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ORIGINAL PAPER

The importance of the nuclear and cytoplasmic signalling in the cellular response to ionizing radiation

Irena Szumiel

Abstract DNA is the universal primary target for ionizing radiation; however, the cellular response is highly diversified not only by differential DNA repair ability. The monitoring system for the ionizing radiation-inflicted DNA damage consists of 3 apparently independently acting enzymes which are activated by DNA breaks: two protein kinases, Atm (ataxia telangiectasia mutated) and DNA-PK (DNA-dependent protein kinase) and a poly(ADP-ribose) polymerase, PARP-1. These 3 enzymes are the source of alarm signals, which affect to various extents DNA repair, progression through the cell cycle and eventually the pathway to cell death. Their functions probably are partly over-lapping. On the side of DNA repair their role consists in recruiting and/or activating the repair enzymes, as well as preventing illegitimate recombination of the damaged sites. A large part of the nuclear signalling pathway, including the integrating role of Tp53 has been revealed. Two main signalling pathways start at the plasma membrane: the MAPK/ERK (mitogen and extracellular signal regulated protein kinase family) "survival pathway" and the SAPK/JNK (stress-activated protein kinase/c-Jun N-terminal kinase) "cell death pathway". The balance between them is likely to determine the cell's fate.
When DNA break rejoining is impaired, the cell is unconditionally radiation sensitive. The fate of a repair-competent cell is determined by the time factor: the cell cycle arrest should be long enough to ensure the completion of repair. Incomplete repair or misrepair may be tolerated, when generation of the death signal is prevented. So, the character and timing of the signals are, to a large part, responsible for the cellular intrinsic radiation sensitivity and depend on the characteristics of the cellular signalling web.

Key words cellular signalling pathways • ionizing radiation • radiation sensitivity

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Introduction

DNA is the universal primary target for ionizing radiation; however, the cellular response is highly diversified not only by differential DNA repair ability. There is an increased understanding of the importance of the signalling pathways for the fate of the irradiated cell.

To establish sensitivity to ionizing radiation, mammalian cells are examined *in vitro* and the usual end-point is the loss of clonogenicity. The loss is connected with various cell death types: apoptosis, necrosis or irreversible cell cycle arrest in G1 or G2 phase of the cell cycle. Late apoptosis and/or necrosis follow such an arrest. Apoptosis requires *de novo* protein synthesis and an adequate level of ATP. The orderly progression of processes leading to a stepwise degradation of macromolecules is characteristic for apoptotic death. The remains, the so-called apoptotic bodies, are mostly taken up by the neighbouring cells. In ATP-depleted cells the prevailing death type is necrosis. In necrotic cells the degradation proceeds in a chaotic manner and inflammation is produced in the surrounding tissue *in vivo*.

Although the relation between DNA damage and cell death is firmly established, the damage may be tolerated to various extents; most differentiated cells (lymphocytes are a notable exception) retain their functional ability after irradiation with doses, which kill proliferating cells. The repair of DNA is vital, but the cell's fate also can be influenced by signals passing through various pathways. Examples of such influence are discussed below.

It should be added that the cellular signalling web is very complicated and differrs between various cell types; so, numerous oversimplifications have been unavoidable. On the other hand, none of the acronims of the main signalling kinases are omitted in the diagrams, to convey the complexity of the signal pathways. To keep the scope of the paper in reasonable limits, the details on signalling concerning apoptosis are not included.

Signalling in the nuclear and cytoplasmic compartments of the irradiated cell

Since DNA is the target of radiation, it is logical to expect that the nucleus is the primary site of signal generation. Indeed, it is so and details on the «alarm signal» are given below. However, also signalling in the cytoplasmic compartment takes place soon (minutes) after irradiation, originating at the plasma membrane. The eventual signal targets are mostly transcription factors which activate transcription of genes specific to the cell's response to the given damaging agent. The role of their protein products is to initiate or maintain the cellular functions that are necessary for recovery from the damage or else, for directing the cell to the death path.

To these goals contribute the signals from both compartments. Fig. 1 diagramatically shows this concept and it will be recalled further in the text, which is organized into sections dealing with the nuclear and cytoplasmic signal transduction pathways.

DNA damage monitoring

The monitoring system for the ionizing radiation-inflicted DNA damage (reviewed in [25]) consists of three apparently independently acting enzymes which are activated by DNA breaks: two protein kinases, Atm (ataxia telangiectasia mutated) and DNA-PK (DNA-dependent protein kinase) and a poly(ADP-ribose) polymerase, PARP-1. These three enzymes are the source of alarm signals, which affect to various extents DNA repair, progression through the cell cycle and eventually the pathway to cell death. On the side of DNA repair their role consists in recruiting and/or activating the repair enzymes, as well as preventing illegitimate recombination of the damaged sites by binding to the free ends of DNA strand breaks. Recently, Wang et al. [29] proposed that a "super complex" of Brcal – associated proteins is a sensor of DNA damage. This complex would include Atm, Blm (mutated in Bloom's syndrome), proteins of mismatch repair, Rad 50, Mre11 and nibrin (mutated in Nijmegen breakage syndrome).

The dual role of the alarm signalling

The alarm signal initiates two chains of events, presented in Fig. 2. One is connected with DNA repair systems and consists of recruiting repair enzymes and "helper" proteins (such as analogues of the yeast proteins Sir 2, 3 and 4; see [8] for a recent review). In Fig. 2, grey blocks and thin arrows represent this pathway. The other one consists of signalling

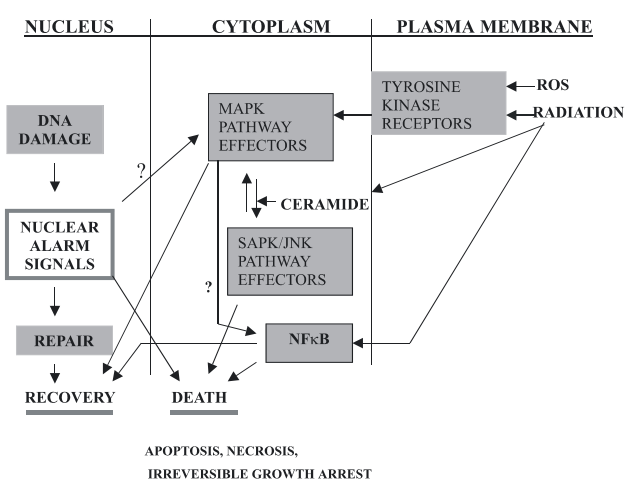


Fig. 1. The main signal transduction pathways that function in the nuclear and cytoplasmic compartments of the irradiated cell. NFκB – nuclear factor κB; MAPK – mitogen activated protein kinase family; ROS – reactive oxygen species; SAPK/JNK – stress-activated protein kinase / c-Jun N-terminal kinase.

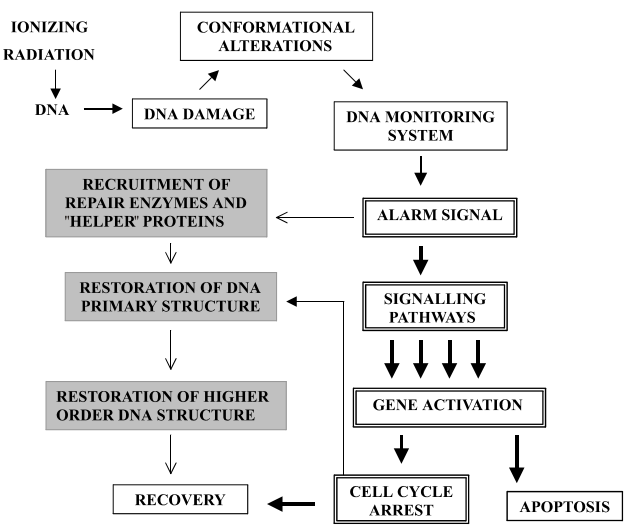


Fig. 2. Events initiated by the DNA damage-monitoring system in the irradiated mammalian cell: two interconnected chains of events take place. One is connected with the enzymatic repair of DNA damage; the other one comprises signalling that leads to the expression of genes necessary for cellular recovery, cell cycle control or cell death programes. See text for explanations.

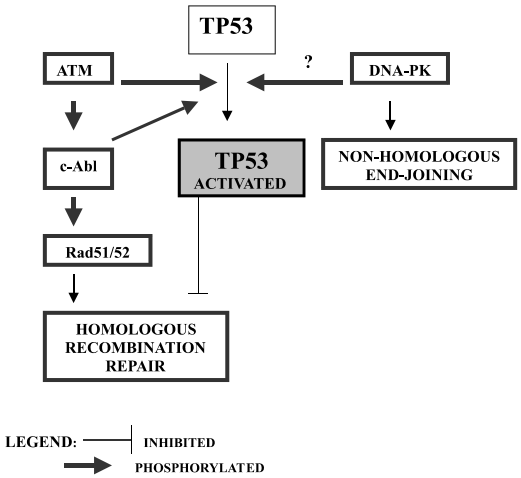


Fig. 3. Post-irradiation signals that pass through Tp53 and are related to DNA repair system.

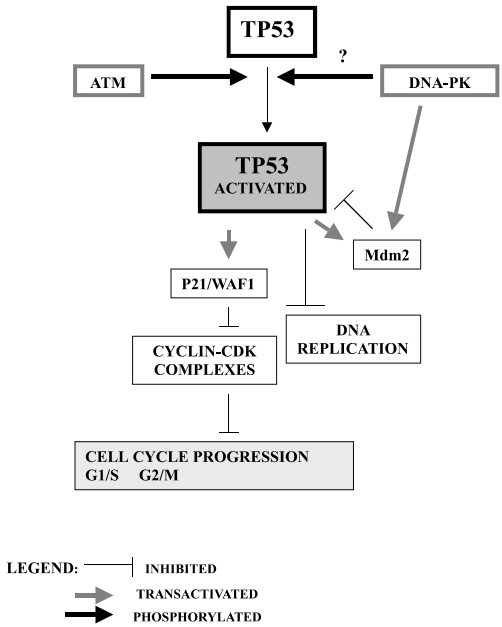


Fig. 4. Post-irradiation signals that pass through Tp53 and are related to cell cycle checkpoint control.

connected with cell cycle control and cell death. In Fig. 2 it is presented by framed blocks and thick arrows. These two groups of post-radiation events are closely interconnected. Since unrepaired DNA damage can be fixed by the cell's passage through certain points of the cell cycle [10] cell cycle arrests at G1/S or G2/M phase boundaries leave time for repair and are important recovery factors. Other signals come from mitogen or other extracellular factor-activated signalling pathways; they may have either anti-apoptotic or pro-apoptotic character, may add to the cell cycle control or change the balance between various DNA repair systems. The details of these aspects of signalling are yet not well understood.

There is a close relation between the DNA damage monitoring system and the signalling connected with cell cycle control. Progression through the cell cycle involves checking whether all conditions for the safe passage from one phase to another are fullfilled (e.g. is DNA replication completed? is DNA unbroken? decatenated? are the necessary enzymes and substrates synthesized?). Thus, the cell "takes a decision" to proliferate, when DNA is complete and undamaged. Alternatively, the decision to die is taken in order to eliminate the potential danger that the damaged cell presents to the organism.

The integrating role of Tp53

A large part of the nuclear signalling pathway, including the integrating role of Tp53 has been revealed. The signals that are down-stream of Tp53 are directed towards DNA repair (Fig. 3), cell cycle control (Fig. 4) and apoptosis (Fig. 5). The Tp53 dependent mechanisms of G1 and G2 arrests are relatively well understood (reviewed in [3]). There is good evidence that expression of potentially lethal damage is related to cell cycle arrests and that these are considerably diminished in cells with inactivated Tp53 [24]. The way by which Tp53 leads to radiation-induced apoptosis involves transactivation of *Bax* (main pro-apoptotic) and transrepression of *Bcl2* (main anti-apoptotic) genes. Also, there are Tp53 transactivation-independent ways to apoptosis, involving a protein-protein interaction (most probably binding to DNA helicases, XPB and XPD (proteins mutated in *xeroderma pigmentosum*), possibly to other ones).

A characteristic feature of cellular signalling is its redundancy. Accordingly, there is also a Tp53-independent pathway to apoptosis. A prominent role in it is played by tyrosine protein kinase, c-Abl. Upon irradiation it is activated by Atm. One of its down-stream effectors in the apoptosis pathway is p73α, a member of Tp53 family, sharing many properties and functions of Tp53 [2]. Likewise, cell cycle progression can to some extent be controlled by Tp53-independent signalling (reviewed in [26, 28]). Thymocytes from Tp53-null rats provide an example of the importance of signalling for the fate of the irradiated cell. In contrast with wild type Tp53 thymocytes, they do not respond to ionizing radiation by apoptosis and show high viability after time intervals, when the wild type Tp53 cells are already dead [6, 14].

Signalling initiated in the cytoplasm

There are two main signalling pathways starting at the plasma membrane that become activated in the irradiated cell [12, 20]: the MAPK/ERK (mitogen and extracellular signal regulated protein kinase family) "survival pathway" and the SAPK/JNK (stress-activated protein kinase/c-Jun N-terminal kinase) "cell death pathway" (Fig. 1). The balance between them is likely to determine to some extent the cell's fate.

The MAPK pathway (shown in greater detail in Fig. 6) can be inhibited at the level of the receptor tyrosine kinases by tyrphostine and similar inhibitors; at the level of the immediate down-stream effectors – by inhibitors of farnesyla-

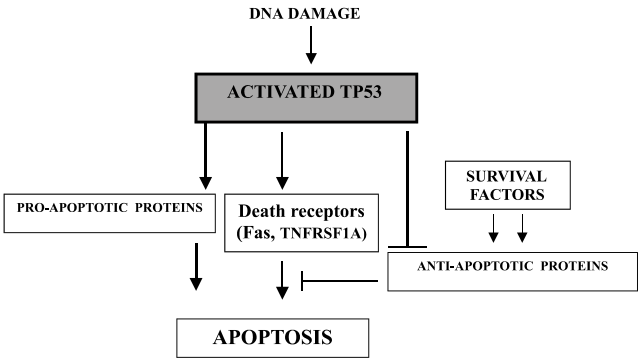


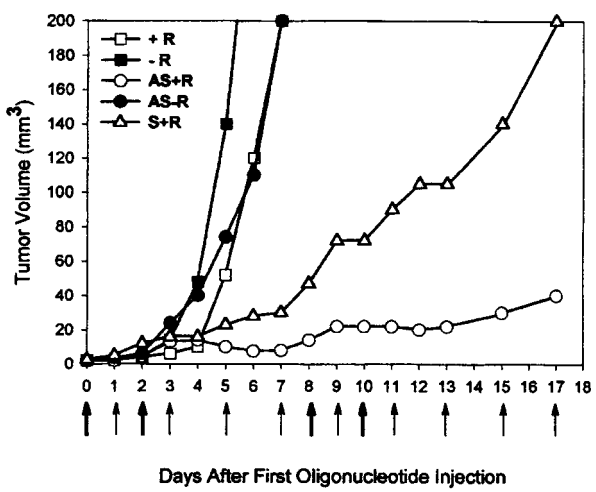
Fig. 5. Post-irradiation signals that pass through Tp53 and are related to apoptosis.

tion which prevent Ras anchoring in the plasma membrane and thus, inactivating it; at the level of Raf-1 by antisense *raf* [16, 19] at the level of MEK1, 2 by PD98059. All these interceptions (reviewed in [23]) stop the "survival signals" and cause radiosensitization. It has been noted that inhibition of this pathway leads to a higher activity of the other (SAPK/JNK) pathway and thus, to a higher death rate (usually apoptosis [21, 27]). The rate of repair of double strand break (DSB) is not affected by such a shift of balance between the two signalling pathways [1].

The importance of this signalling pathway is illustrated by the following observations. Introduction of antisense *raf* (antisense oligonucleotide) blocks Raf synthesis and thus, the pathway becomes inhibited [16]. A similar technique is applied with antisense *ras* [19]. The result is a considerable sensitization of the antisense oligonucleotide-treated cells in culture [16] or *in vivo* [19] to radiation (Fig. 7).

Radiation activates an acidic sphingomyelinase, an enzyme that hydro-lyses sphingomyelin and produces ceramide; *de novo* ceramide synthesis also takes place later (Fig. 8). This molecule is considered as "death-signalling molecule" (some reservations about this view are reviewed in [17]).

The pro-apoptotic role of ceramide, seems to be executed by the shift of balance between the MAPK and SAPK/JNK



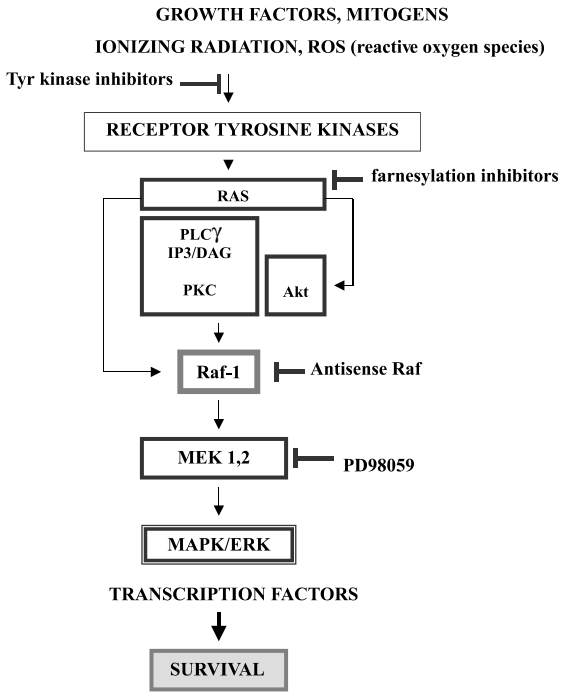


Fig. 6. The MAPK/ERK signalling pathway. See text for explanations.

pathways, as shown in Fig. 1 (reviewed in [23]). The signalling that involves ceramide can give opposite results, depending on the available down-stream effectors. In most cases, however, the SAPK/JNK pathway is activated, leading to apoptosis. Interestingly, some cell types show high or low radiosensitivity depending on the ability to generate ceramide in response to irradiation [5, 15]. This shows that the cells can to some extent tolerate radiation-inflicted damage when the death signal is not generated upon exposure to ionizing radiation. A similar case has been discussed above, namely, thymocytes from Tp53-null rats, unable to enter post-irradiation apoptosis that is Tp53-dependent in these cells [6, 14].

Other examples of the role of cytoplasmic signalling in the cellular radiosensitivity are connected with the mitogen-acti-

Fig. 7. Effect of combination of sense (S) or antisense (AS) partially phosphorothioate (PPS) – modified anti-*ras* ODN (oligodeoxynucleotide) with an additional hydrophobic tail at the 3'-end (PPS-C16 ODN) and radiation on tumor growth in a xenograft mouse model [19]. RS5504 cells were subcutaneously injected on the back of female athymic nude mice. The antisense or sense PPS-C_{16} ODN (250 pmol in 50 µl) were injected directly into the tumors. Beginning 24 h after the first ODN injection, the tumor area was exposed to 2-2.5 Gy doses of X-rays, as indicated by the arrows, to a total of 20 Gy. (-R) – control untreated tumors; (+R) – tumors received only radiation; (AS+R) – tumors treated with AS PPS-C_{16} ODN and radiation; (AS-R) – tumors treated with AS PPS-C_{16} ODN only; (S+R) – tumors treated with control, S PPS-C_{16} ODN and radiation. Bold arrows indicate ODN injections; thin arrows indicate irradiation. Reproduced from Rait et al. [19] by permission of the American Chemical Society.

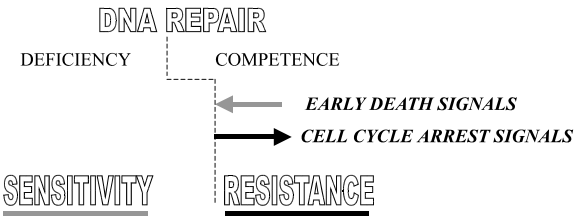


Fig. 8. Diagram illustrating the concept of DNA repair competence and competition between repair and death signalling as determinants of the cellular intrinsic radiosensitivity.

vated signalling pathway (Fig. 6). Activated Ras may, in certain cellular contexts, lead to a higher radiation resistance: REF (rat embryo fibroblasts) transformed by mutation-activated H-*ras* and *c-myc* are more resistant to irradiation in spite of the identical DSB induction and repair to that in the untransformed REF. The possible mechanism lays in prolongation of the G2 arrest and a concomitant decrease in apoptosis [4].

Activation of nuclear factor κB (NFκB) provides yet another example of signalling initiated by various kinds of stress, including ionizing radiation (Fig. 1). NFκB is a member of a large family of transcription factors, NFκB/Rel, with numerous biological functions, among them, modulation of the apoptotic response. It may have both pro- and anti-apoptotic activity, depending on the cell type. NFκB is a heterodimer (p50/p65), sequestered in the cytoplasm by the inhibitory subunit, IκB. It becomes activated by a complex containing IκB kinase and other proteins (called "signalsome"). Degradation of the phosphorylated IκB follows and the p50/p65 heterodimer is free to translocate and to exert its transactivatory function in the nucleus.

A peculiar feature of NFκB is that it can be activated either by signals from the cytoplasm or from the nucleus. The latter activation takes place in response to DNA damage; the pathway is Tp53-dependent and involves the MAPK signalling, discussed above (see Fig. 1) [22]. This recent observation completes the earlier ones, where impaired NFκB activation was observed in *ataxia telangiectasia* cells [11, 13]. Also, this is a convincing example of signalling that supports the hypothesis of signalling loop. This hypothesis was put forward by Weichselbaum and co-workers; it assumes that a signal generated in the nucleus in response to DNA damage, is transduced to the cytoplasm and returns to the nucleus through the cytoplasmic signal transduction pathway [30]. Also the observation that ceramide is synthesised after X-irradiation in a Tp53-dependent way [7] may be interpreted in terms of this hypothesis. The mechanism of signal transduction from Tp53 to the cytoplasm is unknown.

Concluding remarks

Transduction of the various signals generated in the irradiated cell is modulated by the cross-talk among the various pathways, availability and localisation of the down-stream effectors, their organisation into complexes with specific scaffold proteins and the balance between phosphorylation and dephosphorylation. At each transduction step the function is carried out by a number of related proteins rather than by a single protein. Hence, the degree of complexity of the signalling network in the cell necessitates an examination of the signal transduction pathways in a given cell type before making a general conclusion on the ability of a specific factor to modify the radiation response. The recent advances in this field create considerable possibilities for amelioration of cancer radiotherapy as shown by the example in Fig. 7.

When DNA break rejoining is impaired, the cell is unconditionally radiation sensitive. The fate of a repair-competent cell is determined by the time factor. Fig. 8 illustrates this concept. The cell cycle arrest should be long enough to ensure the completion of repair; the respective signalling is favourable for the cell's survival, or increased resistance. When death signal is generated before break rejoining is completed (early death signals in Fig. 8), cell recovery is limited, and radiosensitivity increases, as observed by Radford et al. [18] in a panel of lymphoid cell lines. On the other hand, incomplete repair or misrepair may be tolerated by the irradiated cell, when generation of the death signal is prevented (e.g. [14]). So, the character and timing of the signals are, to a large part, responsible for the cellular intrinsic radiation sensitivity and depend on the characteristics of the "cellular integrated circuit" (a term introduced in [9] to describe the cellular signalling network).

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