No.01503002, March 2015

Journal of Integrated Creative Studies

The DNA damage responses: important determinants of the biological responses to radiation

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Abstract. The actions and biological effects of radiation are diverse and complex, starting from the early stages when radiation energy is deposited into the biological systems and DNA damage is induced, and continuing into the late stages when the actual health effects appear. To understand these processes comprehensively, knowledge from a broad range of fields will be needed, including physics, chemistry, biology and medicine. Ultimate biological consequences of radiation exposure depend not only on the doses, dose rates, fractionation, source and quality of radiation, but also on other factors, such as age, lifestyles, environments, tissue oxygen concentration, and genetic backgrounds that determine the repair capacity for DNA damages in each individual. Responses to the DNA damages are activated upon induction of any type of the DNA damages by radiological and non-radiological causes, and play a critical role in preventing clinically detectable adverse effects due to the DNA damages. Hereditary defects in the DNA damage responses lead to high-risk groups for radiation-induced diseases, including cancer, and in such "radiosensitive" populations, even low-dose radiation exposure can cause severe health effects. Further genetic and molecular biological approaches are needed to identify all the high-risk groups for radiation-induced diseases and to develop effective therapies for these conditions based on the radiosensitivity of each individual.

Keywords: DNA damage responses, Radiation, Health effects, Cancer, Radiosensitivity

1. Introduction

Humans are exposed to radiation through both natural and anthropogenic sources (Thorne, 2003). Natural sources of radiation include potassium-40 (40K) in foods, gas radon-222 (222Rn) and its progeny present in the air, cosmic rays from outer space and from the surface of the sun, and terrestrial radiation from ground and building materials. While some of these exposures can vary widely depending on geological environments, the worldwide average dose rate from these natural backgrounds is about 2.4 mSv/year. Anthropogenic sources include occupational exposure to radiation, medical use of radiation in diagnostic procedures and treatments, and disasters such as the dropping of the atomic bomb at Hiroshima and Nagasaki in 1945, the Chernobyl nuclear power plant accident in 1986, the JCO accident at Tokaimura, Japan in 1999, and the recent Fukushima nuclear power plant accident following the East Japan earthquake and subsequent tsunami on March 11, 2011.

Radiation is genotoxic and produces a variety of DNA damages in human cells, while human cells have signaling pathways that respond to DNA damages. These responses represent *the DNA damage responses*, which are biological mechanisms activated upon DNA damages to prevent adverse biological effects. The DNA damage responses are initiated from recognition of the DNA damage by sensor proteins, and followed by transmission of the damage signals to effector proteins, which induce cell cycle arrest, activation of DNA repair machineries, and apoptotic cell death. Defects in DNA damage responses, which are found in some hereditary disorders (see chapter 8), are associated with hypersensitivity to radiation and increased risk of cancer, suggesting that DNA damage responses are important determinants of the biological responses to radiation.

In this review, we provide an overview of the biological features of early and late effects of radiation, and discuss the future possibility of identifying and treating high-risk groups for radiation-induced diseases based on the capacity of DNA damage responses in each individual.

2. The time evolution of biological responses to radiation

The actions and biological consequences of radiation can be divided into following distinct stages: the first physical stage, which occurs within 10^{-13} second, the subsequent chemical stage lasting from 10^{-12} to 1 second, and the last biological stage ranging from seconds to years (life time) (Adams, 1980; Dingfelder, 2006). The physical stage encompasses the transfer of the radiation energy from the moving particles to atoms or molecules in the biological systems in a stochastic manner, mainly through ionization but also through excitation. The ionization can directly produce physical damages to DNA or to surrounding water, creating ions and radicals. In the following chemical stage, the ions and radicals created in the physical stage react with their surroundings and distribute in space. Importantly, these radicals can attack DNA and other relevant biological molecules, leading to indirect DNA damages (Cornforth, 1993). The last biological stage encompasses biological responses of the system, including enzymatic reactions of the DNA damage response and repair processes (seconds to hours) and early (hours to weeks or months) and late health effects (years).

3. DNA damages induced by radiological and non-radiological causes

Radiation produces a diverse spectrum of DNA damages, including base damages, crosslinks, single-strand breaks (SSBs), and double-strand breaks (DSBs). Among them, DSBs are the most deleterious, and if left unrepaired, they have lethal effects (Khanna, 2001). One Gy exposure of γ-ray to human cells produces 16-40 DSBs in addition to 600-1,000 SSBs (Ward, 1988; Vilenchik, 2000). At lower radiation doses, the number of damaged lesions may be proportional to those induced at higher doses, since there is no threshold doses of radiation that can be considered completely safe from damages.

DNA damages can also be induced by non-radiological causes including DNA-damaging chemicals, endogenous replication errors, oxidative stress and hydrolysis arising during normal cell metabolism. DNA damages can also be formed programmedly during developmentally regulated V(D)J and class switch recombination in lymphocytes and meiotic recombination in germ cells (Sancar, 2004).

4. The DNA damage responses: biological mechanisms that prevent adverse effects due to DNA damage

In order to prevent adverse effects due to the DNA damage and their transmission to daughter cells, the cells possess signaling pathways for DNA damage responses to recognize and repair the DNA damage, which are activated upon production of the DNA damage.

Whether the physical DNA damage observed at the molecular level results in clinically detectable adverse effects or not is dependent on the efficiency and accuracy of the DNA damage responses and repair (figure 1). If the DNA damage is very severe or its accumulation exceeds the levels of its elimination by the DNA damage repair mechanisms, the DNA damage will not be repaired and DNA will be instead destroyed, leading to apoptotic cell death. When accumulation of the DNA damage is within the capacity of repair, it will be repaired. Accurate repair of the DNA damage will result in complete recovery, and no adverse effects will be clinically detectable. However, insufficient or inaccurate repair would lead to survival of cells exhibiting genomic alterations, which are fundamental to various disorders including cancer (Jackson, 2009). This represents the critical importance of efficient DNA damage responses for cell viability and health, and it should be noted that the DNA damage produced by both radiological and non-radiological causes would result in common adverse effects, if not properly repaired.

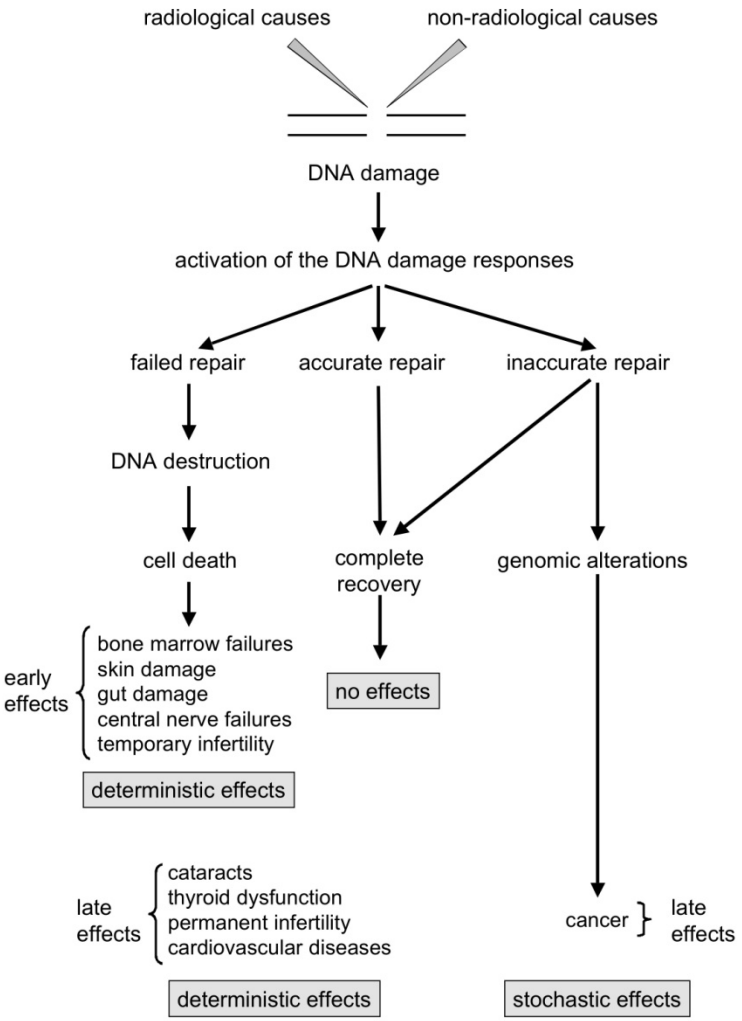

Figure 1. Overview of the mechanisms of biological adverse effects due to DNA damage

5. Biological mechanisms underlying early and late effects of radiation on healthy tissues

Health effects of radiation exposure can be classified into either early or late effects. While early effects may be apparent within hours to weeks or months, late effects may take months to years to be manifested.

Early effects include bone marrow failures, skin damages, gut damages, central nerve failures, and temporary infertility, which appear after relatively high-dose radiation exposure (Mettler, 2012). Acute radiation syndrome occurs after acute high-dose radiation exposure of more than 1 Gy, and is characterized by multiple organ injuries of bone marrow, skin, gut, and nerve, occurring in four phases; the initial reaction, latent period, disease manifestation, and late outcomes (Weisdorf, 2006). The severity and time course of each phase is mainly dependent on the dose and dose rate of radiation exposure. Without medical treatment, survival is likely at doses of < 2 Gy, possible at 2-4.5 Gy and unlikely at doses above 5 Gy as a result of combined organ injuries. With intensive medical treatments including hematopoietic stem cell transplantation, survival might be possible at doses up to 10 Gy. The early effects are deterministic, and there are threshold doses above which the effects increase in both frequency and severity with further increase in the dose (figure 2). The biological mechanism underlying these high-dose deterministic effects of radiation is represented by apoptotic cell death (figure 1). Proliferating cells lead to the apoptosis pathway induced by the tumor suppressor protein p53, whereas non-proliferating cells lead to apoptosis in a p53-independent manner. Apoptotic cell death due to high-dose radiation exposure is an irreversible process, and autologous regeneration of the organs can not be expected when all the stem cells in the organs are completely lost.

Late effects include cataracts, thyroid dysfunction, permanent infertility, cardiovascular diseases, and cancer (Jacob, 2010). Among these effects, cataracts, thyroid dysfunction, permanent infertility, and cardiovascular diseases are deterministic effects. Cancer is regarded as the most important stochastic effect. Epidemiological studies from Japanese atomic bomb survivors in Hiroshima and Nagasaki atomic bomb disasters, who were exposed to relatively high doses and high dose rates, have shown a linear-quadratic (LQ)-dose-response relationship for leukemia incidence and a linear (L)-dose-response relationship for solid cancer incidence at doses of 100-200 mSv or higher (Pierce, 1996; Preston, 2007) (figure 2). Exposure of 1 Sv increases solid cancer incidence by 1.5 times. For radiation protection purposes, the International Commission on Radiation Protection (ICRP) has recommended that the cancer risk for low-dose exposure can be estimated by using a linear-no-threshold (LNT) stochastic model, based on extrapolation from the epidemiological studies of the atomic bomb survivors. This recommendation is based on the hypothesis that the deleterious effects of radiation may proportionally decline as the number of radiation-induced DNA damages will proportionally decrease with lowering doses. The biological mechanism underlying the low-dose stochastic effects of radiation is represented by unsuccessful repair of the DNA damages, which may lead to an accumulation of genomic alterations (figure 1). Since the DNA damage can also be induced by non-radiological causes, it is difficult to distinguish whether the development of cancer is due to radiation exposure or other causes with exception of rare special cases.

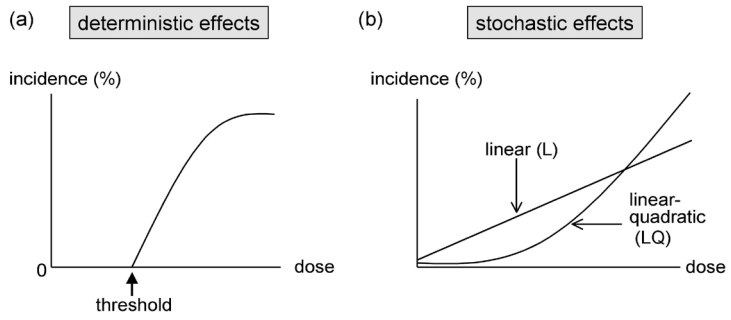

Figure 2. Dose-response relationships in deterministic effects (a) and stochastic effects (b)

6. Factors modulating radiation effects

As described above, at a high-dose radiation exposure, the absorbed dose is a very critical factor that would determine the ultimate biological consequences. In addition, the dose rate, fractionation, source and quality of radiation might also be very important. Low dose rates and fractionated exposure would reduce the biological effects of radiation (Miller, 1989; Howe, 1995). High-linear energy transfer (LET) radiation such as high energy charged particles kills more cells at the same dose as compared with low-LET radiation such as X- or γ-rays (Hunter, 2009) . However, at low-dose radiation exposure, influences from many other background factors such as age, lifestyles, environments, tissue oxygen concentration, and the capacity for DNA damage responses and repair, which would vary among individuals, would become large and should also be taken into account as important modulators of radiation effects. As for age, the sensitivity to radiation is generally considered to be higher in immature proliferating cells, especially those of fetuses and infants (Preston, 2007). Regarding the tissue oxygen concentration, hypoxia is a common tumor condition correlated with resistance to radiation (Rockwell, 2009). With respect to the capacity for the DNA damage responses and repair, both genetic and epigenetic backgrounds may be important. In this regard, mechanistic approaches are also necessary to overcome the limitations of epidemiological approaches in estimating the biological effects of low-dose radiation.

7. Key players of the DNA damage responses

The DNA damage responses are initiated from recognition of the DNA damage by sensor proteins that transmit information to transducer proteins. The responses then transmit the damage signals to numerous effector proteins that induce cell cycle arrest, activation of DNA repair machineries, and apoptotic cell death. For DSBs, the MRE11-RAD50-NBS1 (MRN) complex initially recognizes the DSB ends, and recruits and activates the ataxia telangiectasia-mutated (ATM) kinase (Carney, 1998; Falck, 2005; Williams, 2007). ATM is a member of the phosphoinositide 3-kinase-related protein kinase family and has functional domains that possess a serine/threonine kinase activity (Lavin, 2008). The ATM kinase is activated by autophosphorylation, leading to a conformational change from an inactive homodimer into active monomers that can phosphorylate a variety of downstream proteins (Bakkenist, 2003), including the MRN complex, the histone variant H2AX, the checkpoint mediator MDC1, the checkpoint kinase Chk2, the breast cancer susceptibility protein BRCA1, and p53 (Matsuoka, 2007; Costanzo, 2001; Burma, 2001; Lou, 2003; Stewart, 2003; Kang, 2005; Gatei, 2000; Canman, 1998). Phosphorylations of the MRN complex, H2AX, and MDC1 recruit many proteins required for signal transduction and DNA repair. Phosphorylation of Chk2 can contribute to the cell cycle arrest, and that of BRCA1 leads to the cell cycle arrest and homologous recombination, whereas activation of p53 triggers cell cycle arrest or apoptotic cell death (figure 3).

The two major repair pathways for radiation-induced DSBs are represented by non-homologous end joining and homologous recombination. The former is an error-prone DNA repair pathway which directly rejoins DSB ends by ligation and takes place throughout the cell cycle (Mahaney, 2009), whereas the latter is a repair pathway of greater accuracy and complexity. As the later pathway requires a non-damaged sister chromatid to serve as a template for repair, it acts only during the S and G2 phases of the cell cycle, when the sister chromatid is available (Hartlerode, 2009).

8. Hereditary diseases induced by defects in the DNA damage responses

Defects in the DNA damage responses due to germline mutations in the relevant genes lead to hereditary diseases which share common clinical features, including hypersensitivity to DNA damage and cancer predisposition.

One of the most important examples is ataxia telangiectasia (AT), which is a rare autosomal recessive disease caused by germline mutations in both alleles of the *ATM* gene (Savitsky, 1995). This disorder is characterized by progressive neurodegeneration, immunodeficiency, hypersensitivity to radiation, telangiectasia, and cancer predisposition (Taylor, 1975). Defects in one of the components of the MRN complex also lead to hereditary radiosensitive syndromes such as Nijmegen breakage

syndrome, AT-like disorder, and Nijmegen breakage syndrome-like disorder. These syndromes/disorders are caused by germline mutations in both alleles of the *NBS1*, *MRE11*, and *RAD50* genes, respectively (Waltes, 2009; Matsuura, 1998; Stewart, 1999). Defects in non-homologous end-joining also cause severe combined immunodeficiency associated with hypersensitivity to radiation, whose responsible genes have been identified as *DNA-PKcs*, *Artemis*, and *LIG4* (DNA ligase IV) (van der Burg, 2009; Moshous, 2001; Li, 2002; Riballo, 1999).

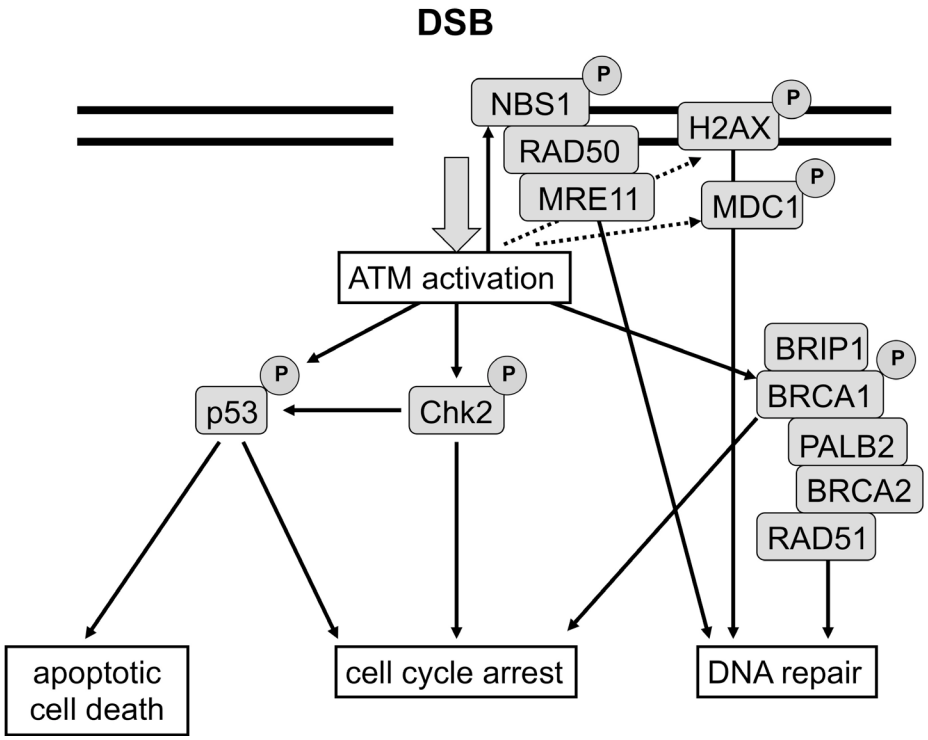

Figure 3. Key players of the DNA damage response signaling pathways

Li-Fraumeni syndrome (LFS) is a cancer susceptibility syndrome characterized by early onset tumors including sarcoma, adrenocortical carcinoma, breast cancer, leukemia, or brain tumor. This syndrome is generally caused by germline mutations in one of the two alleles of the *p53* gene (Malkin, 1990), whereas *Chk2* is also reported to be mutated in cases without *p53* mutations (Bell, 1999), indicating that defects in the DNA damage responses are responsible for this disease. Thus, radiation exposure is likely to worsen the prognosis of the LFS. Gonzalez *et al* reported that germline mutations in the *p53* gene were detected in all of the five patients who developed breast cancer under the age of 30 and had a family history of sarcoma, adrenocortical carcinoma, or brain tumor in the first- or second-degree relatives, indicating that it is possible to predict LFS clinically (Gonzalez, 2009).

The *BRCA1* and *BRCA2* genes were first identified as hereditary breast cancer susceptibility genes (King, 2003). It later became clear that their protein products are involved in the repair of DNA DSBs, by forming a complex with the RAD51 protein, which plays a central role in the early stage of homologous recombination repair (Scully, 1997; Chen, 1998; Venkitaraman, 2002). The BRCA1- or BRCA2-deficient hereditary tumors arise from carriers with germline mutations in one of the two alleles of the *BRCA1* or *BRCA2* gene. While one wild-type allele remains in normal cells, no wild-type

alleles remain in tumor cells developed in the carriers by a mechanism known as "loss of heterozygosity." BRCA1- or BRCA2-defective cells show increased sensitivity to radiation and DNA cross-linking agents compared to the wild-type cells. On the other hand, germline mutations in both of the two alleles of the *BRCA2* gene cause Fanconi anemia, an autosomal recessive disorder characterized by multiple congenital abnormalities, bone marrow failure, cancer susceptibility, and hypersensitivity to DNA cross-linking agents and also to radiation (Howlett, 2002).

Since these rare hereditary diseases constitute high-risk groups for radiation hypersensitivity and cancer susceptibility, unnecessary exposure to radiation even at low doses should be avoided in patients with these diseases.

9. Risk of radiation effects in carriers of mutations in the DNA damage response genes

In addition to the hereditary diseases with defects in the DNA damage responses, recent studies suggest that carriers of one mutated pathogenic allele and one wild-type allele of DNA damage response genes (heterozygotes) may also have an increased risk of cancer following exposure to radiation.

The AT carriers (heterozygotes for the *ATM* gene) constitute approximately 1% of the general population (Thompson, 2005). Although they appear clinically normal, epidemiological studies have demonstrated that they have a 3- to 5-fold increased risk of developing breast cancer. Cells heterozygous for the *ATM* gene show moderate radiation hypersensitivity *in vitro* (Fernet, 2004). In an *in vivo* mouse model, the AT heterozygosity increased the susceptibility to radiation-induced breast cancer (Weil, 2001).

As described in the previous section, normal cells in patients with BRCA1- or BRCA2-deficient hereditary tumors are heterozygotes for the *BRCA1* or *BRCA2* gene, since BRCA1- or BRCA2-deficient tumors arise from the carriers of *BRCA1* or *BRCA2* mutations. *BRCA1/2* carriers are at a very high risk of developing breast or ovarian cancer. Many studies have been performed to test whether carriers of *BRCA1* or *BRCA2* mutations have an increased sensitivity to radiation. Human cells with heterozygous *BRCA1* and *BRCA2* mutations have been shown to exhibit enhanced radiosensitivity compared to wild-type cells (Foray, 1999). A recent study reported that *BRCA1/2* carriers are more susceptible to low levels of radiation exposure by chest X-rays, compared to non-carriers (Andrieu, 2006).

Furthermore, carriers with one mutated allele of other DNA damage response genes are also reported to display an elevated risk of cancer. They include heterozygotes for the *NBS1*, *RAD50*, *MRE11*, and *Chk2* genes as well as for the *BRIP1* and *PALB2* genes. The *BRIP1* gene encodes a protein that interacts with BRCA1 and has an important role in BRCA1-dependent DNA repair and cell cycle checkpoint functions (figure 3), and the *PALB2* gene encodes an integral component of the BRCA complex important for homologous recombination repair (Brooks, 2012; di Masi, 2008), (Dzikiewicz-Krawczyk, 2008; Rahman, 2007) (figure 3). Because all of these genes are implicated in the responses to radiation, it has been hypothesized that heterozygotes for these genes might also be more sensitive to radiation than non-carriers, even at low doses. Mice heterozygous for the *Nbn* gene, a mouse homologue of human *NBS1* gene, showed a dramatically increased occurrence of spontaneous tumors by radiation exposure, providing a clear relationship between *NBS1* heterozygosity, radiation sensitivity, and increased cancer risk (Dumon-Jones, 2003). The pathogenic mechanism for this is presumed to be "haploinsufficiency," which is defined as a moderate loss-of-function phenotype in diploid organisms due to inactivation of one of the two alleles.

In addition to the studies designed to evaluate whether heterozygotes or variants of the known candidate genes implicated in the DNA damage responses are involved in increased radiation sensitivity, some studies have attempted to discover new genetic variants that influence the radiation sensitivity (Amundson, 2003; Correa, 2004; Jones, 2005; Smirnov, 2009), although the number of published reports is small at present. A comprehensive genome-wide analysis using the latest second generation DNA sequencing technique or microarrays would be a powerful approach to identify new genetic determinants of the radiation sensitivity.

10. Bystander effects and adaptive responses

While the role of the DNA damage responses in biological responses to radiation has been unveiled, it is suggested that other phenomena may also modulate health effects associated with low-dose radiation exposure.

The term *bystander effects* is used to describe the phenomenon in which non-irradiated cells whose neighbors are irradiated also respond to radiation and exhibit phenotypes associated with direct radiation exposure (Little, 2006). Two main mechanisms for bystander effects have been identified: direct cell-to-cell communication via specific pores between cells called gap junctions and release of soluble factors from irradiated cells into the surrounding medium. While bystander effects have been demonstrated in both *in vitro* and *in vivo* experiments, the precise role of these effects in radiation exposure in humans remains to be clarified. If human cells really exhibit bystander effects, the biological consequences of low-dose radiation exposure would be more severe than estimated from the LNT model.

The term adaptive responses is used to describe the phenomenon in which cells irradiated with a low priming dose of radiation exhibit reduced effects from a higher challenging dose of radiation given later, and such response responses are considered to be in competition with the bystander effects (Tapio, 2007). The mechanisms underlying the adaptive responses are generally thought to consist of enhancement of DNA damage repair and antioxidant activity, but these mechanisms are still poorly characterized at the molecular level. Since adaptive responses have not been observed in all the cell systems in previous reports (Nagar, 2003; Andersson, 1992; Hain, 1992), the existence of adaptive responses in humans is still uncertain. If human cells really exhibit adaptive responses to radiation, the biological consequences of low-dose radiation exposure would be less severe than estimated from the LNT model, which is in contrast to the case of bystander effects.

11. Implications for treatments for early and late radiation effects

For early effects of radiation that occur upon exposure of high-dose radiation, apoptotic cell death of the stem cells represents the fundamental mechanism. Two therapeutic approaches may therefore be applied to these effects. One is the regenerative therapy using multipotent stem cells that can differentiate into various tissue-cells, and the other is inhibition of apoptosis. Examples of the latter approach include inhibition of the proapoptotic functions of p53 (Komarov, 1999; Komarova, 2004; Strom, 2006; Morita, 2010), mimicry of antiapoptotic Bcl-2 family proteins (Sugioka, 2003), or enhancement of the antiapoptotic pathway by activating the Toll-like receptor 5 signal (Burdelya, 2008). Further investigations are still required to advance these approaches into clinical use, and it should be noted that the inhibition of apoptosis, while ameliorating the effects of radiation, could have the adverse effect of increasing accumulation of DNA-damaged cells, resulting in increased cancer risk.

There are no therapeutic approaches specific for radiation-induced cancer, because radiation-induced cancer has no specific pathogenic features that can distinguish it from cancer arising from other causes. However, it is notable that a new therapeutic strategy has recently been developed for cancer with increasing accumulation of DNA-damaged cells. This strategy is based on the principle that targeted inhibition of the compensatory DNA repair pathway that is activated in tumor cells defective in a particular DNA repair pathway will selectively kill the tumor cells, a phenomenon referred to as *synthetic lethality*. The notable examples are the recent findings that BRCA1- or BRCA2-deficient tumors characterized by defective homologous recombination repair are sensitive to therapies that utilize poly(ADP-ribose) polymerase (PARP) inhibitors, which leads to synthetic lethality (Martin, 2008; Helleday, 2008). The PARP protein plays a critical role in DNA base-excision repair (Durkacz, 1980), and inactivation of this protein increases the number of SSBs, leading to DDBs that must be repaired by homologous recombination mediated by BRCA1 and BRCA2. The treatment of BRCA1- or BRCA2-defective tumor patients with PARP inhibitors is tumor-specific, because only the tumors with no wild-type alleles of *BRCA1* or *BRCA2* are completely defective in homologous recombination, whereas normal cells in the same patients possessing one wild-type allele

of *BRCA1* or *BRCA2* are still able to perform homologous recombination repair. This therapeutic strategy can be applied to other cases with increasing accumulation of DNA-damage due to defects in other DNA damage response pathways, if inhibition of the compensatory DNA repair pathway can achieve synthetic lethality. Since tumors with increasing accumulation of DNA-damaged cells may constitute high-risk groups for radiation-induced diseases, this strategy would also be applicable to future treatments of radiation-induced cancer.

12. Conclusions

Advances in radiation biology have led to a better understanding of the DNA damage responses and their importance in prevention of the adverse effects due to DNA damages induced by both radiological and non-radiological causes. Identification of hereditary diseases that are caused by germline mutations in the DNA damage response genes has provided insights into the association between defects in the DNA damage responses with radiation hypersensitivity and cancer. The heterozygotes for the DNA damage response genes have also turned out to constitute high-risk groups for radiation-induced diseases, including cancer. The development of further genetic and molecular biological approaches could have important implications for the identification of genetic or epigenetic high-risk factors for radiation hypersensitivity and for the management and treatment of radiation-induced diseases.

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