

Therapeutic strategies for head and neck cancer based on *p53* status (Review)

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Abstract. Squamous cell carcinomas of the head and neck (HNSCC) are one of the most common types of cancers worldwide, and despite advances in treatment, they still represent a clinical challenge. Inactivation of one or more components in the *p53* signaling pathway is an extremely common event in human neoplasia, including HNSCC. The loss of *p53* function is responsible for increased aggressiveness in cancers, while tumor chemoresistance and radioresistance can depend on deleted *p53* expression, or on the expression of mutated-*p53* proteins. Thus, consideration and manipulation of the *p53* status during HNSCC cancer therapy should be considered. This review discusses the *p53* signaling pathways activated by various cellular stresses, including exposure to cancer therapies. The recognition of the *p53* status in cancer cells is a significant factor and could provide valuable assistance during the selection of an effective therapeutic approach.

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1. Introduction

HNSCC is the sixth most common type of cancer worldwide. More than 49,000 new cases of HNSCC cancer are predicted to have occurred in the US in 2010 (1). The disease is multifactorial in its pathogenesis and is associated with smoking, alcohol and infection with the human papillomavirus (HPV) (2). However, the abrogation of *p53* function is one of the most common molecular alterations in human cancer cells, including HNSCC (3-5). The prognosis for patients with tumors bearing *p53* mutations is often worse than for those with tumors lacking a wild-type *p53* (*wt-p53*) gene (6). For predictive assays, which can be used to evaluate prognosis in cancer therapy, the genetic status of the *p53* gene is one of the most critical candidates among various cancer-related genes (7). In addition, the spectrum of *p53* deletions or mutations observed among tumor cells suggests that the mutations vary in their prognostic power. Disruptive *p53* mutations in tumor DNA are reported to be associated with reduced survival following surgical treatment of HNSCC (2).

It has been previously reported that the radio-, heat- and chemo-sensitivities of HNSCC cells are *p53*-dependent, and are closely correlated with induction of apoptosis *in vitro* (8-10) and *in vivo* (11-13). Consequently, the restoration of *wt-p53* function and *p53*-independent therapy have been developed as therapeutic strategies to target tumors with abrogated *wt-p53* functions.

In this review, cancer therapies aimed at targeting signaling pathways controlled by *p53* are described. These include *p53*-gene therapy, chemical chaperones, *p53* C-terminal peptides and small molecules that can target *p53*. In addition, therapeutic strategies independent of *p53* status in cancer cells are discussed. These include high-linear energy transfer (LET) heavy-ion radiation, and enhancement of cancer therapies with other strategies, including an RNA-silencing therapy targeted at DNA repair pathways, and a molecular-targeting therapy for the survival pathway Akt-mTOR.

2. The *p53* signaling pathway is activated by various cellular stresses

The *p53* protein was identified in simian virus 40 (SV40) transformed cells where it is associated with the large T antigen (14),

and was initially considered to be an oncogene. Subsequently, the *p53* gene was revealed to be mutated in various human tumors (15), while its protein product was reported to act as a tumor suppressor (16). *p53*-deleted and *p53*-mutated cells make up approximately 50% of the cells in advanced cancers (17).

p53 is normally in 'standby' mode. The *p53* protein is a powerful transcription factor and plays a pivotal role in the pathway controlling apoptosis, cell growth and cell proliferation in response to cellular stresses. These include genotoxic and non-genotoxic stresses, such as DNA damage, hypoxia, oncogene overexpression and viral infection (18-20).

The p21/WAF1 (wtp53 activated fragment 1) gene product, a *p53* target gene, inactivates the proliferating cell nuclear antigen (PCNA), which can regulate DNA replication (21), and induce a *p53*-dependent G1 arrest through the inhibition of cyclin/CDK activity (22,23). During cell cycle arrest, *p53*-regulated pathways, including those involving growth arrest and DNA damage inducible 45 (*Gadd45*) and the *p53* ribonucleotide reductase small subunit 2 (*p53R2*), are significant in the repair of damaged DNA (24,25). In the absence of competent repair activity, DNA damage induces apoptosis through interactions with other genes in *p53*-regulated pathways, including the Bcl-2-associated X protein (Bax) (26), *p53*-upregulated modulator of apoptosis (PUMA) (27), Fas/APO-1 (28) and *p53*-activated gene 608 (PAG608) (29). By contrast, the *p53*-regulated MDM2 (murine double minute 2) (30) functions to produce negative feedback, which regulates *p53* activity (26).

In the presence of cellular stresses, *p53* is subjected to a complex and diverse array of covalent post-translational modifications. These include phosphorylation (31), acetylation (32), poly(ADP-ribosyl)ation (33), ubiquitylation and sumoylation (34,35). In response to cellular stress, Ser15/Ser20 in *p53* are phosphorylated and MDM2 is separated from the phosphorylated *p53*, leading to the stabilization and activation of *p53* (36-38). Therefore, *p53* can bind to the promoter of the *p21* or *p53R2* genes associated with DNA repair, and induce their expression. However, if there are numerous DNA lesions or too much cellular damage, G1 arrest and DNA repair will not be successful. In this situation, *p53* can be phosphorylated at Ser46, and bind to the promoter of the *p53*-regulated apoptosis-inducing protein 1 (*p53AIP1*) gene, leading to apoptosis (39,40). Moreover, PUMA is reported to be required not only for *p53*-dependent apoptosis induced by DNA damage, hypoxia and oncogenes, but also for apoptosis induced by *p53*-independent stimuli, including serum withdrawal, glucocorticoids, kinase inhibitors and phorbol esters (27). By contrast, *p53* molecules are inactivated and degraded by activated MDM2 molecules, which are phosphorylated at multiple sites by other protein kinases. In addition, *p53* is reported to bind to other proteins, including heat shock proteins (HSPs), functioning as stress proteins (41,42), and Bcl-X (43). Consequently, these modifications of *p53* molecules can regulate or affect the fate of cells following exposure to stresses, including cancer therapies.

3. *p53*-dependent cancer therapy via the restoration of *p53* function

Recently, Poeta *et al* reported an association between a *p53* mutation in a patient with HNSCC and survival following

surgical treatment (2). The results demonstrated that *p53*-deleted and *p53*-mutated HNSCC patients were significantly associated with short survival periods. These data indicate that *p53* mutations could be a useful evaluation or stratification factor in prospective clinical trials. However, in the study, chemotherapy was administered only as an adjuvant measure in combination with postoperative radiation therapy, or prior to study entry in a few cases. There are no data on tumor response to chemotherapy. It would be clinically useful to determine whether *p53* mutations are associated with a response to treatments that attack *p53*-specific pathways. A study described that sensitization to radiation, heat and chemical therapies was observed in cells containing wtp53, but not in cells containing mutated *p53* (mp53) *in vitro* and *in vivo* (8-10). Furthermore, in attempts to treat cancer using more than one treatment modality, a synergistic depression of tumor growth was found only in tumors containing wtp53 (44). These findings suggest that hyperthermic enhancement of tumor growth inhibition with irradiation may result in *p53*-dependent apoptosis via caspase-3 activation in HNSCC cells. Therefore, an analysis of *p53*-gene status in cancer cells could be considered as a useful predictive assay for estimating the possible effectiveness of combined therapies involving radiation, heat and anti-cancer agents. Thus, it is very reasonable to enhance *p53*-dependent apoptosis pathways through the restoration of *p53* function even for mp53 HNSCC cells as a more effective therapeutic strategy. A number of approaches have been employed to achieve this outcome (illustrated in Fig. 1).

A p53 gene therapy-based approach. As previously mentioned, the activation of endogenous wtp53 by radiation and/or chemotherapy in wtp53 cancer cells leads to *p53*-mediated apoptosis. In recent years, the introduction of exogenous wtp53 into cancer cells, either by gene delivery or by direct protein delivery, has been explored. Although preliminary studies in cell cultures and in animal models have indicated the effectiveness and the low toxicity of these approaches (45-47), their efficacy in clinical trials is currently controversial. Clinical studies in lung, bladder, ovarian and breast cancer revealed the absence of additional beneficial effects compared to conventional treatments (48). On the other hand, encouraging results were reported for phase II and III clinical trials on 135 patients with advanced HNSCC. In this study, patients were treated with a combination of recombinant adenovirus-*p53* (Gendicine) administration and radiotherapy. The results demonstrated that 64% of the patients achieved complete regression, and 32% achieved partial regression. No serious side effects were observed (49). Although such results are encouraging, further improvements in methods are required to accomplish the safe and effective delivery of wtp53 *in vivo* (50).

Onyx-015, an adenovirus based therapy. In the absence of wtp53 activity in cancer cells, the generation of a mutated viral vector for tumor cell lysis (Onyx-015) was exploited. McCormick *et al* from Onyx Pharmaceuticals hypothesized that an adenovirus with the *E1B* region deleted could only replicate and generate cellular lysis in cells lacking functional *p53*, due to the putative requirement for *p53* inactivation for adenoviral replication. Accordingly, the Onyx-015 reagent, a *p53*-targeting oncolytic mutant adenovirus, has been

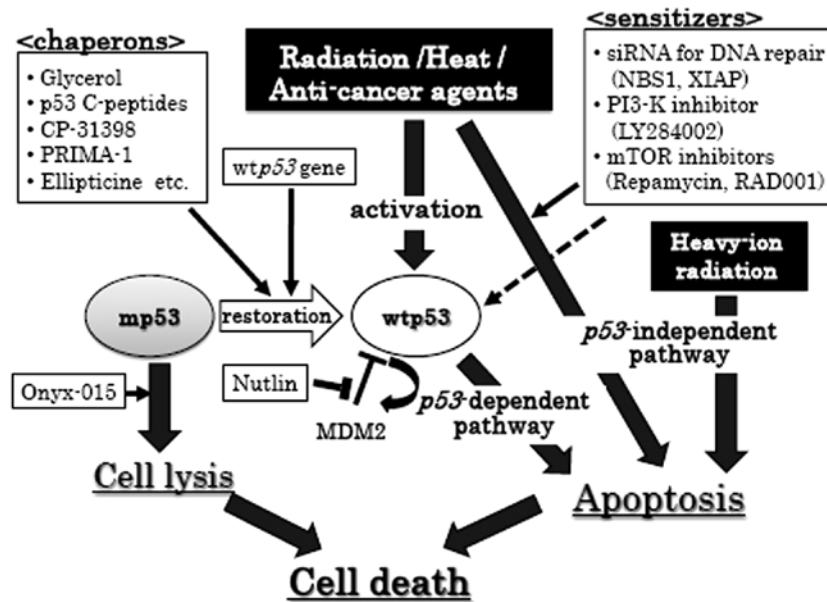


Figure 1. *p53*-dependent and -independent therapeutic strategies for cancer cells. Circles, *p53* status of cancer cells; black squares, cancer therapeutic tool; white squares, enhancer for cancer therapeutic tool; thin arrows, enhancement; dashed arrows, partial enhancement; thick arrows, therapeutic pathway. MDM2, murine double minute 2; XIAP, X-chromosome-linked inhibitor of apoptosis protein; wtp53, wildtype *p53*; mp53, mutant *p53*; siRNA, small interference RNA.

developed for clinical application (51). However, evaluation of numerous clinical trials performed thus far have indicated that the administration of Onyx-015 as a single agent produces only a marginal benefit, whereas its administration in combination with conventional therapy is more effective (52).

Chaperones for the restoration of the *p53* molecule

Glycerol. Another approach in preclinical development involves restoring tumor-suppressing function to mp53. Studies have demonstrated that glycerol, as a chemical chaperone, can restore normal *p53* function in mp53 HNSCC cells (53). Glycerol has been previously reported to act as a chemical chaperone due to its ability to refold proteins and restore normal activity; this type of activity was able to alter or restore a functional protein conformation from conformation forms found in a human disease state (54,55). Consistent with this observation, glycerol is capable of restoring *p53*-dependent radiosensitivity in mp53-HNSCC cells via Bax-mediated induction of apoptosis (53,56,57). Glycerol can also restore heat-induced *p53*-dependent apoptosis in A-172/mp53 cells (58) and CDDP-induced tumor growth inhibition in 8305c (59) and SAS/mp53 cells (13), by binding to *p53* consensus sequences (*p53*CON) located upstream of *p53*-regulated genes. These results suggest that glycerol could be effective in causing conformational changes that restore wtp53 functioning to mp53, leading to enhanced results with radio-, hyperthermic- and/or chemo-therapies through the induction of apoptosis via a restored wtp53 function.

***p53* C-terminal peptides.** One of the significant features of *p53* tumor-suppressor activity is its ability to bind to *p53*CON; the majority of mutations in *p53* are localized in the binding domain of *p53*CON (60,61). This sequence-specific DNA-binding ability of *p53* is allosterically regulated by its C-terminal domain (62,63), and can be activated *in vitro*

by C-terminal truncation or anti-*p53* monoclonal antibody PAb421 binding, which recognizes a C-terminal epitope (64). In addition, small peptides corresponding to the C-terminal residues 369-383 of *p53* are capable of activating latent *p53*, and permit specific DNA-binding *in vitro* (65). Thus, the sequence-specific DNA binding activity of mp53 proteins could be rescued by PAb421 (62,66,67). The inhibition of cell proliferation (68) and the induction of apoptosis (69) were effectively induced in mp53 cancer cells transfected with C-terminal peptides following X-ray-irradiation or heat-treatment (70,71).

CP-31398 and other small molecules. In other studies evaluating binding to *p53*CON sequences, CP-31398 was identified as a small molecule with the ability to restore the wild-type conformation to mp53 protein by stabilizing the active conformation of the DNA binding domain (72,73). Further studies have confirmed that CP-31398 treatment causes: i) stabilization of wtp53 levels; ii) apoptosis-related changes; and iii) induction of *p53* target genes. Moreover, CP-31398 was demonstrated to increase the levels of wtp53 protein by inhibiting the MDM2-mediated ubiquitylation and degradation of *p53* (73). The observation that CP-31398 stabilizes wtp53 suggests that CP-31398 interacts with newly synthesized *p53* molecules *in vivo* and changes its folding behavior (74). Subsequently, PRIMA-1 (75) and ellipticine (76) were also found to be capable of inducing mp53-dependent cell death. On the other hand, Nutlin was developed to rescue wtp53 from degradation mediated by MDM2 (77). More recently, *p53* family members could be activated, were capable of serving as substitutes for *p53* in tumor cells, and were able to induce cell death. These observations may provide a novel tool for the correction of mp53 conformation and loss of function, and may be applicable to *p53*-targeted cancer therapy.

4. *p53*-independent cancer therapy

High-LET heavy-ion radiation. High-LET charged particle radiation has several potential advantages over X-rays, including an excellent dose distribution, a higher relative biological effectiveness (RBE), a reduction in the oxygen enhancement ratio, less variation in cell cycle-related radiosensitivity, and the existence of less efficient repair mechanisms for cellular radiation injury (78-80). As a result, high-LET charged particle radiation could have serious lethal effects, even on radioresistant tumors (81). Heavy ion beams can also allow a high radiation dose to be delivered to a tumor with minimal irradiation of the surrounding normal tissues. High-LET radiation also induces apoptosis effectively regardless of the genetic status of the *p53* gene in cancer cells (82,83). Heavy ion radiotherapies consequently appear to be attractive for use in numerous types of human cancer. The lack of a *p53*-regulated pathway is a common feature in a large number of tumors, suggesting that it is a significant factor in the pathogenesis of human cancers. As previously reported, cellular sensitivity to radiation and/or heat depends on the *p53*-gene status in HNSCC cells (9,18) and other cells (82,84). Therefore, the aim of a number of studies has been to induce apoptosis by reinstating normal functioning of the *mp53* gene (58). However, it has not been practical in the clinic to monitor the status of the *p53* gene or other useful genetic markers. Thus, attention has been given to therapies using high-LET radiation, which have highly lethal effects on radioresistant tumors (81), and which can induce apoptosis effectively regardless of the *p53*-gene status (82,83). It has been suggested that high-LET radiation delivered to HNSCC cells may enhance apoptosis through the activation of caspase-3 through caspase-9, even in the presence of *mp53* (85).

RNA-silencing therapy targeting DNA repair pathway. RNA interference has become a valuable tool for the selective suppression of the expression of a target gene. The mRNAs produced by a targeted gene are cleaved by an RNA-induced silencing complex, which includes small interference RNAs (siRNAs) and a nuclease. Cell cycle signaling or DNA repair proteins, including ATM, ATR and DNA-PKcs, have become the targets of interest in investigations involving the siRNA-mediated enhancement of radiation sensitivity (86,87). Studies have demonstrated the potential use of siRNA as a novel radiation sensitizer for improving the effectiveness of radiation therapy in cancer.

The NBS1 protein is essential for the initial processing of the DNA double-strand break via the homologous recombination (HR) repair pathway (88,89). NBS1 forms a complex with MRE11 and RAD50 in the nucleus. This complex binds to ATM-phosphorylated γ H2AX and is recruited to the area surrounding damaged DNA ends (90). MRE11/RAD50/NBS1 complexes can be visualized in the form of foci in an irradiated area of the nucleus (91). Studies indicate that NBS1 appears to regulate radiation sensitivity in cells through its role in the HR repair system. In addition, it has recently been demonstrated that the siRNA-mediated reduction of NBS1 appears to lead to an increase in radiation-induced mutagenesis in human cells (92).

Ionizing radiation induces a signaling pathway which activates the transcription factor NF- κ B. NF- κ B then regulates the

transcriptional activation of a number of genes involved in cell proliferation, angiogenesis, metastasis and the suppression of apoptosis (93). Therefore, the radiation-induced activation of NF- κ B could promote oncogenesis and resistance to cancer therapy (94,95). Moreover, it is possible that the NBS1 protein may play a role in the NF- κ B pathway, which is activated by radiation; NBS1-deficient cells exhibit a defective activation of NF- κ B following exposure to radiation (96). Thus, inhibition of NBS1 could result in depression of NF- κ B activation and in the transcriptional activation of NF- κ B-regulated genes. One of the proteins regulated by NF- κ B, X-chromosome-linked inhibitor of apoptosis protein (XIAP), plays a pivotal role in cancer progression, and is a strong candidate among cancer therapy targets (97).

Sensitization to radiation results from *NBS1*-siRNA mediated suppression of DNA repair functions and X-ray-induced cell survival signaling pathways, which operate through NF- κ B and XIAP (98). NBS1 is also involved in the heat-induced cellular responses to DNA damage, and it has been suggested that *NBS1*-siRNA is a potential candidate for a sensitizer for heat treatments, which could be effective regardless of cellular *p53*-gene status (99,100). Moreover, siRNA that can target *XIAP* can lead to an effective enhancement of X-ray-induced apoptosis in human cancer cells with *mp53* (98,101). The results described in these studies suggest that siRNA designed to target DNA repair functions could lead to novel methods that could increase radiation and/or heat sensitivity, even in human *mp53*-bearing cancer cells.

Molecular-targeting therapy for Akt-mTOR pathway. The PI3K/Akt pathway is a major cell survival pathway and plays a critical role in oncogenesis and tumor cell growth (102). Recent studies have reported that Akt activation contributes to resistance to radiation, chemotherapy and tyrosine kinase inhibitors by promoting survival signals, which protect cancer cells from undergoing apoptosis (103,104). The inhibition of PI3K/Akt through pharmacological or genetic means induces anti-proliferative effects in HNSCC cells *in vitro* and *in vivo* (105,106). Akt is activated by heat and radiation through a phosphatidylinositol-3-kinase (PI3K)-mediated phosphorylation pathway (107). Radio-sensitization induced by LY294002, a specific inhibitor of PI3K, has been reported in *in vitro* (108) and *in vivo* experiments (109). LY294002 inhibits anti-apoptosis signaling through the induction of Hsp27 and Hsp72, and cell survival signaling through Akt and survivin. LY294002 appears to be a noteworthy candidate as a *p53*-independent heat sensitizer in hyperthermic cancer therapy (110).

The mammalian target of rapamycin, mTOR, is a 289-kDa serine-threonine kinase, which acts as a downstream effector for Akt (111). It regulates key processes, including cell growth and proliferation, cell cycle progression and protein translation through two distinct pathways; one involving the ribosomal p70S6 kinase (p70S6K), and one involving the eukaryotic translation initiation factor 4E (eIF4E) binding proteins (4E-BPs) (112). Akt activation is closely associated with the upregulation of mTOR activity. It has been suggested that dysregulation of mTOR contributes to cancer progression (111), and therefore, mTOR may be a potential therapeutic target which could inhibit or block the PI3K/Akt pathway. Inhibitors

of mTOR are currently under development; rapamycin and its derivatives CCI-770, AP23573 and RAD001. The anti-proliferative effects of mTOR inhibitors have been observed in various tumor cells *in vitro* and *in vivo* (113-115). These mTOR inhibitors are generally regarded as cytostatic agents, since they induce G1 cell cycle arrest, but not apoptosis (113). However, recent studies have demonstrated that mTOR inhibitors can enhance the cytotoxic effects of chemotherapeutic agents and radiation in a number of human cancers (116-118). Furthermore, a study has demonstrated that rapamycin in combination with radiation was able to augment the cytostatic effects of radiation regardless of cellular *p53*-gene status in lung cancer and HNSCC cells, suggesting that inhibition of the mTOR signaling may be a promising strategy for radio-sensitization regardless of *p53*-gene status, with respect to cell lethality and cell growth depression (119).

5. Conclusion

Over the past few decades, despite the introduction of new multimodal therapies, there has been a failure to achieve a high efficacy in tumor therapy. This may be caused by a primary or acquired resistance to the DNA damaging agents used in chemotherapy and radiotherapy, and remains a formidable and poorly understood problem. This review discussed the effect of *p53*-targeting cancer therapies on *p53* signaling pathways, including *p53*-gene therapy, chemical chaperones, *p53* C-terminal peptides, *p53*-targeting chemicals and inhibitors targeting several signaling pathways, in an effort to induce cell death in cancer cells. High-LET radiation can induce apoptosis effectively regardless of the genetic status of the *p53*-gene in cancer cells. Identification of the *p53* status in the target cells is imperative, and the knowledge of additional oncogenic events contributing to specific types of cancer could significantly aid in the selection of appropriate therapeutic protocols. The restoration of *p53* functioning could be helpful when pathways upstream of *p53* expression are defective, but not if defects are downstream of *p53* signaling. The re-expression and re-activation of *p53* in human cancer cells could increase tumor susceptibility to radiation or chemotherapy, enhance the efficacy of standard therapeutic protocols, and lead to individually designed therapies (52). Further investigations in this area will hopefully lead to more effective cancer treatments in the near future.

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References

- Jemal A, Siegel R, Xu J and Ward E: Cancer statistics, 2010. *CA Cancer J Clin* 60: 277-300.
- Poeta ML, Manola J, Goldwasser MA, *et al*: TP53 mutations and survival in squamous-cell carcinoma of the head and neck. *N Engl J Med* 357: 2552-2561, 2007.
- Vogelstein B, Lane D and Levine AJ: Surfing the *p53* network. *Nature* 408: 307-310, 2000.
- Guimaraes DP and Hainaut P: TP53: a key gene in human cancer. *Biochimie* 84: 83-93, 2002.
- Gasco M and Crook T: The *p53* network in head and neck cancer. *Oral Oncol* 39: 222-231, 2003.
- Lowe SW: Cancer therapy and *p53*. *Curr Opin Oncol* 7: 547-553, 1995.
- Velculescu VE and El-Deiry WS: Biological and clinical importance of the *p53* tumor suppressor gene. *Clin Chem* 42: 858-868, 1996.
- Ota I, Ohnishi K, Takahashi A, *et al*: Transfection with mutant *p53* gene inhibits heat-induced apoptosis in a head and neck cell line of human squamous cell carcinoma. *Int J Radiat Oncol Biol Phys* 47: 495-501, 2000.
- Takahashi A: Different inducibility of radiation- or heat-induced *p53*-dependent apoptosis after acute or chronic irradiation in human cultured squamous cell carcinoma cells. *Int J Radiat Biol* 77: 215-224, 2001.
- Ohnishi K, Ota I, Takahashi A, Yane K, Matsumoto H and Ohnishi T: Transfection of mutant *p53* gene depresses X-ray- or CDDP-induced apoptosis in a human squamous cell carcinoma of the head and neck. *Apoptosis* 7: 367-372, 2002.
- Asakawa I, Yoshimura H, Takahashi A, *et al*: Radiation-induced growth inhibition in transplanted human tongue carcinomas with different *p53* gene status. *Anticancer Res* 22: 2037-2043, 2002.
- Tamamoto T, Yoshimura H, Takahashi A, *et al*: Heat-induced growth inhibition and apoptosis in transplanted human head and neck squamous cell carcinomas with different status of *p53*. *Int J Hyperthermia* 19: 590-597, 2003.
- Yuki K, Takahashi A, Ota I, *et al*: Sensitization by glycerol for CDDP-therapy against human cultured cancer cells and tumors bearing mutated *p53* gene. *Apoptosis* 9: 853-859, 2004.
- Lane DP and Crawford LV: T antigen is bound to a host protein in SV40-transformed cells. *Nature* 278: 261-263, 1979.
- Nigro JM, Baker SJ, Preisinger AC, *et al*: Mutations in the *p53* gene occur in diverse human tumour types. *Nature* 342: 705-708, 1989.
- Finlay CA, Hinds PW and Levine AJ: The *p53* proto-oncogene can act as a suppressor of transformation. *Cell* 57: 1083-1093, 1989.
- Hollstein M, Sidransky D, Vogelstein B and Harris CC: *p53* mutations in human cancers. *Science* 253: 49-53, 1991.
- Efeyan A and Serrano M: *p53*: guardian of the genome and policeman of the oncogenes. *Cell Cycle* 6: 1006-1010, 2007.
- Slee EA, O'Connor DJ and Lu X: To die or not to die: how does *p53* decide? *Oncogene* 23: 2809-2818, 2004.
- Vousden KH and Lu X: Live or let die: the cell's response to *p53*. *Nat Rev Cancer* 2: 594-604, 2002.
- Bambara RA and Jessee CB: Properties of DNA polymerases delta and epsilon, and their roles in eukaryotic DNA replication. *Biochim Biophys Acta* 1088: 11-24, 1991.
- El-Deiry WS, Tokino T, Velculescu VE, *et al*: WAF1, a potential mediator of *p53* tumor suppression. *Cell* 75: 817-825, 1993.
- Deng C, Zhang P, Harper JW, Elledge SJ and Leder P: Mice lacking p21CIP1/WAF1 undergo normal development, but are defective in G1 checkpoint control. *Cell* 82: 675-684, 1995.
- Kastan MB, Zhan Q, el-Deiry WS, *et al*: A mammalian cell cycle checkpoint pathway utilizing *p53* and GADD45 is defective in ataxia-telangiectasia. *Cell* 71: 587-597, 1992.
- Smith ML, Kontny HU, Zhan Q, Sreenath A, O'Connor PM and Fornace AJ Jr: Antisense GADD45 expression results in decreased DNA repair and sensitizes cells to u.v.-irradiation or cisplatin. *Oncogene* 13: 2255-2263, 1996.
- Miyashita T and Reed JC: Tumor suppressor *p53* is a direct transcriptional activator of the human bax gene. *Cell* 80: 293-299, 1995.
- Yu J and Zhang L: No PUMA, no death: implications for *p53*-dependent apoptosis. *Cancer Cell* 4: 248-249, 2003.
- Owen-Schaub LB, Zhang W, Cusack JC, *et al*: Wild-type human *p53* and a temperature-sensitive mutant induce Fas/APO-1 expression. *Mol Cell Biol* 15: 3032-3040, 1995.
- Israeli D, Tessler E, Haupt Y, *et al*: A novel *p53*-inducible gene, PAG608, encodes a nuclear zinc finger protein whose overexpression promotes apoptosis. *EMBO J* 16: 4384-4392, 1997.
- Haupt S, Louriya-Hayon I and Haupt Y: *p53* licensed to kill? Operating the assassin. *J Cell Biochem* 88: 76-82, 2003.
- Barak Y and Oren M: Enhanced binding of a 95 kDa protein to *p53* in cells undergoing *p53*-mediated growth arrest. *EMBO J* 11: 2115-2121, 1992.
- Brooks CL and Gu W: Ubiquitination, phosphorylation and acetylation: the molecular basis for *p53* regulation. *Curr Opin Cell Biol* 15: 164-171, 2003.

33. Valenzuela MT, Guerrero R, Nunez MI, *et al*: PARP-1 modifies the effectiveness of p53-mediated DNA damage response. *Oncogene* 21: 1108-1116, 2002.
34. Schmidt D and Muller S: Members of the PIA5 family act as SUMO ligases for c-Jun and p53 and repress p53 activity. *Proc Natl Acad Sci USA* 99: 2872-2877, 2002.
35. Melchior F and Hengst L: SUMO-1 and p53. *Cell Cycle* 1: 245-249, 2002.
36. Siliciano JD, Canman CE, Taya Y, Sakaguchi K, Appella E and Kastan MB: DNA damage induces phosphorylation of the amino terminus of p53. *Genes Dev* 11: 3471-3481, 1997.
37. Shieh SY, Ahn J, Tamai K, Taya Y and Prives C: The human homologs of checkpoint kinases Chk1 and Cds1 (Chk2) phosphorylate p53 at multiple DNA damage-inducible sites. *Genes Dev* 14: 289-300, 2000.
38. Urban G, Golden T, Aragon IV, *et al*: Identification of a functional link for the p53 tumor suppressor protein in dexamethasone-induced growth suppression. *J Biol Chem* 278: 9747-9753, 2003.
39. Oda K, Arakawa H, Tanaka T, *et al*: p53AIP1, a potential mediator of p53-dependent apoptosis, and its regulation by Ser-46-phosphorylated p53. *Cell* 102: 849-862, 2000.
40. Saito S, Goodarzi AA, Higashimoto Y, *et al*: ATM mediates phosphorylation at multiple p53 sites, including Ser(46), in response to ionizing radiation. *J Biol Chem* 277: 12491-12494, 2002.
41. Ohnishi T, Matsumoto H, Takahashi A, Shimura M and Majima HJ: Accumulation of mutant p53 and hsp72 by heat treatment, and their association in a human glioblastoma cell line. *Int J Hyperthermia* 11: 663-671, 1995.
42. Matsumoto H, Wang X and Ohnishi T: Binding between wild-type p53 and hsp72 accumulated after UV and gamma-ray irradiation. *Cancer Lett* 92: 127-133, 1995.
43. Petros AM, Gunasekera A, Xu N, Olejniczak ET and Fesik SW: Defining the p53 DNA-binding domain/Bcl-x(L)-binding interface using NMR. *FEBS Lett* 559: 171-174, 2004.
44. Takahashi A, Ota I, Tamamoto T, *et al*: p53-dependent hyperthermic enhancement of tumour growth inhibition by X-ray or carbon-ion beam irradiation. *Int J Hyperthermia* 19: 145-153, 2003.
45. Fujiwara T, Cai DW, Georges RN, Mukhopadhyay T, Grimm EA and Roth JA: Therapeutic effect of a retroviral wild-type p53 expression vector in an orthotopic lung cancer model. *J Natl Cancer Inst* 86: 1458-1462, 1994.
46. Scardigli R, Bossi G, Blandino G, Crescenzi M, Soddu S and Sacchi A: Expression of exogenous wt-p53 does not affect normal hematopoiesis: implications for bone marrow purging. *Gene Ther* 4: 1371-1378, 1997.
47. Bossi G, Mazzaro G, Porrello A, Crescenzi M, Soddu S and Sacchi A: Wild-type p53 gene transfer is not detrimental to normal cells in vivo: implications for tumor gene therapy. *Oncogene* 23: 418-425, 2004.
48. Vecil GG and Lang FF: Clinical trials of adenoviruses in brain tumors: a review of Ad-p53 and oncolytic adenoviruses. *J Neurooncol* 65: 237-246, 2003.
49. Pearson S, Jia H and Kandachi K: China approves first gene therapy. *Nat Biotechnol* 22: 3-4, 2004.
50. Bossi G, Lapi E, Strano S, Rinaldo C, Blandino G and Sacchi A: Mutant p53 gain of function: reduction of tumor malignancy of human cancer cell lines through abrogation of mutant p53 expression. *Oncogene* 25: 304-309, 2006.
51. Bischoff JR, Kirn DH, Williams A, *et al*: An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science* 274: 373-376, 1996.
52. Bossi G and Sacchi A: Restoration of wild-type p53 function in human cancer: relevance for tumor therapy. *Head Neck* 29: 272-284, 2007.
53. Ohnishi K, Ota I, Takahashi A and Ohnishi T: Glycerol restores p53-dependent radiosensitivity of human head and neck cancer cells bearing mutant p53. *Br J Cancer* 83: 1735-1739, 2000.
54. Welch WJ and Brown CR: Influence of molecular and chemical chaperones on protein folding. *Cell Stress Chaperones* 1: 109-115, 1996.
55. Thomas PJ, Qu BH and Pedersen PL: Defective protein folding as a basis of human disease. *Trends Biochem Sci* 20: 456-459, 1995.
56. Ohnishi K, Ota I, Yane K, *et al*: Glycerol as a chemical chaperone enhances radiation-induced apoptosis in anaplastic thyroid carcinoma cells. *Mol Cancer* 1: 4, 2002.
57. Imai Y, Ohnishi K, Yasumoto J, *et al*: Glycerol enhances radiosensitivity in a human oral squamous cell carcinoma cell line (Ca9-22) bearing a mutant p53 gene via Bax-mediated induction of apoptosis. *Oral Oncol* 41: 631-636, 2005.
58. Ohnishi T, Ohnishi K and Takahashi A: Glycerol restores heat-induced p53-dependent apoptosis of human glioblastoma cells bearing mutant p53. *BMC Biotechnol* 2: 6, 2002.
59. Yuki K, Takahashi A, Ota I, *et al*: Glycerol enhances CDDP-induced growth inhibition of thyroid anaplastic carcinoma tumor carrying mutated p53 gene. *Oncol Rep* 11: 821-824, 2004.
60. Bargonetti J, Friedman PN, Kern SE, Vogelstein B and Prives C: Wild-type but not mutant p53 immunopurified proteins bind to sequences adjacent to the SV40 origin of replication. *Cell* 65: 1083-1091, 1991.
61. Cho Y, Gorina S, Jeffrey PD and Pavletich NP: Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. *Science* 265: 346-355, 1994.
62. Halazonetis TD and Kandil AN: Conformational shifts propagate from the oligomerization domain of p53 to its tetrameric DNA binding domain and restore DNA binding to select p53 mutants. *EMBO J* 12: 5057-5064, 1993.
63. Hupp TR, Meek DW, Midgley CA and Lane DP: Regulation of the specific DNA binding function of p53. *Cell* 71: 875-886, 1992.
64. Hupp TR and Lane DP: Allosteric activation of latent p53 tetramers. *Curr Biol* 4: 865-875, 1994.
65. Hupp TR, Sparks A and Lane DP: Small peptides activate the latent sequence-specific DNA binding function of p53. *Cell* 83: 237-245, 1995.
66. Hupp TR, Meek DW, Midgley CA and Lane DP: Activation of the cryptic DNA binding function of mutant forms of p53. *Nucleic Acids Res* 21: 3167-3174, 1993.
67. Abarzua P, LoSardo JE, Gubler ML, *et al*: Restoration of the transcription activation function to mutant p53 in human cancer cells. *Oncogene* 13: 2477-2482, 1996.
68. Selivanova G, Iotsova V, Okan I, *et al*: Restoration of the growth suppression function of mutant p53 by a synthetic peptide derived from the p53 C-terminal domain. *Nat Med* 3: 632-638, 1997.
69. Kim AL, Raffo AJ, Brandt-Rauf PW, *et al*: Conformational and molecular basis for induction of apoptosis by a p53 C-terminal peptide in human cancer cells. *J Biol Chem* 274: 34924-34931, 1999.
70. Ohnishi K, Inaba H, Yasumoto J, Yuki K, Takahashi A and Ohnishi T: C-terminal peptides of p53 molecules enhance radiation-induced apoptosis in human mutant p53 cancer cells. *Apoptosis* 9: 591-597, 2004.
71. Selivanova G, Ryabchenko L, Jansson E, Iotsova V and Wiman KG: Reactivation of mutant p53 through interaction of a C-terminal peptide with the core domain. *Mol Cell Biol* 19: 3395-3402, 1999.
72. Foster BA, Coffey HA, Morin MJ and Rastinejad F: Pharmacological rescue of mutant p53 conformation and function. *Science* 286: 2507-2510, 1999.
73. Takimoto R, Wang W, Dicker DT, Rastinejad F, Lyssikatos J and el-Deiry WS: The mutant p53-conformation modifying drug, CP-31398, can induce apoptosis of human cancer cells and can stabilize wild-type p53 protein. *Cancer Biol Ther* 1: 47-55, 2002.
74. Rippin TM, Bykov VJ, Freund SM, Selivanova G, Wiman KG and Fersht AR: Characterization of the p53-rescue drug CP-31398 in vitro and in living cells. *Oncogene* 21: 2119-2129, 2002.
75. Bykov VJ, Issaeva N, Shilov A, *et al*: Restoration of the tumor suppressor function to mutant p53 by a low-molecular-weight compound. *Nat Med* 8: 282-288, 2002.
76. Peng Y, Li C, Chen L, Sebt S and Chen J: Rescue of mutant p53 transcription function by ellipticine. *Oncogene* 22: 4478-4487, 2003.
77. Vassilev LT, Vu BT, Graves B, *et al*: In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* 303: 844-848, 2004.
78. Blakely EA and Kronenberg A: Heavy-ion radiobiology: new approaches to delineate mechanisms underlying enhanced biological effectiveness. *Radiat Res* 150: S126-S145, 1998.
79. Guida P, Vazquez ME and Otto S: Cytotoxic effects of low- and high-LET radiation on human neuronal progenitor cells: induction of apoptosis and TP53 gene expression. *Radiat Res* 164: 545-551, 2005.
80. Demizu Y, Kagawa K, Ejima Y, *et al*: Cell biological basis for combination radiotherapy using heavy-ion beams and high-energy X-rays. *Radiother Oncol* 71: 207-211, 2004.
81. Debus J, Jackel O, Kraft G and Wannenmacher M: Is there a role for heavy ion beam therapy? *Recent Results Cancer Res* 150: 170-182, 1998.
82. Takahashi A, Matsumoto H, Yuki K, *et al*: High-LET radiation enhanced apoptosis but not necrosis regardless of p53 status. *Int J Radiat Oncol Biol Phys* 60: 591-597, 2004.

83. Takahashi A, Matsumoto H, Furusawa Y, Ohnishi K, Ishioka N and Ohnishi T: Apoptosis induced by high-LET radiations is not affected by cellular p53 gene status. *Int J Radiat Biol* 81: 581-586, 2005.
84. Takahashi A, Ohnishi K, Wang X, *et al*: The dependence of p53 on the radiation enhancement of thermosensitivity at different LET. *Int J Radiat Oncol Biol Phys* 47: 489-494, 2000.
85. Yamakawa N, Takahashi A, Mori E, *et al*: High LET radiation enhances apoptosis in mutated p53 cancer cells through caspase-9 activation. *Cancer Sci* 99: 1455-1460, 2008.
86. Peng Y, Zhang Q, Nagasawa H, Okayasu R, Liber HL and Bedford JS: Silencing expression of the catalytic subunit of DNA-dependent protein kinase by small interfering RNA sensitizes human cells for radiation-induced chromosome damage, cell killing, and mutation. *Cancer Res* 62: 6400-6404, 2002.
87. Collis SJ, Swartz MJ, Nelson WG and DeWeese TL: Enhanced radiation and chemotherapy-mediated cell killing of human cancer cells by small inhibitory RNA silencing of DNA repair factors. *Cancer Res* 63: 1550-1554, 2003.
88. Tauchi H, Kobayashi J, Morishima K, *et al*: Nbs1 is essential for DNA repair by homologous recombination in higher vertebrate cells. *Nature* 420: 93-98, 2002.
89. Sakamoto S, Iijima K, Mochizuki D, *et al*: Homologous recombination repair is regulated by domains at the N- and C-terminus of NBS1 and is dissociated with ATM functions. *Oncogene* 26: 6002-6009, 2007.
90. Tauchi H, Matsuura S, Kobayashi J, Sakamoto S and Komatsu K: Nijmegen breakage syndrome gene, NBS1, and molecular links to factors for genome stability. *Oncogene* 21: 8967-8980, 2002.
91. Nelms BE, Maser RS, MacKay JF, Lagally MG and Petrini JH: In situ visualization of DNA double-strand break repair in human fibroblasts. *Science* 280: 590-592, 1998.
92. Zhang Y, Lim CU, Williams ES, *et al*: NBS1 knockdown by small interfering RNA increases ionizing radiation mutagenesis and telomere association in human cells. *Cancer Res* 65: 5544-5553, 2005.
93. Lee SJ, Dimtchev A, Lavin MF, Dritschilo A and Jung M: A novel ionizing radiation-induced signaling pathway that activates the transcription factor NF-kappaB. *Oncogene* 17: 1821-1826, 1998.
94. Orlowski RZ and Baldwin AS Jr: NF-kappaB as a therapeutic target in cancer. *Trends Mol Med* 8: 385-389, 2002.
95. Yamagishi N, Miyakoshi J and Takebe H: Enhanced radiosensitivity by inhibition of nuclear factor kappa B activation in human malignant glioma cells. *Int J Radiat Biol* 72: 157-162, 1997.
96. Habraken Y, Jolois O and Piette J: Differential involvement of the hMRE11/hRAD50/NBS1 complex, BRCA1 and MLH1 in NF-kappaB activation by camptothecin and X-ray. *Oncogene* 22: 6090-6099, 2003.
97. LaCasse EC, Baird S, Korneluk RG and MacKenzie AE: The inhibitors of apoptosis (IAPs) and their emerging role in cancer. *Oncogene* 17: 3247-3259, 1998.
98. Ohnishi K, Scuric Z, Schiestl RH, Okamoto N, Takahashi A and Ohnishi T: siRNA targeting NBS1 or XIAP increases radiation sensitivity of human cancer cells independent of TP53 status. *Radiat Res* 166: 454-462, 2006.
99. Ohnishi K, Scuric Z, Yau D, *et al*: Heat-induced phosphorylation of NBS1 in human skin fibroblast cells. *J Cell Biochem* 99: 1642-1650, 2006.
100. Okamoto N, Takahashi A, Ota I, *et al*: siRNA targeted for NBS1 enhances heat sensitivity in human anaplastic thyroid carcinoma cells. *Int J Hyperthermia* 27: 297-304.
101. Ohnishi K, Nagata Y, Takahashi A, Taniguchi S and Ohnishi T: Effective enhancement of X-ray-induced apoptosis in human cancer cells with mutated p53 by siRNA targeting XIAP. *Oncol Rep* 20: 57-61, 2008.
102. Nicholson KM and Anderson NG: The protein kinase B/Akt signalling pathway in human malignancy. *Cell Signal* 14: 381-395, 2002.
103. Vivanco I and Sawyers CL: The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nat Rev Cancer* 2: 489-501, 2002.
104. Bjornsti MA and Houghton PJ: The TOR pathway: a target for cancer therapy. *Nat Rev Cancer* 4: 335-348, 2004.
105. Wang F, Arun P, Friedman J, Chen Z and Van Waes C: Current and potential inflammation targeted therapies in head and neck cancer. *Curr Opin Pharmacol* 9: 389-395, 2009.
106. Raimondi AR, Molinolo A and Gutkind JS: Rapamycin prevents early onset of tumorigenesis in an oral-specific K-ras and p53 two-hit carcinogenesis model. *Cancer Res* 69: 4159-4166, 2009.
107. Shaw M, Cohen P and Alessi DR: The activation of protein kinase B by H₂O₂ or heat shock is mediated by phosphoinositide 3-kinase and not by mitogen-activated protein kinase-activated protein kinase-2. *Biochem J* 336: 241-246, 1998.
108. Rosenzweig KE, Youmell MB, Palayoor ST and Price BD: Radiosensitization of human tumor cells by the phosphatidylinositol3-kinase inhibitors wortmannin and LY294002 correlates with inhibition of DNA-dependent protein kinase and prolonged G2-M delay. *Clin Cancer Res* 3: 1149-1156, 1997.
109. Gupta AK, Cerniglia GJ, Mick R, *et al*: Radiation sensitization of human cancer cells in vivo by inhibiting the activity of PI3K using LY294002. *Int J Radiat Oncol Biol Phys* 56: 846-853, 2003.
110. Ohnishi K, Yasumoto J, Takahashi A and Ohnishi T: LY294002, an inhibitor of PI-3K, enhances heat sensitivity independently of p53 status in human lung cancer cells. *Int J Oncol* 29: 249-253, 2006.
111. Guertin DA and Sabatini DM: Defining the role of mTOR in cancer. *Cancer Cell* 12: 9-22, 2007.
112. Hay N and Sonenberg N: Upstream and downstream of mTOR. *Genes Dev* 18: 1926-1945, 2004.
113. Beuvink I, Boulay A, Fumagalli S, *et al*: The mTOR inhibitor RAD001 sensitizes tumor cells to DNA-damaged induced apoptosis through inhibition of p21 translation. *Cell* 120: 747-759, 2005.
114. Majumder PK, Febbo PG, Bikoff R, *et al*: mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. *Nat Med* 10: 594-601, 2004.
115. Boulay A, Zumstein-Mecker S, Stephan C, *et al*: Antitumor efficacy of intermittent treatment schedules with the rapamycin derivative RAD001 correlates with prolonged inactivation of ribosomal protein S6 kinase 1 in peripheral blood mononuclear cells. *Cancer Res* 64: 252-261, 2004.
116. Mabuchi S, Altomare DA, Cheung M, *et al*: RAD001 inhibits human ovarian cancer cell proliferation, enhances cisplatin-induced apoptosis, and prolongs survival in an ovarian cancer model. *Clin Cancer Res* 13: 4261-4270, 2007.
117. Cao C, Subhawong T, Albert JM, *et al*: Inhibition of mammalian target of rapamycin or apoptotic pathway induces autophagy and radiosensitizes PTEN null prostate cancer cells. *Cancer Res* 66: 10040-10047, 2006.
118. Albert JM, Kim KW, Cao C and Lu B: Targeting the Akt/mammalian target of rapamycin pathway for radiosensitization of breast cancer. *Mol Cancer Ther* 5: 1183-1189, 2006.
119. Nagata Y, Takahashi A, Ohnishi K, *et al*: Effect of rapamycin, an mTOR inhibitor, on radiation sensitivity of lung cancer cells having different p53 gene status. *Int J Oncol* 37: 1001-1010.