EFFECTIVE NEW CANCER THERAPIES WHICH ARE INDEPENDENT OF *P53* GENE STATUS

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Abstract: The gene product of the tumor suppressor gene *p53* is known to play an important role in cancer therapy. The p53 molecule induces cell-cycle arrest, apoptosis and DNA repair after cells are subjected to cancer therapies involving ionizing radiation, hyperthermia and anti-cancer drugs. Patients with cancers bearing mutated (m) *p53* or deleted *p53* gene often have a poorer prognosis than those with cancers bearing wild-type (wt) *p53* gene. We reported that efficient cell lethality by ionizing radiation, hyperthermia and anti-cancer drugs was observed in wt*p53* cells, but not in cells bearing m*p53* or deleted *p53* genes in human cultured cancer cells. This review summarizes the contribution of *p53* in these cancer therapies and demonstrates the strategy for tailor-made therapies for cancer cells with a different *p53* gene status. The application of potential new therapies, such as chemical chaperon therapy with glycerol and *p53* C-terminal peptides could be effective even for m*p53* bearing cancers. Some sensitizers such as small interference RNA and targeting inhibitors, and heavy ion beams could be effective regardless of *p53* gene status. These new therapies would be expected to be high efficacy treatments regardless of *p53* gene status.

Key words : *p53*, radiation, hyperthermia, anti-cancer drug, sensitizer, cancer therapy

INTRODUCTION

If cancer therapies using ionizing radiation (IR), hyperthermia, and anti-cancer drugs are to be effective, these therapies must be designed to produce a high level of cell death in cancer cells, and simultaneously to maximize protection for normal non-malignant cells. With these conditions in mind, recent advances in molecular biology have led to progress in cancer research from basic research at the lab bench to the clinic. Advances in molecular biology research have provided new knowledge which can permit searches for predictive indicators among cancer-related genes such as oncogenes and tumor suppressor genes. This type of search could provide useful information to select the most appropriate high efficacy cancer therapy for specific patients.

We have focused on the functions of the *p53* tumor suppressor gene over the last 15 years. Mutations and deletions of the *p53* gene have been shown to provide important information concerning the clinical course for several human cancers. Patients with cancers bearing mutated *p53* (m*p53*) gene or deleted *p53* gene often have a poorer prognosis than patients with cancers bearing a wild-type *p53* (wt*p53*) gene[1,2]. Wt*p53* molecules have multiple functions in complex signal transduction pathways leading to cell death, cell cycle regulation, DNA repair, angiogenesis (Fig. 1), and also in responses to environmental changes

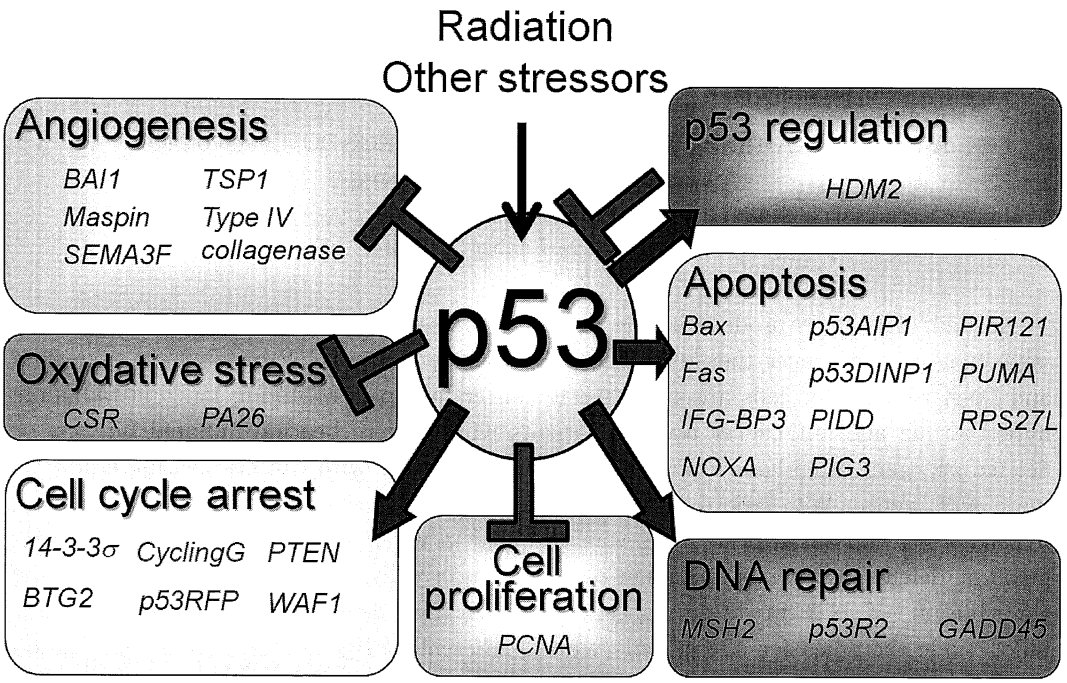

Fig. 1. Multifunctional p53 molecules. p53 acts as a transactivation factor for *p53*-related gene expression and binds to many proteins. p53 is called a guardian of the genome because of its protective functions. Many of the genes indicated here are controlled by p53.

leading to genotoxic or non-genotoxic stresses[3]. These p53 molecules have two primary activities. One is transcriptional activity which regulates gene expression through binding to p53-responsive elements (p53RE; p53 consensus genes, pCON) at locations upstream of *p53*-regulated genes, and another primary activity is the ability to bind to many kinds of proteins. Therefore, *p53* studies are expected to make effective contributions to cancer research. Here, *p53*-centered cancer therapies and new applications for non-wt*p53* patients are described from the viewpoint of molecular biology.

1. IR therapy

1-1. IR-induced DNA damage and its repair

IR induces many types of DNA lesions, either directly, or indirectly through free radicals. The most prominent types of DNA lesions are base damage, single strand breaks (SSBs) and double strand breaks (DSBs)[4]. SSBs are frequently occurring endogenous DNA lesions in human cells (10^4/cell/day), and are induced directly by free radicals, or more commonly as a consequence of the repair of apurinic sites generated by the depurination or repair of deaminated cytosine or other damaged bases[5]. In normal human cells, it is estimated that approximately 1% of these DNA single-strand lesions are converted to approximately 50 endogenous DSBs per cell per cell cycle[6]. This number is similar to the

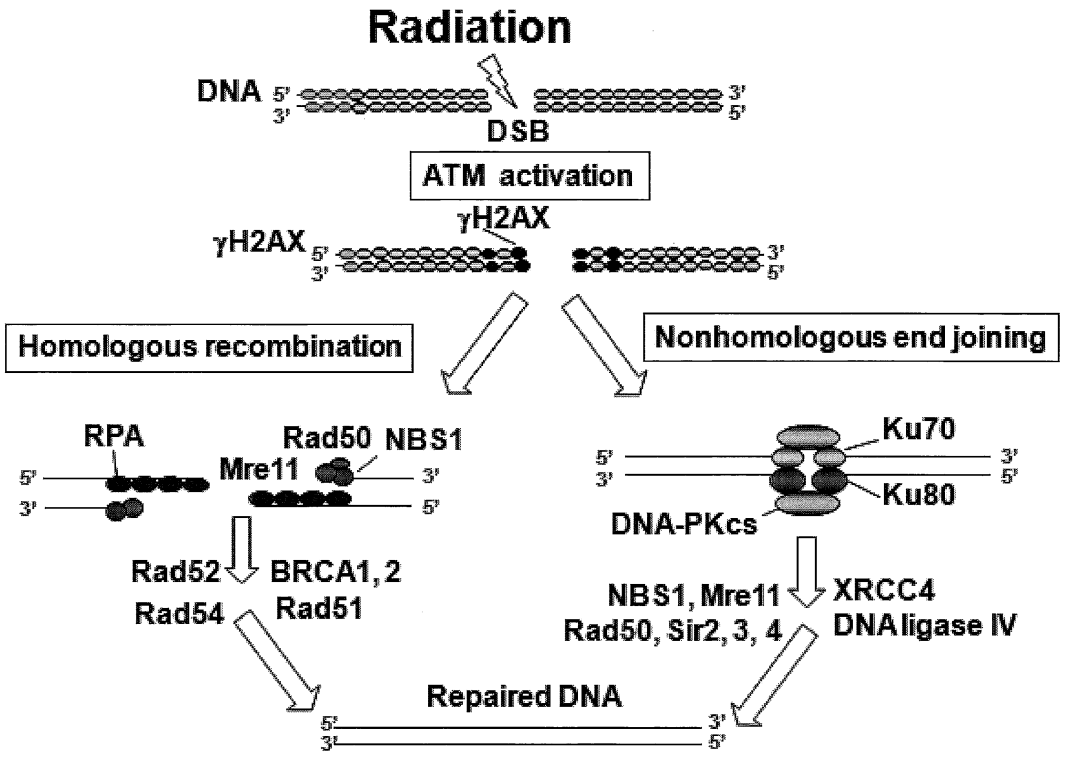

Fig. 2. DNA repair systems for DSBs. IR induces DSBs and activates the phosphorylation of H2AX by ATM. Two major repair processes are shown here. The enzymes involved are indicated for each process.

estimate of the number of exogenous DSBs produced by IR at 1.5-2.0 Gy. DSBs are highly cytotoxic lesions, and to ensure that they are repaired with a minimal impact on genome stability, cells initiate a complex DNA-damage response which includes the spatial reorganization of DSB repair and signaling proteins into sub-nuclear structures: these are IR-induced foci which surround DSB sites[7]. The formation of most IR-induced foci depends on the phosphorylation of the H2A histone family member X (H2AX) at residue serine 139 by ataxia telangiectasia mutated (ATM) and DNA-dependent protein kinase (DNA-PK)[8]. Recently, it was reported that another important residue of H2AX, tyrosine 142, is phosphorylated. Dephosphorylation of tyrosine 142 regulates the main trigger for the DSB-induced chromatin pathway choices: repair and survival, or cell death[9,10].

Failure to repair DNA lesions such as DSBs can lead to mutations, genomic instability, and cell death. Due to the severe consequences of DSBs, cells have developed two major repair pathways for this type of lesion: homologous recombination (HR) and non-homologous end joining (NHEJ) (Fig. 2)[11]. HR is usually an error-free repair pathway which uses DNA homology to direct DNA repair. A DSB can be accurately repaired by using the undamaged sister chromatid strand as a template for the repair of the broken sister chromatid strand. HR in eukaryotes is carried out by the RAD52 epitasis group of proteins. This name arose

from the fact that these genes were originally identified through the genetic analysis of IR hypersensitive mutants. In human cells, the proteins in this group include the members of the MRN (MRE11/RAD50/NBS1) complex, RAD51, the RAD51 paralogs (RAD51B, RAD51C, RAD51D, XRCC2 (X-ray repair cross-complementing group 2), XRCC3, RAD54 and RAD54B[12]. The products of the breast cancer susceptibility genes, BRCA1 and BRCA2 (also known as Fanconi anemia complementation group D1 (FANCD1)), are also involved in the functioning of the HR pathway[13].

Conceptually, NHEJ would be the simplest way of repairing DSBs; this is the straightforward re-ligation of the broken DNA ends without any requirement for a template. NHEJ plays a major role in the elimination of DSBs during the G_1-phase of the cell cycle since HR is not efficient in this phase due to the lack of sister chromatids[14]. After DSB formation, the Ku70/80 heterodimer binds to the damaged DNA ends. This facilitates the recruitment of the DNA-PK catalytic subunit (cs) to the DSB. The sequential binding of these proteins activates the phosphorylation function of DNA-PKcs, which phosphorylates itself, the Ku heterodimer, and other proteins involved in cell cycle regulation[15]. It has been speculated that Ku70/80 might also function as an alignment factor which binds DSB ends, creating an easy access for, and greatly stimulating the functioning of the DNA ligase IV (Lig4)-XRCC4 complex. This can increase the efficiency and accuracy of NHEJ[16]. The Lig4-XRCC4 complex then ligates the juxtaposed DNA ends.

1-2. p53-dependent death signal transduction

A series of cancer cell lines was established from tongue[17], lung[18,19] and other cancer cell types[20]. These cell lines were designed to have a wt*p53*, deleted-*p53*, or m*p53* gene status, although two other genes which are homologous to *p53*, *p63* and *p73*, were present (Fig. 3). When cells were exposed to IR, apoptosis was induced at a high frequency only in wt*p53* cells, and not in m*p53* and *p53*-deleted cells. During the process of cellular apoptosis, the enhanced induction of the *Bax* gene with the cleavage of Caspase-3 and poly (ADP-ribose) polymerase[17,21] was confirmed. It was also confirmed that Bax activation and Bcl-2 inactivation in cancer cells from human cervical cancer patients who had taken radiotherapy occurred only in wt*p53*, but not in m*p53* patients[2]. These results provide support for the idea that IR treatment induces cell death signal transduction pathways regulated by wt*p53*. Therefore, it was proposed that *p53* gene status could be an important predictive indicator for radio-cancer therapies.

1-3. Chemical chaperons in mp53 cancer cells

To design efficient therapies which can be effective in m*p53* patients, a chemical chaperon therapy with IR, heat and anti-cancer drugs was suggested. Chaperon activity can lead to the restoration of protein conformation and subsequent protein activity. In this case the aim is to transform mp53 molecules to wtp53 molecules[22]. Targeted mp53 molecules restored through chaperon activity can bind upstream of p53-responsive genes and induce their gene expression[23]. Therefore, it could theoretically be possible to induce apoptosis after cancer treatments in m*p53* cells. It has been suggested that such chaperon activity could be provided by glycerol and specific p53 C-terminal peptides containing about 15 peptides of the p53 molecule[24]. Reports describing investigations of chemical chaperons list about 10 candidates. In the near future, such new therapies could be applicable to cancer

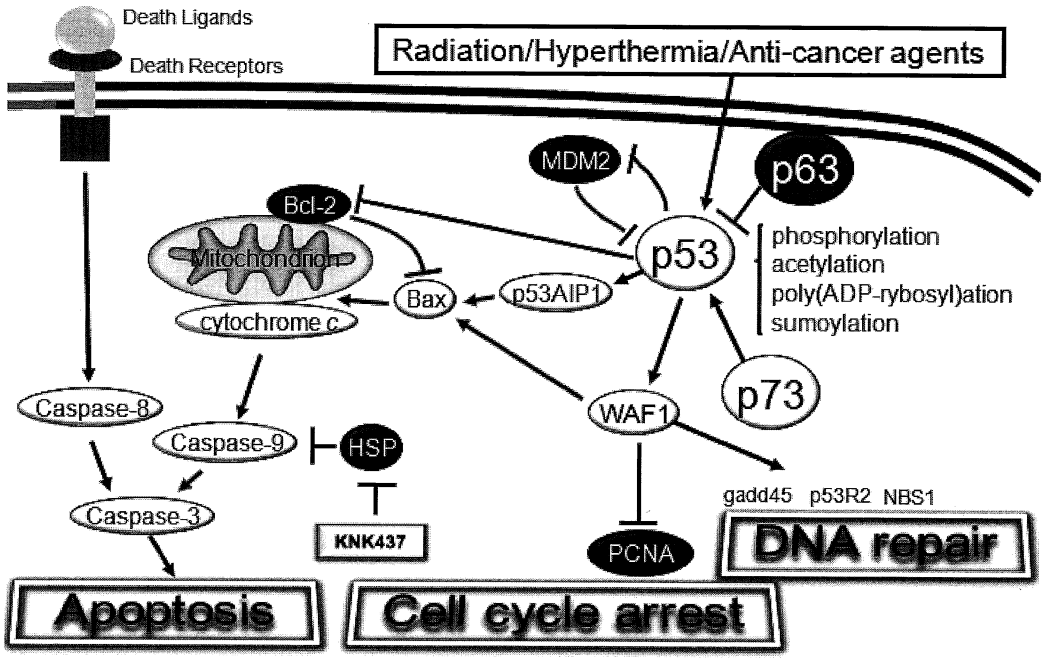

Fig. 3. *p53*-centered signal transduction pathway with simple modifications. The three primary biological responses controlled by *p53* are apoptosis, cell cycle arrest and DNA repair. Cancer therapies induce cytotoxic damage. Arrows indicate enhancement, and – indicates depression of gene activity. *p63* and *p73* are genes which are homologous to *p53*. Aberrations are described in the text.

patients. Unfortunately this method would not be applicable to patients with a deleted *p53* gene, because, in these cases, there would be no p53 molecules to restore in these cancer cells. For *p53*-deleted cancer cells, gene therapy may be possible by introducing a *p53* gene with a Cytomegalo virus promoter region to ensure excess *p53* gene expression.

1-4. Radio-sensitizers

For over the past 30 years, radio-sensitizers have been studied for their ability to produce oxygen radicals in hypoxic tumors. However, there have been very few attempts to utilize these agents in conjunction with IR therapy. In patients with cancers bearing m*p53* and/or *p53*-deleted, investigators have looked for new compounds or agents capable of sensitizing patients to the effects of IR. One approach is to inhibit DNA repair processes in order to block repair of DNA damage induced by therapies. IR produces numerous types of DNA lesions such as base damage, oxygenic bases, and strand breaks such as SSBs and DSBs. The most critical lesions leading to cell lethality are DSBs. Currently, details of recombination repair for HR and NHEJ, excision repair mechanisms for base lesions, and base repair for base excision modifications are all well understood. Therefore, attempting to deactivate repair enzymes with some type of targeting agent could be expected to lead to a highly effective cancer therapy. Wortmannin is a protein kinase inhibitor, and depresses

DSB repair through its ability to depress the phosphorylation of p53 proteins and DNA repair enzymes in pathways involving *ATM* and *DNA-PK*. LY294002 [2-(4-morpholnyl)-8-phenyl-4H-1-benzo-pyran- 4-one] is another inhibitor which is effective against phospho-inositide-3-kinase (PI3K)[25].

Other potential future methods could include the application of small interference RNA (siRNA) techniques. Therapies which would be independent of *p53* gene status include suppressing repair processes for IR induced DSBs, and the depression of cell survival signal pathways. The ability of siRNA was reported to suppress expression of the *NBS1* and *Lig4* genes involved in DNA repair processes[26-28]. The application of siRNA to suppress survival promoting pathways involving *XIAP* (X-linked inhibitor of apoptosis) and *NF-kB* (Nuclear factor of kappa light polypeptide gene enhancer in B-cells 2) in IR-irradiated cells has also been reported[27,28].

1-5. High linear energy transfer (LET) radiation

Generally, γ-rays and X-rays, species of radiation with low LET values, are used for therapy. In contrast, high LET radiation has high relative biological effectiveness (RBE) values, and thus high LET radiation such as carbon ion beams has been used for effective cancer therapy[29]. Carbon ion beams lead to high rates of apoptotic cell death, even in cells which have a deleted *p53* gene or contain m*p53* gene[30]. With this type of radiation the RBE value is 3. In contrast, wt*p53* cells have an RBE value of 2. Recently, it has been suggested that the induction of *p53*-independent apoptosis takes place through the activation of Caspase-9 which results in the cleavage of Caspase-3 and PARP[31]. Many patients have been treated at two facilities at Chiba and Harima in Japan, and treatments at these facilities were effective. Moreover, a new facility will soon open at Gunma University in Japan. It is not necessary to check the *p53* gene status of patients or tumors as a predictive indicator for this heavy particle therapy, because all patients with any *p53* gene status are responsive to this type of therapy.

2. Hyperthermic therapy

2-1. Heat-induced cell death signaling

Hyperthermic therapy is intended to elevate temperatures over approximately 42°C, and Arrhenius plots show a critical action point for temperatures occurs at 42.5°C[32]. High temperatures induce structural denaturation in most proteins which form cellular components. Thermal effects result from the breaking of hydrogen bonds in these proteins. However, an important observation showed that *p53*-dependent apoptosis was induced by heat-treatment at high temperatures over 42.5°C[17]. The activation of the Bax gene and Caspase-3, DNA degradation, and apoptotic body formation was observed in wt*p53* cells but not in m*p53* cells or in *p53*-deleted cancer cells[17]. A clinical report described a *p53*-dependent high efficacy for hyperthermia therapy in cervical cancer patients through induction of apoptosis involving one of the Bax pathways[33].

2-2. Heat-induced DNA damage

Hyperthermic effects result from protein denaturation through the breakage of heat labile hydrogen bonds. Recent reports describe DSB formation in DNA molecules subsequent to a heat treatment[32]. DSBs were detected using a new immuno-cytochemical

method to observe γH2AX positive foci induced by heat treatment[32,34-36]. This method is capable of specifically recognizing γH2AX foci, and has become the gold standard for the detection of DSBs[37,38]. This assay is considered to be an extremely sensitive and specific indicator for the existence of DSBs; specifically, one γH2AX focus correlates with one DSB[39-41]. The frequency of γH2AX-foci formation corresponds closely to the efficiency of cell killing induced after exposure to heat (Fig. 4**A**). It is suggested that high temperature leads to the formation of DNA DSBs indirectly through heat-induced radicals, base modifications such as thymine glycol (Tg), 8-oxoGuanine (G) and Uracil (U), incision steps by a glycosylase and apurinic/apyrimidinic (AP)-endonuclease, and during a DNA synthesis step which uses the DNA polymerase β (pol β) (Fig. 4**B*a***)[32,42,43]. When these steps are performed successfully, DSBs are removed. When the cells are exposed to heat, heat-labile pol β is denatured. When DNA synthesis subsequently occurs under these conditions, DSBs can be induced in the presence of existing single strand DNA lesions (Fig. 4**B*b***). However, when cells are conditioned by mild heat or placed into a thermo-tolerant state by an exposure to mild heat, heat shock proteins (HSPs) are induced. These induced HSPs have molecular chaperon activity and are capable of restoring or rescuing damaged or denatured pol β molecules to a normal structure[44]. Consequently the formation of DSBs is reduced when cells gain thermo-tolerance (Fig. 4**B*c***). Thermo-tolerance was observed in conjunction with a depression in the number of γH2AX foci formed after heating, if cells were conditioned by exposure to a mild heat treatment (Fig. 4**A**)[32]. A high degree thermo-tolerance was not

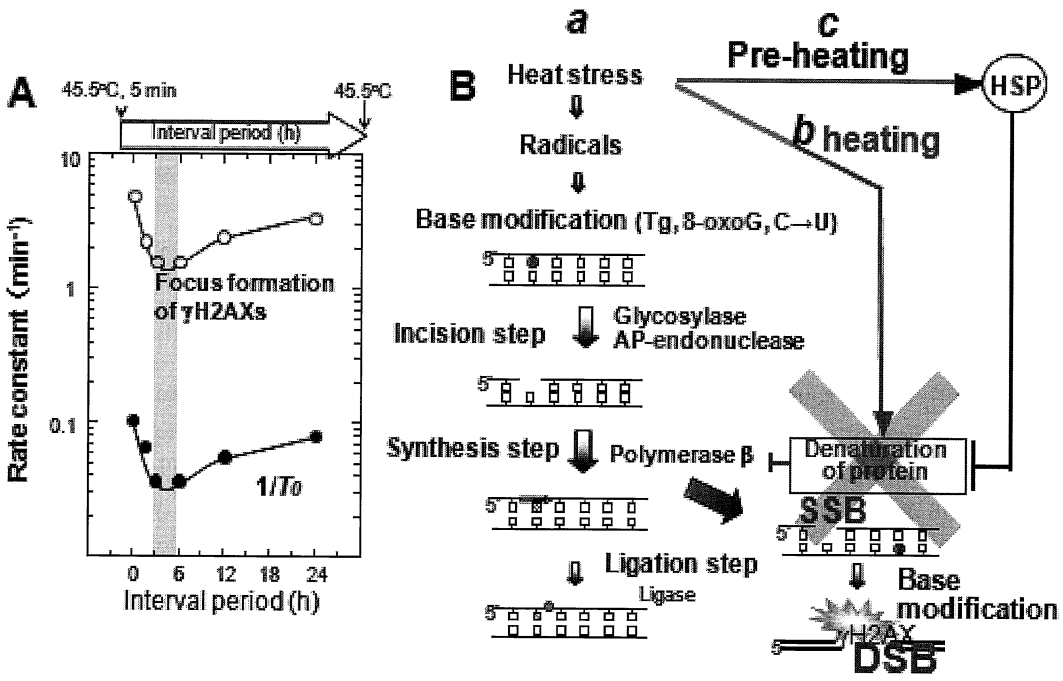

Fig. 4. DSB formation by heat and thermo-tolerance. **A**, A conditioning or pre-heat treatment depressed γH2AX formation and cell killing induced by a challenging heat-treatment; **B**, heat-induced DNA damage and repair (*a*); DSB formation (*b*); thermo-tolerance through HSPs (*c*).

observed in *pol β*-defective cells when compared to parental wild-type *pol β* mouse embryonic fibroblast cells[44]. This suggests that only part (approximately half) of the thermo-tolerance effect was induced through a functioning pol *β*. However, from these results, it appears that a conditioning mild heat treatment induces HSPs which are capable of rescuing denatured pol *β* molecules which result from a subsequent severe heat treatment[44].

2-3. Sensitizers leading to heat-induced cell death

Thermal sensitivity in cancer cells was reported to be enhanced by an inhibitor of HSPs, KNK437[45]. HSPs are stress responsive proteins which can be induced by environmental changes produced by cancer therapies. HSPs are classified by their molecular weights as Hsp110, 90, 70, 60, 40, 27, and there are additional smaller proteins. These proteins possess numerous biological functions which enable them to have chaperon activities for denatured proteins and unfolded proteins. Hsp27 and Hsp70 also depress apoptosis induced by cancer therapies through the conformational inhibition of apoptosomes which consist of cytochrome *c*, Caspase-9 and Apaf1. Thus, in designing effective therapies for cancer cells, it is desirable to depress anti-apoptosis function of HSPs. For this reason, KNK437, which inhibits the induction of HSPs, and especially of Hsp27, was studied. KNK437 depressed the binding of heat-activated heat-shock factor 1 (HSF1) to the heat-shock element (HSE) upstream of *Hsp27*[45]. Since the cellular content of Hsp27 in m*p53* cells was higher than that in wt*p53* cells, m*p53* cells were more resistant to heat than wt*p53* cells. Exposing cells to KNK437 showed that m*p53* cells were more effectively sensitized to heat than were wt*p53* cells, although KNK437 was effective regardless of *p53* gene status.

Sensitization to heat, but not to IR, by Tween 80 has also been reported. This chemical was effective in both m*p53* and wt*p53* cells through the depression of Akt activation by PTEN. After heat treatment, Tween 80 introduced cell cycle arrests at the G_2/M stage without apoptosis regardless of *p53* gene status. Depression or arrest of tumor cell growth was found to be at the G_2 phase in transplanted tumors in nude mice.

Hyperthermia has also been reported to indirectly induce immunological activity against cancer cells. This observation suggests the possibility that the enhancement of heat-induced immune activities could be developed to use with immunotherapy regardless of *p53* gene status.

3. Anti-cancer drug-induced damage and its repair

Currently, numerous types of anti-cancer drugs are utilized in the treatment of cancer patients. Progress in drug delivery has been developed to deliver DNA damaging agents, cell cycle control agents, and targeting agents for cell survival and cell death signal transduction pathways. Important factors have been mentioned, especially with regard to cell survival and cell death. Here, the use of alkylating agents for glioblastomas is discussed.

DNA alkylating agents, including temozolomide (TMZ) and 1-(4-amino-2-methyl-5-pyrimidinyl) methyl-3-(2-chloroethyl)- 3-nitrosourea hydrochloride (ACNU) are chemicals commonly used for chemotherapy for glioblastoma cells. These chemicals cause base modifications and DSBs indirectly through mismatch repair and DNA-DNA crosslinks (Fig. 5). To clarify repair mechanisms involved in alkylating agent sensitivity in DNA repair deficient cells with different repair capacities, several cell lines were used for studies. These

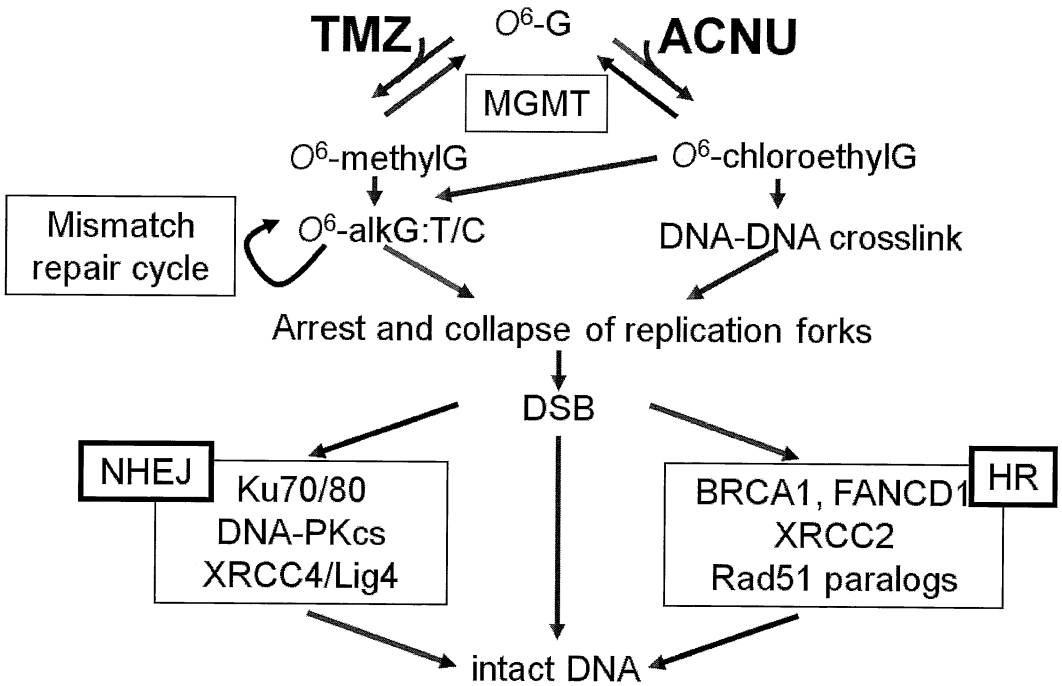

Fig. 5. DNA repair systems responding to TMZ and ACNU. DSB repair sytems play an important role in cell survival.

cell lines included embryonic fibroblasts from knockout mice deficient in the methyl-guanine methyl transferase (*MGMT*) gene; the DSB recognition gene *H2AX*; the HR-related genes *XRCC2* and *Rad54*; the NHEJ-repair related genes *Lig4* and *DNA-PKcs*. The parental cell lines were also studied. *Lig4-/-* cells were quite sensitive to both TMZ[28] and ACNU. The difference in sensitivity between *Lig4-/-* cells and the parental cells to TMZ was larger than differences in sensitivity to ACNU. The difference in sensitivity between *Rad54-/-* cells and their parental cells to ACNU was larger than differences in sensitivity to TMZ. Using siRNA against the *Lig4* gene efficiently enhanced sensitivities to TMZ[28] and ACNU. Analysis of the number of γH2AX foci indicated that the introduction of DSBs induced by TMZ[28] or ACNU in *Lig4-/-* cells was very slow when compared with the parental cells. These findings suggest that targeting DSB repair pathways which are active against TMZ and ACNU induced damage might be useful for sensitization during chemotherapy for brain tumors. Since sensitivities to these chemicals are *p53*-independent, the use of these agents may be effective regardless of the *p53* gene status of tumors.

Cisplatin (CDDP) is also widely used as an anti-cancer drug. Reports have shown that wt*p53* cancer cells are sensitive to CDDP when compared to m*p53* cancer cells. A high frequency of apoptosis was detected only in wt*p53* cells through Caspase-3 activation, but not in m*p53* cells. CDDP also led to an effective depression of tumor growth and apoptosis in a transplanted wt*p53* tumor-nude mouse system[46,47]. On the other hand, chemical chaperon induction by glycerol in m*p53* cancer cells was also effective with in vitro cultured cells and

with *in vivo* nude mouse systems[46,47].

4. Combination therapies using IR, hyperthermia and anti-cancer drugs

When combination treatments using IR and hyperthermia have been used, a high efficacy for cancer therapy has been reported. The mechanism underlying these results appears to be based on the fact that DNA damage induced by IR is irreparable in heated cells because DNA repair enzymes, and particularly pol β, are heat-labile proteins[48,49]. This appears reasonable because a mild conditioning or pre-heat treatment depresses the combined effects of IR and heat when these are used as a subsequent challenging treatment[50].

Hyperthermia, even at mild temperatures between 39-42°C, induced effective levels of cell death when anti-cancer drugs such as bleomycin[51] and CDDP[52] were used to treat tumors transplanted into nude mice. CDDP, used as an anti-cancer drug, was efficiently incorporated into cancer cells, and subsequently far greater levels of DNA damage were produced when compared to control tumors which were not treated with hyperthermia[53]. In this situation, an effective suppression of tumor growth was seen when cells were exposed to a combination therapy using CDDP and hyperthermia. Judging from recent work, it appears clear that mild hyperthermia enhances the effective incorporation of some agents into tumor cells and depresses the incorporation of agents into normal tissues, when heat treatment is targeted or restricted to a tumor. Such an enhanced effect of anti-cancer drugs by local hyperthermia can compensate for the side-effects of whole body exposure to anti-cancer drugs in patients.

Combination therapies using IR and anti-cancer drugs are already commonly applied and are called "Chemo-Radi therapy". Not only IR but also the chemicals used in these therapies induce numerous types of DNA damage in DNA molecules to produce tumor cell death and a depression of tumor growth. Thus, these treatments show a relatively high efficacy in cancer therapy when compared to results using each single treatment alone. The additive DNA damage generated in cancer cells using the combination therapy can induce a higher rate of cell death in tumor cells.

Since these combination therapies using IR, hyperthermia and anti-cancer drugs will be able to induce high efficacy treatments for wt*p53* patients, the combination with *p53*-independent new cancer therapies is expected for all patients with any *p53* gene status.

CONCLUSION

High efficacy cancer therapies are required for the treatment of cancer patients. Tools and methods to improve the effectiveness of these therapies can be obtained from current research in molecular and cell biology. Information about patient genetic backgrounds with regard to cancer-related genes such as oncogenes and tumor suppressor genes is now important. In this review, discussions were presented concerning the tumor suppressor gene *p53*, and how the status of this gene affects sensitivity towards IR, hyperthermia and anti-cancer drugs (Fig. 6). These therapies and the combination therapies of them will be able to induce high efficacy treatments for wt*p53* patients. On the other hand, the application of potential new chemical chaperon therapy could be effective even for m*p53* patients. Some

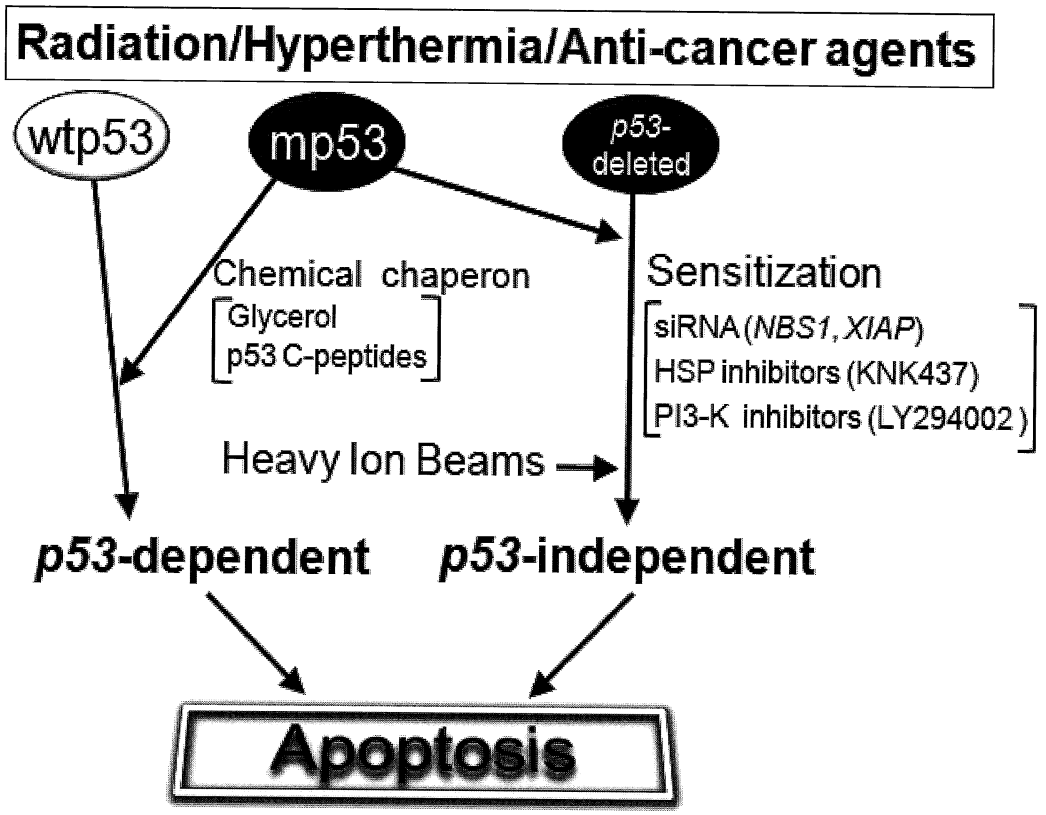

Fig. 6. Strategy for tailor-made therapy for cancer cells with a different *p53* gene status. Apoptosis was induced independently of *p53* status in wt*p53* cancer cells, but not in m*p53* cells. However, chemical chaperoning with glycerol and p53 C-peptides affected apoptosis rates even in m*p53* cells. Some sensitizers and heavy ion beams induced *p53*-independently apoptosis.

sensitizers such as siRNA and targeting inhibitors, and heavy ion beams could be effective regardless of *p53* gene status. Thus, different *p53* gene status may serve as a relevant indicator for a predictive assay to help select the most effective cancer treatments for individual patients.

ABBREVIATIONS

14-3-3 σ, modulator of protein kinase and phosphatase; Msh2, mismatch repair protein MutS homolog 2; BAI1, brain-specific angiogenesis inhibitor 1; Bax, Bcl-2 associated x protein; Bcl-2, B-cell CLL/lymphoma 2; BTG2, B-cell translocation gene 2; CSR, cellular stress response; Fas, FS-7-associated-surface antigen; GADD45, growth arrest and DNA-damage-inducible gene 45; Hdm2, human homolog of Mdm2; IFG-BP3, insulin-like growth factor binding protein 3; KNK437, *N*-formyl-3,4-methylenedioxy-−-butyrolactam; Maspin, mammary serpin; MDM2, mouse p53 binding protein homolog; MSH2, mutS homologue of chromosome 2q gene; NOXA, noxious stresses inducible pro-apoptotic gene; p53AIP1, *p53*-regulated apoptosis induced-protein 1; p53DINP1, *p53*-dependent damage-inducible nuclear protein 1; p53R2, p53-

induced R2 protein; p53RFP, p53-inducible RING-finger protein; PA26, 26S proteasome; PCNA, proliferating cell nuclear antigen; PIDD, p53-induced death-domain-containing protein; PIG3, p53-inducible genes 3; PIR121, 121F-specific p53 inducible RNA; PTEN, phosphatase and tensin homologue deleted on chromosome 10; PUMA, p53-upregulated modulator of apoptosis; RPA, replication protein A; RPS27L, S27-like ribosomal protein; SEMA3F, semaphorin 3F; T0, the mean lethal heating periods (min); TSP1, thrombospondin-1; Ub, ubiquitin; WAF1, wild-type p53 activating factor 1.

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