

# High LET Heavy Ion Radiation Induces p53-Independent Apoptosis

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## Heavy ion beams/LET/Apoptosis/p53/Caspase-9.

Conventional clinical treatments with X-rays provide an effective modality for widely various human cancers, however, therapeutic results are sometimes poor. Many mutations have been reported to be in the *p53* gene in advanced human cancers. The *p53* plays a pivotal role in the pathway which controls apoptosis, cell growth and cell proliferation, and mutations or deletions in the *p53* gene lead to resistance to cancer therapy. The involvement of the *p53* gene in determining the sensitivity of many cell types toward low linear energy transfer (LET) radiation is now well established. In contrast to low LET radiation, high LET radiation has several potential advantages over X-rays, one of which is the fact that its effects may be independent of cellular *p53* gene status. It is conceivable that effective future therapeutic strategies may be designed on the basis of genetic and biochemical events involved in cell death. Therefore, the accurate characterization and quantification of the mode of cell death, such as apoptosis and necrosis, has become increasingly important for the further understanding of the biological effectiveness of high LET radiation. This review discusses the mechanisms of *p53*-independent apoptosis by high LET radiation.

## INTRODUCTION

Understanding the specific biological effects of high linear energy transfer (LET) heavy ion radiation on cancer cells could provide valuable insights for the design of novel therapeutic applications for the treatment of cancers which are resistant to many types of therapies. In the light of recent work, an accurate characterization and quantification of the mode of cell death, such as apoptosis and necrosis, is becoming an increasingly important consideration in the further understanding of the biological effects of high LET radiation.

High LET charged particle radiation has several potential advantages over X-rays such as (*i*) an excellent dose distribution (Fig. 1a), (*ii*) a higher relative biological effectiveness (RBE) (Fig. 1b), (*iii*) a reduction in the oxygen enhancement ratio, (*iv*) less variation in cell cycle-related radiosensitivity, and (*v*) cells have a decreased ability to repair radiation injury<sup>1,2)</sup> when compared to their repair abilities in response to low LET radiation. High LET charged particle radiation has highly lethal effects, even on radioresistant tumors.<sup>3–7)</sup>

This means that these heavy ion beams can severely damage a tumor, and simultaneously, produce fewer deleterious effects and a reduction of damage to the surrounding normal tissues. In other words, heavy ion beams can permit a high dose to be delivered to a tumor while minimizing the amount of radiation delivered to the surrounding normal tissues. It may be possible to design more effective therapeutic strategies based on the genetic and biochemical events involved in cell death.

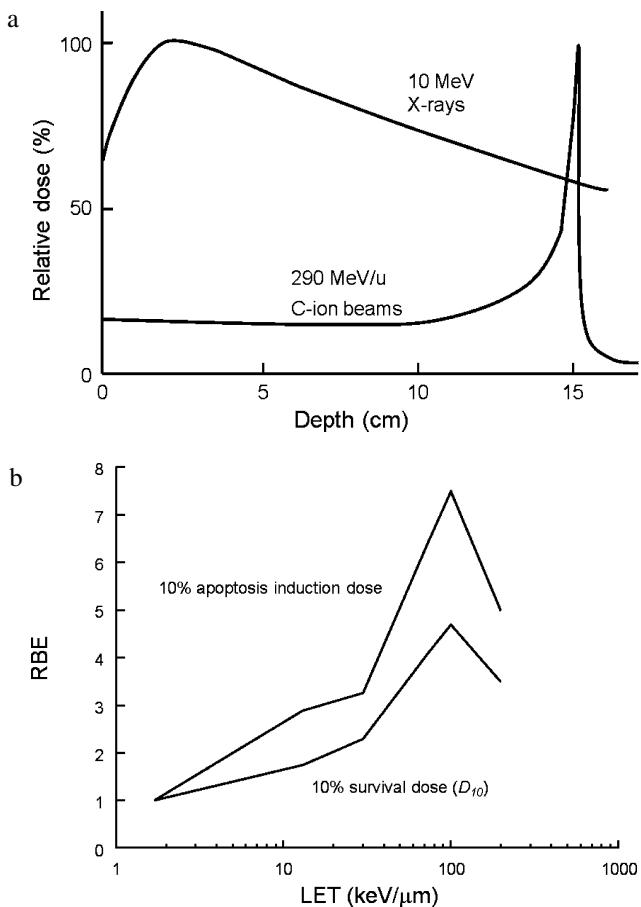
Heavy ion radiotherapy appears to be a promising tool for the treatment of many kinds of human cancers. Among several types of ion species used for heavy ion therapy beams, carbon ions are often chosen for cancer therapy because they have optimal properties with regards to producing effective dose-localization distributions in the body.<sup>8,9)</sup> *i.e.* carbon ion radiotherapy has the potential ability to provide a sufficient dose to the tumor with an acceptable morbidity in the surrounding normal tissues. Tumors which appear to respond favorably to carbon ions include locally advanced tumors and tumors containing histologically non-squamous cell types such as adenocarcinoma, adenoid cystic carcinoma, malignant melanoma, hepatoma, and bone/soft tissue sarcomas.<sup>10–12)</sup>

## LOW LET RADIATION INDUCES P53-DEPENDENT APOPTOSIS

Although conventional treatment with X-rays is an effective treatment modality for widely various human cancers,

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doi:10.1269/jrr.08075



**Fig. 1.** Characteristics of high LET charged particle radiation. a, Dose distributions of X-rays and carbon-ion beams. The carbon-ion beams can produce a high dose in a narrow region when compared to the broad high dose region produced by low LET X-rays.<sup>58)</sup> b, RBE using a  $D_{10}$  dose and a 10% apoptosis induction dose. High LET radiation at a 10% apoptosis dose has a higher RBE than a  $D_{10}$  dose.<sup>34)</sup>

these treatments sometimes produce poor or ineffective results. In tumors harboring mutated *p53* (*mp53*), resistance to radiation therapy may result from the failure of X-rays to induce a sufficient level of apoptosis. This is an important consideration because the induction of apoptosis in cancer cells is part of the mechanism involved in the lethal effect of X-rays. The involvement of the *p53* gene in the sensitivity of many cell types to low LET radiation is well established.<sup>13–18)</sup>

The wild-type *p53* (*wtp53*) protein has multiple functions, including activity as a sequence-specific transcription factor which targets genes capable of inducing growth arrest and apoptosis.<sup>19)</sup> The *p53* gene can thus play a pivotal role in the pathways which control apoptosis, cell growth and cell proliferation.<sup>20–22)</sup> In *p53*-dependent apoptosis, many *p53*-targeted gene products have been identified such as WAF1 (*wtp53* activated fragment 1), PCNA (proliferating cell

nuclear antigen), cyclin/CDK, gadd45 (growth arrest and DNA damage inducible 45), p53R2 (ribonucleotide reductase small subunit 2), Bax (Bcl-associated X protein), Fas/APO-1, PAG608, and p53AIP1 (p53-regulated apoptosis-inducing protein 1).<sup>23)</sup> Mutations in the *p53* tumor suppressor gene have recently been shown to have important consequences for the clinical course of several human cancers, and many mutations have been observed to occur in the *p53* gene in advanced human cancers.<sup>24)</sup> The absence of a *p53*-regulated pathway is a common feature observed in a large number of these cancers, suggesting that this might be an important factor in the pathogenesis of human cancers. Indeed, patients with cancers harboring *mp53* often have a poorer prognosis than those with cancers harboring a *wtp53* gene.<sup>25)</sup> Thus the genetic and functional status of the *p53* gene may be an important factor in guiding therapeutic strategies for patients with cancer.<sup>26)</sup>

The *p53* gene is involved in the sensitivity of many cell types towards low LET radiation. Previously reported cellular sensitivities to radiation and/or heat were shown to depend on the cellular *p53* gene status in human tongue squamous cell carcinomas (SAS cells),<sup>27,28)</sup> human glioblastoma cells (A172 cells),<sup>29)</sup> and human non-small lung cancer cells (H1299 cells).<sup>30)</sup> In view of these findings, the aim of many investigators is to induce apoptosis in cancer cells by restoring normal function to a defective or *mp53* gene.

## HIGH LET RADIATION INDUCES P53-INDEPENDENT APOPTOSIS

In the clinic, it is usually not practical to determine the status of the *p53* gene or other relevant markers. Thus, therapies using high LET radiation appear to be promising, because this type of radiation can have highly lethal effects on radioresistant tumors,<sup>3–7)</sup> and can inhibit tumor growth *in vivo*.<sup>31)</sup> Moreover, high LET radiation can induce apoptosis effectively regardless of cellular *p53* gene status.<sup>30,32)</sup> The RBE values for the surviving fraction and apoptosis induction were increased in a LET-dependent manner. Both RBE curves reached a peak at 100 KeV/μm, and then decreased at values over 100 KeV/μm (Fig. 1b). To date, various approaches have been used to target the apoptotic pathway and utilize it to induce cancer cell death.<sup>33)</sup> Thus heavy ions appear to be capable of providing an effective tool to target cancer cells, because apoptosis induced by high LET radiation is *p53*-independent. 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.<sup>34)</sup>

## HIGH LET RADIATION ACTIVATES CASPASE-9

Caspases function as a component in cell signaling pathways which are involved in events such as apoptosis, cell

growth and differentiation.<sup>35)</sup> Two distinct pathways upstream of the Caspase cascade have been identified; death receptor-induced apoptosis and mitochondrial stress-induced apoptosis.<sup>36-38)</sup> Death receptors (*e.g.* CD95/APO-1/Fas, TNF-R, TRAIL-R) trigger Caspase-8, and the mitochondria subsequently release apoptogenic factors (cytochrome *c*, Apaf-1, AIF) leading to the activation of Caspase-9. The mitochondrial and death receptor apoptotic pathways are intimately connected. However, under most conditions, any cross communication or crosstalk is minimal, and the 2 pathways operate largely independently of each other.<sup>39)</sup>

While Caspases serve as the main effectors of apoptosis, the mechanisms involved in the activation of the Caspase system after exposure to high LET radiation (regardless of *p53* gene status) are largely unknown. Even cells with *mp53* exhibit a high sensitivity to high LET radiation by exhibiting Caspase-3 activation and the cleavage of PARP.<sup>30,32,34)</sup> Caspase-3 activation and apoptosis induction by high LET radiation were suppressed by inhibitors of Caspase-9 and Caspase-3 when compared to the effects of a Caspase-8 inhibitor.<sup>34)</sup> These observations suggest that Caspase-9 may contribute to Caspase dependent apoptosis after exposure to high LET radiation, *i.e.* high LET radiation may activate the mitochondrial-associated apoptotic pathway in a *p53*-independent manner.

In view of the above observations, a model is proposed here for high LET radiation induced *p53*-independent apoptosis (Fig. 2). Apoptotic pathways triggered by high LET radiation do not require *p53*. In the model proposed here,

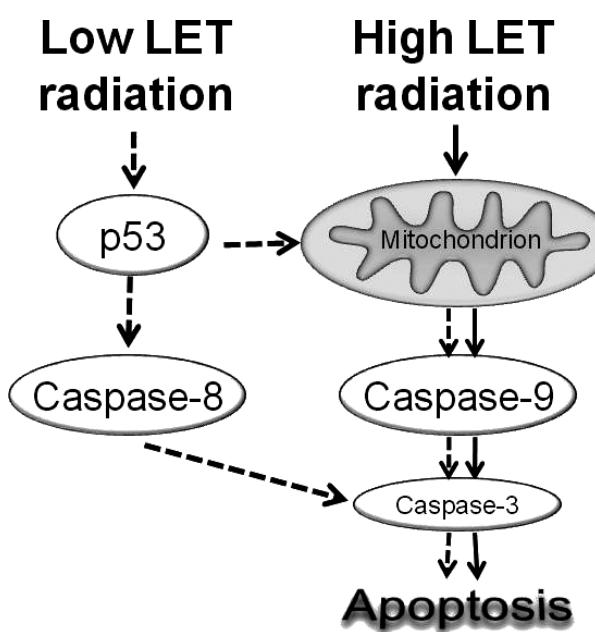
damage induced by high LET radiation acts as a trigger for the activation of the Caspase-9 related apoptotic pathway, rather than the Caspase-8 apoptotic pathway. After activation of Caspase-9 by high LET radiation, Caspase-3 is activated by Caspase-9, and this leads to *p53*-independent apoptosis. Caspase-8 would not be activated in this situation because *p53* is defective and not functional. Activation of the death receptor pathways would make only small contributions to apoptosis induction in this situation.

### POTENTIAL PATHWAYS INVOLVED IN P53-INDEPENDENT APOPTOSIS

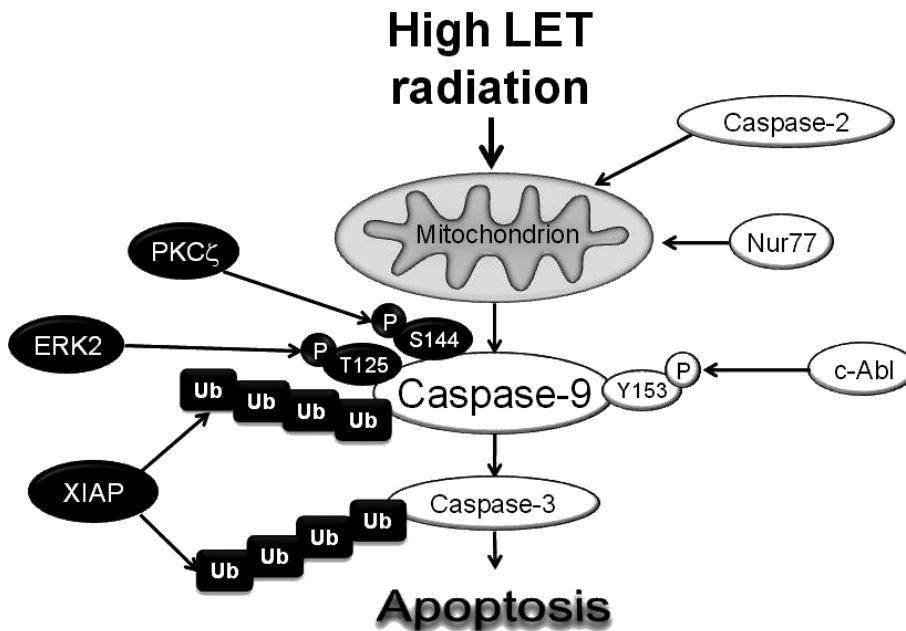
The question of whether high LET radiation triggers the mitochondrial apoptosis pathway directly, or by activating upstream effectors of the mitochondria pathway remains to be addressed. A model is proposed here for high LET radiation induced *p53*-independent apoptosis controlling Caspase-9 mediated signal transduction (Fig. 3). The induction of *p53*-independent DNA damage-induced apoptosis has been attributed to CHK2 acting through the nuclear PML (promyelocytic leukaemia) protein.<sup>40)</sup> It has also been suggested that Nur77 and Caspase-2 can act as transducers to conduct *p53*-independent damage signals from the nucleus to the mitochondria.<sup>41)</sup> Following its activation in response to DNA damage, Caspase-2 is involved in the release of apoptotic effectors from the mitochondria.<sup>42-45)</sup> Alternatively, *p63* and *p73*, members of the *p53* family, may provide a substitute for the activities of *p53* in cells with *mp53* or without *wtp53*. Several lines of evidence suggest that *p73* can induce tumor cell apoptosis in a *p53*-dependent and *p53*-independent manner.<sup>46)</sup> Some tumors exhibit resistance to the *p53*-dependent apoptotic program, therefore *p73*, which can induce apoptotic cell death through *p53*-independent mechanisms, could be particularly important.<sup>47)</sup>

Post-transcriptional modifications of Caspase-9 mediate the fate of cells targeted for death. Some phosphorylation sites appear to have especially important roles in the activation of Caspase-9: these are Thr125, Ser144, Tyr153. Phosphorylation at the Thr125 and Ser144 sites has inhibitory effects on activity, but phosphorylation at the Tyr153 site leads to the activation of Caspase-9, which leads to efficient apoptosis. Caspase-9 is phosphorylated at Thr125, a conserved MAPK consensus site targeted by ERK2, in a MEK-dependent manner.<sup>48)</sup> Moreover, phosphorylation of this site is specific for these classical MAPKs but not when JNK and p38 $\alpha/\beta$  MAPKs are activated.<sup>49)</sup> The predominant kinase which targets Ser144 is PKC $\zeta$  (protein kinase C isoform  $\zeta$ ).<sup>50)</sup> The c-Abl tyrosine kinase binds directly to Caspase-9 and phosphorylates Caspase-9 at the Tyr153 site *in vitro* and in cells treated with DNA damaging agents, and leads to apoptosis.<sup>51)</sup>

The IAP (inhibitor of apoptosis) family of anti-apoptotic proteins regulates programmed cell death.<sup>52)</sup> Of the six



**Fig. 2.** A model for apoptosis pathways by low and high LET radiation. Dot line, low LET radiation-induced apoptosis pathways; closed line, high LET radiation-induced apoptosis pathways.



**Fig. 3.** A model for apoptosis pathways controlling Caspase-9 mediated signal transduction. High LET radiation is postulated to act at Caspase-9 to lead to apoptosis. Black letters, activators of apoptosis; white letters, suppressors of apoptosis.

known human IAP-related proteins, the X-linked inhibitor-of-apoptosis protein (XIAP, also known as MIHA) is the most potent inhibitor.<sup>53)</sup> XIAP functions downstream of pro caspase-9 cleavage as an inhibitor of both proteolytically processed Caspase-9 and Caspase-3 during cellular responses to genotoxic stress.<sup>54)</sup> XIAP prevents apoptosis by inhibiting Caspase-9 and Caspase-3 activation. Unlike Bcl-2, XIAP functions after the release of cytochrome *c* and Smac from the mitochondria and is able to bind to both processed Caspase-9 and processed Caspase-3 to prevent feedback activation of their zymogen forms.

Some IAPs have a C-terminal RING finger domain that has been identified as an essential motif required for the activity of ubiquitin ligase (E3). The large subunit of mature Caspase-9 was poly-ubiquitinated by XIAP *in vitro*, while pro caspase-9 was not.<sup>55)</sup> These ubiquitination reactions require the RING finger domain of XIAP. XIAP disruption by siRNA increases radiation sensitivity independently of *p53* gene status, and effectively enhances radiation-induced apoptosis in cells with *mp53*,<sup>56,57)</sup> suggesting that XIAP is a strong candidate for the control of apoptosis regardless of *p53* gene status, and acts through Caspase-9 activation.

## CONCLUSION

High LET radiation can induce apoptosis effectively regardless of *p53* gene status. Thus, it appears that cells treated with high LET radiation can enter apoptosis through the action of downstream effectors of *p53*-centered signal

transduction pathways, regardless of the presence or absence of a functioning *p53*. Further studies should provide new insights into high LET radiation enhanced apoptosis which is observed in response to the selective activation of the mitochondrial apoptotic factor, Caspase-9, in a *p53*-independent manner.

## ACKNOWLEDGMENTS

This work was supported by Grants-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan. This work was also funded in part by a grant from the Central Research Institute of the Electric Power Industry of Japan, and by a grant for Exploratory Research for Space Utilization from the Japan Space Forum.

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*Received on July 7, 2008**Revision received on August 8, 2008**Accepted on August 12, 2008**J-STAGE Advance Publication Date: October 29, 2008*