wt	Sensitive	34
A2780 Transfectants	mt	Increased resistance	
Bladder			
MGH-U1	?	Resistant	34
RT112	mt	Resistant	
Teratoma			
SUSA	Assumed wt	Sensitive	34
GCT27	Assumed wt	Sensitive	
Neuroblastoma			
HX142			34
NB1	Assumed wt	Sensitive	
SK-N-SH	Assumed wt	Sensitive	
Glioma/glioblastoma			
U251	Assumed mt	Resistant	34
MOG-G-CCM	Assumed mt	Resistant	
MOG-G-UV	Assumed mt	Resistant	
IP-SB18	Assumed mt	Resistant	
T98G	Assumed mt	Resistant	
U87-MG	wt	Sensitive	36
U87-LUX.8	wt	Sensitive	
U87-175.4	mt	Increased resistance (role for loss of G_1 checkpoint suggested)	
U87-MG	wt	No difference in sensitivity	35
U87-LUX.8	wt	No difference in sensitivity	
U87-LUX.4	wt	No difference in sensitivity	
U87-175.4	mt	No difference in sensitivity	
U87-MG	mt	Variable response (apoptosis and cell growth)	104
A172	mt ⇒ wt		
U373 MG	mt ⇒ wt		
Head and neck			
24 different	wt and mt	No correlation with p53 status	105
16 different	wt and mt	wt more sensitive than mt	106
Normal human fibroblast cell lines			
Li-Fraumeni family members	wt/mt heterozygous	Increased resistance	32, 33
SV40 transformants			
AG1522	wt	Sensitive	107
AG1522-d10			
AG 1522-U24	wt/SV40		
AG 1522-SVYOT	(p53 binding deficient)	Increased resistance	

Continued

Table 3. *Continued*

	p53 status	Radiation response	Reference
Non-human cell lines			
murine thymocytes (transgenic)	wt wt/mt mt/mt	Sensitive Some increased resistance Resistant	29
murine thymocytes (transgenic)	wt wt/mt Null	Sensitive Some increased resistance Resistant	12
murine bone marrow/spleen (transgenic)	wt mt/mt	Sensitive Increased resistance	30
murine embryo fibroblasts (EIA/RAS transfectants)	wt wt/mt mt/mt	Sensitive Increased resistance Resistant	11
rat embryo fibroblasts (HPV E7/RAS transfectants)	wt mt	Sensitive Increased resistance	108
rat embryo fibroblasts (mt p53 transfectants)	wt mt	Sensitive Increased resistance	31

association between mtp53 and poor prognosis leading to the suggestion of Kovach *et al*. that p53 status may currently be the most clear-cut indicator of tumor recurrence in breast cancer.[41] A number of studies have also observed increased levels of mtp53 in patients with advanced, metastatic and hormone refractory prostate cancer who failed external beam radiotherapy.[42,43]

In cancers of the head and neck/upper aerodigestive tract, an association between p53 status and response to radiotherapy, as measured by treatment failure and survival,[44–48] has also been shown, indicating a higher rate of treatment failure in tumors carrying mtp53. There are also reports with similar findings in, among others, colon and lung cancers (Table 4).

At the same time, as indicated in Table 4, there are a few conflicting reports with prostate and head and neck cancers, stating that either there is no correlation between IHC p53(+) tumors and radiation responsiveness or that mtp53 results in an increased response to radiation therapy.[49–52]

Is the evidence strong enough to support a relationship between p53 and response to anticancer therapies?

Despite these disparate findings, the majority of evidence available in the literature and from the clinic supports a role for loss of functional p53 in resistance to cytotoxic anticancer therapies. There are a number of possible reasons for these apparent discrepancies.

One possible reason for the disparities may stem from the methodologies used to demonstrate the presence of mtp53.[53] Many of the reports examining the prognostic and therapeutic relationship between p53 status and chemotherapeutic and/or radiation response employ IHC to detect mtp53. However, positive IHC is not always an accurate gauge of p53 mutations, missing as many as 30%, including deletion, frameshift or nonsense mutations.[54] In addition, wtp53 protein accumulates in the nucleus in response to DNA damage, thus the elevated protein detected by IHC may reflect activation of wtp53 rather than mutation. Therefore, this misclassification may result in erroneous conclusions.

p53 status alone cannot determine if the pathway is intact. Other factors to be considered include the influence of alterations in other components of the pathway, as well as tumor stage, prior therapeutic treatment and the treatment regimen. These factors can play a significant role in the therapeutic response, particularly for chemotherapeutic studies. There may also be influence from tumor type and agent. Also yet to be established is the influence of other p53 family members.[55]

Another hypothesis to explain the discrepancies between the findings for and against a role for mtp53 in the resistance to anticancer drugs and radiation, suggests that since short-term assays are often used, the results of many of the published studies are not representative of true cell killing.[56] These short-term assays, such as dye uptake, growth inhibition or

Table 4. Correlation between p53 status and radiation responsiveness in clinical studies

	No. of samples in study	p53 analysis	Response	Comments	Reference
Colorectal carcinoma	27	IHC	Decreased radiation response	p53 expression detected in increased percent of tumors post-irradiation	109
Colon carcinoma	141	PCR sequencing	Decreased radiation response	Survival	110
Epidermoid carcinoma	64	IHC	Decreased radiation response	Chemo/radiotherapy, inferior outcome associated with p53 overexpression	111
Pancreatic	–	IHC	Decreased radiation response	Literature review	112
NSCLC[a]	34	SSCP[b]	Decreased response		113
NSCLC[a]	65	IHC	Decreased response	2 year local control	114
NSCLC[a]	30	SSCP[b]/seq.	No correlation with p53 status	Paclitaxel/radiotherapy	52
Cervical carcinoma	52	IHC	Decreased radiation response	Survival	115
Cervical carcinoma	101	IHC/mt-specific immunoabsorbance	No correlation with p53 status	Positive correlation with bcl-2 status	116
Breast	316	Sequencing	Decreased response	Survival	39
Breast	35	IHC	Decreased response	5-FU/radiotherapy	40
Prostate	26	IHC	Decreased response	Recurrent tumors	43
Prostate	54	IHC	Decreased response	Treatment failure	42
Prostate	60	IHC	No correlation with p53 status	Survival	49
SCCHN[c]	110	Sequencing	Decreased response	Increased failure	44
SCCHN[c]	69	IHC	Decreased response	Decreased survival; decreased time to recurrence	46
SCCHN[c]	73	IHC	Decreased response	Decreased survival; chemo/radiotherapy prognosis distinct from response	47
SCCHN[c]	111	IHC	No correlation with p53 status	Chemo/radiotherapy	51
SCCHN[c]	79	IHC	No correlation with recurrence	Trend to decreased survival	48
Laryngeal	70	IHC	Decreased response	Decreased survival	117
Laryngeal	44	IHC/SSCP[b]	Decreased response	Decreased survival; no correlation between IHC and SSCP	118
Laryngeal	178	IHC	No correlation with p53 status	Chemo/radiotherapy	119
Laryngeal	20	IHC/DGGE[d]	No correlation with p53 status	Discordance between IHC and DGGE	50
Esophageal	95	IHC	Decreased response	Chemo/radiotherapy	120
Esophageal	56	IHC	Decreased response	Chemo/radiotherapy	98

[a]Non-small cell lung carcinoma.
[b]Single-strand conformation polymorphism analysis.
[c]Squamous cell carcinoma of the head and neck.
[d]Denaturing gradient gell electrophresis.

viability, as exemplified by the XTT assay, are more influenced by rate rather than the overall level of cell death. As these assays do not take into account kinetic differences in cell death, they may lead to an incorrect assessment of overall cell killing, leading to the conclusion that apoptosis and the genes controlling it, in particular p53, play little or no role in the sensitization of these cells to chemotherapeutic agents and radiation.

In addition, the presence of p53-independent DNA damage response pathways also serve to make assessment of a relationship between p53 and the therapeutic response a complex issue.

p53 gene therapy sensitizes tumors

The development over the last few years of methods for the introduction of genes into cells *in vivo* has made gene therapy interventions a possibility. There are currently a number of approaches being taken towards the development of p53-based anticancer therapies (reviewed in 57) with the goal being to induce tumor growth inhibition, and to resensitize tumor cells to conventional radiation and chemotherapy (Figure 2).

One of the major obstacles to effective gene therapy is the development of an efficient gene delivery system. Current somatic gene therapy approaches employ either viral or non-viral vector systems. The introduction of wtp53 by various viral delivery systems, in particular retroviral and adenoviral vectors, has been reported to suppress, both *in vitro* and in mouse xenograft models, the growth of various types of malignancies, e.g. leukemia, prostate, head and neck, colon, cervical, glioblastoma, breast, liver, ovarian kidney and lung tumor cells.[58–68] However, p53 alone, while being able to partially inhibit tumor growth, has not been able to eliminate established tumors long-term. To augment the effects of p53, researchers are now combining p53-adenovirus gene therapy with traditional cytotoxic anticancer therapies.[1] For instance, the introduction of exogenous wtp53 into radiation-resistant tumor cells can affect the radiation survival level of these cells both *in vitro* and *in vivo*. Using adenovirus-mediated intratumoral delivery, it was shown that exogenous wtp53 could sensitize radiation resistant squamous cell carcinoma of the head and neck (SCCHN) cells to ionizing radiation *in vitro*.[69] This radiosensitivity carried over to an *in vivo* mouse xenograft model where complete xenograft tumor regression was maintained for up to 6 months post-treatment in animals receiving a combination of adeno-p53 and radiation.[69] This radiosensitization correlated with restoration of the G_1 checkpoint and apoptosis.[70] Similar results were found with intratumorally delivered adeno-p53 in a human colorectal cancer model.[71] In addition to reducing radiation survival *in vitro*, a significant increase in apoptosis was observed. This combination therapy also resulted in *in vivo* tumor growth delay in this xenograft mouse model.[71] Vaccinia virus bearing wtp53 (rVV-p53) has also been used in combination with radiation to treat s.c. tumors derived from radioresistant C6 rat glioma cells, resulting in significantly slower tumor progression compared to either rVV-p53 or radiation treatment alone.[72] Additionally, Fujiwara *et al.*[73] and Roth *et al.*[74] have demonstrated chemosensitization of lung cancers by restoration of wtp53. A synergistic effect of the combination of adenoviral-p53 and various chemotherapeutic agents in multiple tumor types in scid mice has also recently been reported by Gurnani *et al.*[75]

One of the principal drawbacks of viral delivery systems is their lack of specificity for cancer cells. Currently, none have tumor targeting capability, although investigations are ongoing to develop new

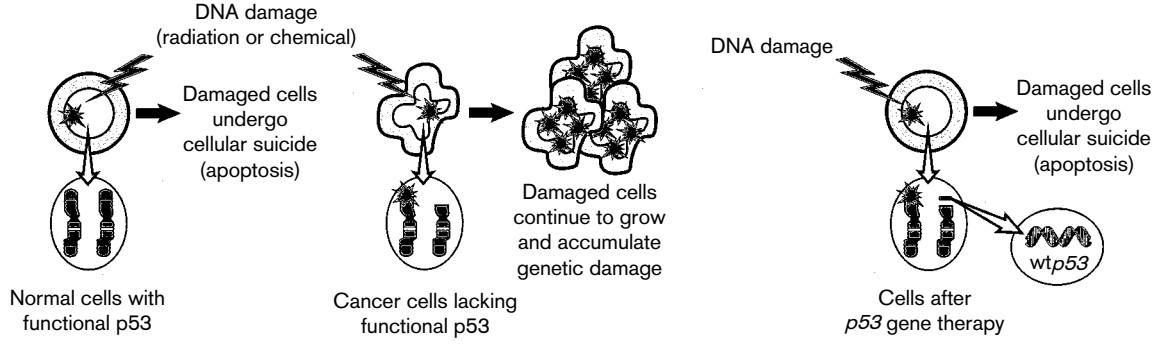

Figure 2. Restoration of p53 function via gene therapy restores sensitivity to DNA-damaging agents.

ways to alter viral coat proteins to increase tumor specificity.[76–79] The gene transfer efficiency of adenoviral vectors has been improved upon in two instances by taking advantage of receptor-mediated endocytosis.[80,81] Efforts are also underway using a bi-functional antibody directed against the adenoviral knob protein and fibroblast growth factor, to redirect adenoviral vectors to fibroblast growth factor receptors.[82,83]

Another promising approach to p53-directed gene therapy is ONYX-015, which is currently in phase I/II clinical trials.[1] This modified adenovirus contains a deletion in the E1B protein, required for viral replication in cells with functional p53. Thus, this adenovirus should be able to replicate within and lyse only those cells containing mtp53. In an *in vivo* nude mouse model, ONYX-015, in combination with cisplatin or 5-FU, proved to be more effective than either agent alone and was capable of lysing cells with acquired resistance to cytotoxic chemotherapy.[84]

Non-viral gene transfer vectors could circumvent some of the problems associated with using viral vectors (recently reviewed in 85). In particular, cationic liposome-mediated gene transfer systems appear to hold great promise. From the perspective of human cancer therapy, cationic liposomes have already been proven to be safe and efficient for *in vivo* gene delivery. More than 20 clinical trials are now underway using cationic liposomes for gene delivery[86] and liposomes for delivery of small molecule therapeutics (e.g. chemotherapeutic and antifungal agents) are already on the market.

One disadvantage of cationic liposomes is that they also lack tumor specificity and have relatively low transfection efficiencies as compared to viral vectors. However, by taking advantage of receptor-mediated endocytosis, this can be dramatically increased when the liposomes bear a ligand recognized by a cell surface receptor (Figure 3). A variety of ligands have

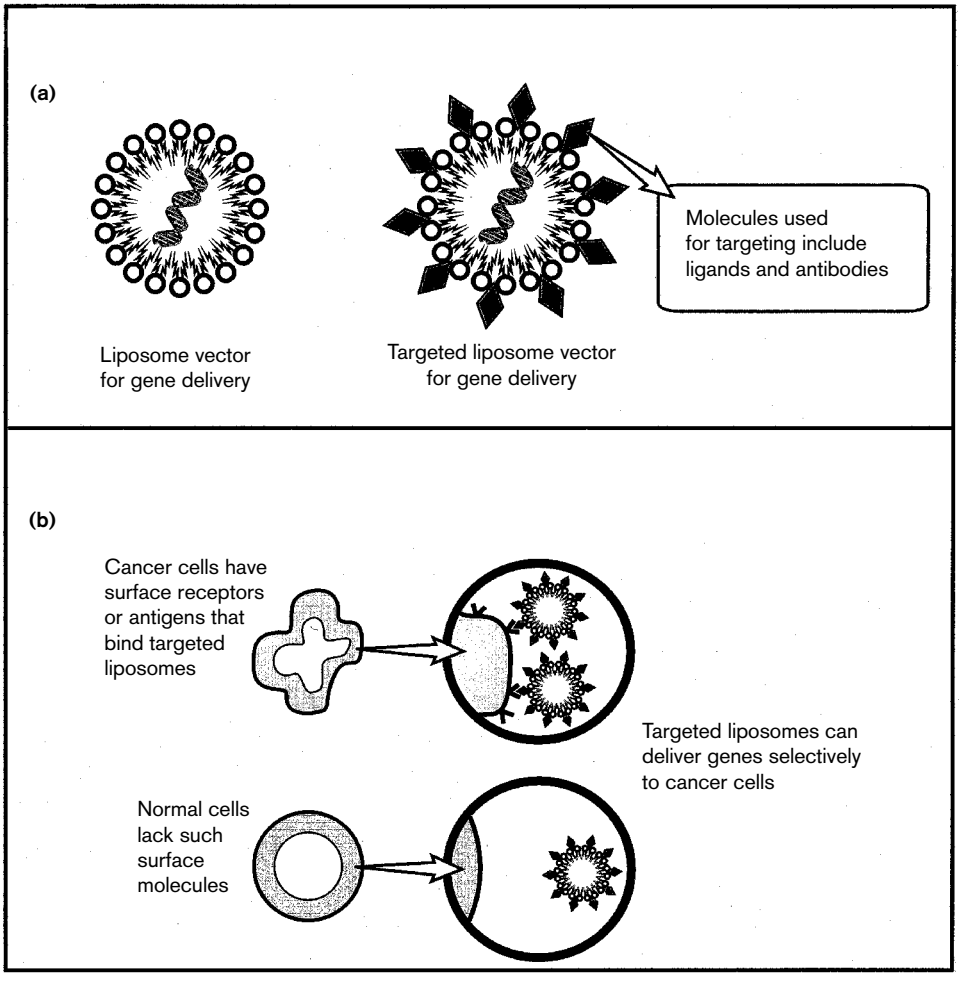

Figure 3. (a) Liposomes for gene therapy. (b) Targeting gene delivery via cellular receptors.

been examined for their liposome-targeting ability, including folate, a vitamin necessary for DNA synthesis, and the iron transport molecule transferrin. Both transferrin and folate receptor levels are elevated in various types of cancer including prostate, ovarian, oral, colon and breast, and correlate with the aggressiveness or proliferative ability of tumor cells. The folate and transferrin ligands have been successfully used to direct systemically delivered cationic liposome complexes preferentially to tumor cells *in vitro* and *in vivo*.[87–89] In *in vivo* studies the ligand–liposome complex carrying wtp53 was used in combination with radiotherapy resulting in total regression of a head and neck xenograft tumor for up to 18 months.[88,89] This increased radiation response correlated with an increase in p53-dependent apoptosis.[89]

Taken together, a majority of the literature currently available supports the premise that p53 is a critical factor in the response of cancer cells to cytotoxic anticancer therapies. Consequently, these findings point to the immense clinical potential of p53 replacement gene therapy as a means to enhance conventional anticancer therapies leading, in the foreseeable future, to new more effective treatment modalities.

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