Review

Typical Cell Signaling Response to Ionizing Radiation: DNA Damage and Extranuclear Damage

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ABSTRACT

To treat many types of cancer, ionizing radiation (IR) is primarily used as external-beam radiotherapy, brachytherapy, and targeted radionuclide therapy. Exposure of tumor cells to IR can induce DNA damage as well as generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which can cause non-DNA lesions or extracellular damage like lipid perioxidation. The initial radiation-induced cell responses to DNA damage and ROS like the proteolytic processing, as well as synthesis and releasing ligands (such as growth factors, cytokines, and hormone) can cause the delayed secondary responses in irradiated and unirradiated bystander cells through paracrine and autocrine pathways.

Key words: Radiation; Bystander effect; DNA damage; Extranuclear damage

Introduction

Radiation-induced bystander-type biological responses were first described in overlooked literature in the late 1940s. At that time, most radiobiologists still believed that only the directly irradiated cells suffered the effects of radiation exposure through direct ionization or the action of water radiolysis products. Recently, the finding of Nagasawa and Little[1] in 1992 sparked people's interest in radiation-induced bystander effect. Their results showed that when the monolayer cells were exposed to low-dose α-particles, some cells (30% of the cells) showed biological damage in sister chromatid exchanges (SCEs), and less than 1% of the cells were estimated to undergo a nuclear traversal based on the microdosimetric principle. Though not recognized initially, in the past 17 years, the significance of radiation-induced bystander effects has been widely accepted. Occurrences of the bystander effect after various qualities, doses, and dose-rates of radiation have been recently demonstrated in studies of both *in vitro* and a few *in vivo* models. Bystander communication has been shown both in the systems where irradiated cells are in contact with each other through gap junction pathways and in the systems where the cells are at considerable distances apart from each other via secreted factors[2]. It is compelling to speculate that, when cells are in close contact, signaling processes mediated through soluble factors in the medium may play a predominant role. Several soluble factors have been considered as potential candidates in the bystander response. However, very little is known about the nature of the signaling mediators, their targets in non-irradiated cells, their mechanism of maintaining sustained communication, or the duration of the communication after irradiation.

Overall, bystander effects are manifested as the expression of a wide range of endpoints, such as mutagenesis, chromosomal aberrations, micronucleation, neoplastic transformation, proliferation, and differentiation. Radiation-induced bystander effects refer to the responses of cells that were not subjected to ionizing radiation (IR) exposure. In other words, the damages caused by radiation in irradiated cells are augmented by subsequent damage to non-irradiated bystander cells. These bystander cells may have been neighbors of irradiated cells or may have been physically separated but subject to soluble secreted signals from irradiated cells.

Surviving tumor cells at the treatment site after radiation therapy may elicit signaling mechanisms that may be responsible for clonal selection, tumor cell proliferation/tumor growth, and metastasis. Hence, it is imperative to understand the relationship between tumor

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re-growth and those altered responses following radiation exposure. If this was to occur, either the cells hit by radiation should be viable and non-responsive to radiation, which is very unlikely, or a sub group of tumor cells should develop resistance and maintain functional integrity to elicit communication in both irradiated and non-targeted bystander neighboring cells. This might allow the cancer cells that are surviving the radiation exposure to develop a clone (clonal selection), re-grow (tumor cell proliferation and growth), and cause tumor relapse at the treatment site. Simultaneous angiogenic support through intercellular communication between the surviving tumor cells and the surrounding endothelium would further augment the tumor growth and increase the risk of distant metastasis.

DNA Damage and Activation of Nuclear Sensory Protein

There are many types of DNA damage induced by radiation, such as single-strand breaks (SSBs) and double-strand breaks (DSBs), sugar and base modifications, and DNA-protein cross-links[3]. The damaged DNA can be not only recognized by the sensory proteins, leading to recruitment of DNA repair enzymes, but also generate signals to delay cell cycle progression until the DNA damage is repaired. Some important proteins implicated in the surveillance to DNA damage and the activation of the damage checkpoint and cell cycle arrest are phosphatidylinositol 3-kinase Family (PI3K-like kinase), like ataxia telangiectasia mutated (ATM), and ATM, Rad3-related protein (ATR). ATM, a serine/threonine-specific protein kinase, is named for the disease, ataxia telangiectasia, caused by mutations of ATM[4]. Activated ATM can phosphorylate and regulate various downstream target proteins, including tumor suppressor proteins P53 and breast cancer type 1 susceptibility protein (BRCA1), checkpoint kinase 2 (CHK2), checkpoint proteins RAD17 and RAD9, and DNA repair protein Nibrin (NBS1), leading to cell cycle arrest, DNA repair or apoptosis[5]. ATR is also a serine/threonine-specific protein kinase, and mutations in ATR are responsible for a rare human disease, Seckel syndrome. ATR has a similar function to ATM that once it is activated, it can phosphorylate downstream proteins like serine/threonine-protein kinase (CHK1), initiating a signal transduction cascade that culminates in cell cycle arrest[6,7]. However, there is difference between ATM and ATR on the kinetics of activation and the types of damage to which they respond best. ATM is preferred in response to DSBs, while ATR is activated in response to persistent single-stranded DNA, which usually occur at stalled replication forks as an intermediate in DNA detection and repair pathways such as nucleotide excision repair (NER) and homologous recombination[8]. Recent studies also support that ATM is the main determinant of the early cell cycle checkpoint response to IR-induced damage, whereas ATR responds later to processed damage induced by IR[9,10]. P53 is a tumor suppressor protein, and is usually called as "the guardian of the genome" to describe its important role in conserving stability by preventing genome mutation. In normal cells, P53 is highly unstable due to the fact that Mdm2 (Hdm2 in humans) binds to P53 to promote its ubiquitylation and destruction in proteasomes, so P53 usually presents at very low concentration. DNA damage activates the protein kinases that cause the phosphorylation of P53, then reduce its binding to Mdm2 and decrease the P53 degradation. As a result, P53 accumulates to high concentration level and stimulate the gene transcription. The P53 functions through two main mechanisms: It can activate DNA repair protein and/or promote transcription of genes that induce cell cycle arrest (especially *p21*, it is transcriptionally activated by P53, and can suppress G1/S-Cdk and S-Cdk complexes, and keep the cell cycle arrest in G1). Alternatively, if the DNA damage cannot be reparable any more, it can initiate apoptosis, the programmed cell death (Figure 1)[11].

It is not limited that, ATR, ATM, CHk1, CHk2 as we mentioned above, are implicated in the genome integrity checkpoint or other responses to several forms of DNA damage (induced by either ultraviolet (UV), IR or chemical agents, such as hydrogen peroxide)[12,13], another group of protein kinase, mitogen-activated protein kinase (MAPK), including c-Jun N-terminal kinases (JNK1/3), extracellular-signal-regulated kinases (ERK1/2), ERK5 and P38 mitogen-activated protein kinases (p38 MAPK), can also respond to several types of stress, such as membrane damage, oxidative stress, osmotic shock, and heat shock, through transcriptionally activating *p53*.

Extranuclear Damage and Activation

IR can directly interact with water, then generate small amounts of reactive oxygen species (ROS), which are amplified by mitochondria, generating large amount of ROS and reactive nitrogen species (RNS). ROS and RNS can inhibit protein tyrosine phosphatase (PTPase) activities. PTPase can remove phosphate groups from phosphorylated tyrosine residues on proteins. PTPase and tyrosine kinase work together to regulate the phosphorylation state balance of many important tyrosine phosphorylation signaling molecules. Hence, the inhibition of PTPase induced by radiation through the ROS and RNS increase the potential of tyrosine phosphorylation of the downstream proteins. Recent data also showed that the epidermal growth factor receptor (EGFR; ErbB-1; HER1 in humans) can be rapidly activated by IR in many tumor cells *in vitro*[14,15]. EGFR, a family of four structurally related receptor tyrosine kinases, is high affinity cell surface receptors for various growth factors, cytokines and hormones. Insufficient EGFR signaling in humans is associated with the