

## MOLECULAR MECHANISMS UNDERLYING CANCER CELL RADIORESISTANCE

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### Summary

Radioresistance of the tumor cells remains a significant obstacle for the radiotherapy treatment of cancer. Radioresistance involves multiple genes, factors, and mechanisms that adapt cancer cells or tissues to radiotherapy-induced changes and develop resistance to ionizing radiation. The major studies of the effect of radiation on cells reported include the following areas: 1) the study of DNA damages and their repair; 2) mutations in tumor suppressor genes and radiation-induced oncogene expression; 3) the role of growth factors and cytokines; 4) violation of the cell cycle; 5) elucidation of the mechanisms of apoptosis and necrosis.

This review aimed to provide a theoretical basis, which may improve the sensitivity of cancer cells to radiotherapy. It focuses on the roles of tumor metabolism, DNA repair capacity, cell cycle checkpoints, and the tumor microenvironment in the development of radioresistance of cancer cells. Understanding the molecular alterations that lead to radioresistance may provide new diagnostic markers and therapeutic targets to improve radiotherapy efficacy.

**Keywords:** ionizing radiation, DNA repair, cell cycle, proliferation, radiotherapy.

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### 1. Introduction

According to the latest studies, the main factors involved in the development of radioresistance are the following: 1) DNA damage repair; 2) cell cycle arrest; 3) oncogene and tumor suppressor alterations; 4) changes in the tumor microenvironment; 5) autophagy (a proteasome-independent degradation of cellular components); 6) the generation of cancer stem cells (undifferentiated cancer cells with high oncogenic activity, with the self-renewal ability and multi-directional differentiation potential); 7) tumor metabolism alterations (*Chen et al., 2017; Fengsheng et al., 2016; Tang et al., 2018; Yoshida et al., 2016; Zhang et al., 2016*).

Ionizing radiation induces a number of DNA damage, including single-strand breaks (SSB) and double-strand breaks (DSB), damage of nitrogenous bases and sugar residues, the formation of inter-strand and intra-strand crosslinks of DNA-DNA and DNA-protein, the appearance of chromosomal aberrations (*Liu et al., 2021*). Radiotherapy directly induces DNA damage or indirectly causes oxidative stress. Free hydroxyl radicals are considered the ionizing radiation-induced mediators of DNA damage, including SSB and DSB, which disturb the DNA structure, triggering cell death (*Tang et al., 2018*). Like most normal cells, tumor cells have reparative systems capable of repairing damage and restoring the native structure of DNA through specific mechanisms and enzymatic complexes. DNA damage may be recognized by detectors specific to different types of repair.

Moreover, additional detector proteins are involved in the regulation of the cell cycle and are involved in the arrest or slowing of the cell cycle in the presence of DNA damage at various stages of the cell cycle. Here, an overview is provided to elucidate the interplay between tumor cell metabolism, DNA repair ability, microenvironment, and radioresistance.

## 2. Cell cycle redistribution in irradiated cells

Initial biological responses to radiation damage occur at the molecular, cellular, subcellular levels and at intervals ranging from seconds to hours or days. Among the many manifestations of the effect of ionizing radiation on cell viability, the most important is replicative cell death (suppression of the ability to divide).

The second type of replicative death of irradiated cells is the formation of so-called giant cells, which arise from the fusion of two neighboring, often "sister" cells. Such cells are capable of no more than 2-3 divisions, after which they die. Giant cells can form without fusion with a long delay in cell division of irradiated cells or their offspring. This method of cell death is inherent in aging cells and is observed after exposure to ionizing radiation. It is known that the sensitivity to radiation may also depend on the rate of proliferation of target cells (*Erenpreisa et al., 2001; Suzuki et al., 2001*).

As a result of DNA damage, cell growth may stop (*Sia et al., 2020*). At the heart of the events leading to cycle arrest in the G1 phase are reactions that increase the level of p53 protein, which, as a transcription factor, activates genes involved in cell cycle blockade (p21), induction of apoptosis (Bax), and repair (Gadd45). Ionizing radiation-induced ATM-dependent serine phosphorylation was found in p53 protein (*Khanna et al., 1998*). In addition, Chk2-catalyzed phosphorylation of serine 20 (*Chechab et al., 2000*) is also necessary for p53 stabilization, which inhibits p53 binding to MDM2. Chk2 kinase is activated by ATM (*Ward et al., 2001*), and thus, directly and indirectly, ATM is involved in p53 stabilizing by phosphorylation. Also, by phosphorylation of serine 395 in the MDM2 protein, ATM influences the ability of MDM2 to bind p53 (*Khosravi et al., 1999*). The final result of phosphorylation is the destabilization of p53-MDM2 binding, stabilization of p53 protein, and its activation as a transcription factor, which allows blocking the cell cycle in the G1 phase.

p21, an inhibitor of cyclin-dependent Cdk2 kinases, plays a fundamental role in blocking the cell cycle in the G1 phase. Inhibition of Cdk2/cyclin E kinases prevents phosphorylation of the Rb (Retinoblastoma) protein and the release of the transcription factor E2F. It blocks the transcription of many proteins needed to initiate DNA synthesis, thereby blocking the cell cycle at the G1 stage. In addition to induction of p21, the p53 protein also activates the transcription of other regulatory proteins: the 14-3-3 protein, responsible, among other things, for the removal of Cdc2 kinase from the nucleus, and the Gadd45 protein, which, apart from participating in the repair of DNA damage, also regulates the activity of Cdc2 kinase (*Al Bitar et al., 2019*). It was found that the different members of the 14-3-3 family of proteins could control p53 activity, and they could be alternative targets for the reactivation of p53 (*Falcicchio et al., 2020*). 14-3-3 $\sigma$ , a member of the 14-3-3 protein family, was closely associated with the radioresistance development by arresting cancer cells in the G2/M phase (*Chen et al., 2017*).

Mammalian cells exposed to ionizing radiation can be blocked in the G2/M phase by inactivating and translocating the protein phosphatase Cdc25C, an activator of CDK kinases, resulting from its phosphorylation (serine 216) by Chk2 kinase (*O'Connell et al., 2000*). Chk2 activation requires the presence of ATM and Nbs1 (*Buscemi et al., 2001*). It is impaired in AT (Ataxia-Telangiectasia) cells and in cells with inactive Nbs1 protein that is also defective at the G2/M checkpoint. The association of ATM with BRCA1 and the phosphorylation of BRCA1 by ATM increases under the influence of ionizing radiation (*Gatei et al., 2000*) and contributes to cell cycle arrest in the G2 phase.

The arrest of cells in the G1 phase under ionizing radiation occurs due to the transactivation of p21CIP/WAF1 by the p53 protein. p21CIP/WAF1 protein, in turn, inhibits

cyclin-dependent kinases and prevents the transition of cells to the S phase of the cell cycle, thus allowing DNA repair. (Al Bitar *et al.*, 2019; Morgan *et al.*, 2015; Shu *et al.*, 2007).

Cell arrest in the G1 phase of the cell cycle in response to ionizing radiation can also occur via the p53-independent signaling pathway. Thus, activated ATM kinase phosphorylates and activates c-ABL kinase, which, in turn, can activate p73, leading to transactivation of p21 and GADD45 (Dasika *et al.*, 1999; Lee *et al.*, 1999).

Irradiation of cells causes a slowdown in the S phase of the cell cycle. ATM, NBS1, CHK2, Cdc25A, BRCA1, SMC1, MRE11, and others are involved in this process (Falck *et al.*, 2002; Kastan *et al.*, 2001). It has been reported that BRCA1 phosphorylation by ATM kinase is necessary for the radiation-induced arrest of cells in the S phase of the cell cycle (Xu *et al.*, 2002).

Considering all the above, we can say that stopping cell growth due to exposure to ionizing radiation is also associated with either DNA repair or the apoptosis process required to eliminate cells that can't repair damaged DNA.

### 3. DNA damage and repair in irradiated cells

A signaling cascade is triggered in response to DNA damage, consisting of sensory, transducer, and effector proteins, which can stop cell growth, repair DNA or, trigger cell death (apoptosis). Sensory proteins (RAD9, RAD1, RAD17, HUS1, and others) recognize DNA damage and are likely to respond to it by directly linking double-stranded DNA breaks in mammalian cells. The RAD17 protein promotes the recognition of DNA damage by the RAD1-RAD9-HUS1 complex. ATM/ATR kinase phosphorylates the effector kinases CHK1 and CHK2, which phosphorylate the p53 protein on the Ser20 residue and other target proteins in mammalian cells (Liu *et al.*, 2021). A model was proposed, according to which changes in the structure of chromatin after DNA damage may contribute to the rapid activation of ATM kinase (Bakkenist *et al.*, 2003).

It has been reported that histone H2AX phosphorylation occurs at the sites of double-stranded DNA breaks with the participation of ATM kinase. H2AX is involved in creating specific DNA-repair complexes on damaged DNA (Sharma *et al.*, 2012). At the same time, p53 and the MDM2 protein, which mediates the degradation of p53, are phosphorylated directly by ATM kinase on Ser15 and Ser395 residues, respectively (Maya *et al.*, 2001). The phosphorylation sites of p53 (Ser15 and Ser20) and MDM2 (Ser395) are localized in the region of interaction of these proteins with each other. Thus, phosphorylation disrupts the binding of MDM2 to p53 and thus protects the p53 protein from ubiquitination and degradation (Marine *et al.*, 2010). It has been discovered that in the offsprings of human fibrosarcoma cells of the HT1080 line, survived after exposure to X-ray irradiation at a dose of 6 Gy, slow down of the activation of p53 protein occurs (Suzuki *et al.*, 2003).

Cells have many mechanisms that enable them to survive under genotoxic stress. The most important of these is the repair of DNA damage and blockage of the cell cycle. Restoration of a variety of DNA damage is possible thanks to the development of specialized repair systems, each of which is responsible for removing specific types of damage. Depending on the type of DNA damage, there are several main mechanisms of repair. Excision repair of nitrogenous bases is necessary to correct modified bases formed by the action of free radicals on DNA. This type of violation occurs by indirect damage of DNA by ionizing radiation. There are several stages of excision repair. In the first stage, the damaged nitrogenous base is cleaved by DNA glycosylases, which hydrolyze the N-glycosidic bond between the damaged base and the deoxyribose residue. Deoxyribose without a nitrogenous base, the so-called apurinic/aprimidinic

site (AP site), is excised by AP endonucleases. In the second stage, the formed “gap”, which has the size of one base, is repaired to the original sequence by the combined action of exonucleases, polymerase, and DNA ligase. Repair of single-stranded DNA breaks occurs using a complementary DNA strand as a template (*Jasin et al., 2013; Marsden et al., 2017*). The protein poly-ADP-ribose polymerase (PARP) plays an essential role in the initiation of enzymes of excision repair. Even before the activation of repair enzymes, PARP binds to the rupture of DNA and prevents the destruction of free end regions of DNA. In addition, PARP, along with DNA-dependent protein kinase (DNA-PC) and ATM protein, is involved in providing a delay in the cell cycle, which is necessary to complete the repair process (*Javle et al., 2011*).

Single strand breaks (SSB) may arise via direct radiation-induced DNA damage or as intermediaries formed during the repair of other single strand lesions (e.g., base excision repair of oxidized bases) (*Morgan et al., 2015*).

Double strand breaks (DSB) are the most dangerous type of DNA damage for a cell. They lead to the development of mutations and chromosomal rearrangements (aberrations), cell death, and the formation of genetic instability (*Vitor et al., 2020*). In eukaryotic organisms, two main mechanisms of repair double-strand DNA breaks have been identified: homologous recombination (HR) and non-homologous end-joining (NHEJ). In the HR, using the undamaged homologous DNA fragment as a template with a sequence complementary to at least one broken end occurs, whereas NHEJ does not require a homologous template (*Brandsma et al., 2012; Frankenberg-Schwager et al., 2009; Mao et al., 2008*).

The complex of proteins involved in double-stranded DNA breaks repairing by the HR mechanism includes ATM, BLM, BRCA1/2, c-ABL, RAD51/51C/51D/52/54, XRCC2/3, and others. Extensive studies have reported that radioresistance of hypoxic cancer cells is induced by the ATM-dependent DNA damage repair pathway, mainly through upregulation of HR mechanism of repair (*Bencokova et al., 2009; Hashimoto et al., 2018; Olcina et al., 2014*). In response to DNA strand breaks, the activated form of ATM phosphorylates some HR-related factors, such as BRCA1 and FANCD2, triggering the DNA damage repair pathway. It has been suggested that ATM also plays a pivotal role in the radioresistance of cancer cells under hypoxic conditions (*Suwa et al., 2021*).

A non-homologous joining occurs between ends of DNA that have non-homologous sequences or very short homologous regions. This reparation is not infallible and is often a source of genetic rearrangements (*Mladenov et al., 2011; Morgan et al., 2015*). Restoration of the original structure of DNA is possible only if the joining between the ends of the same strand of DNA occurs. If a process occurs between different molecules, it causes chromosomal aberrations, such as deletions, insertions, translocations, and inversions. NHEJ mechanism is carried out by a complex of proteins, including DNA-dependent protein kinase (DNA-PK), Ku70, Ku86, DNA ligase IV, XRCC4, and others. In addition, proteins MRE11, RAD50, and NBS1 (MRN complex) are involved in DNA damage repair by both the mechanism of HR and NHEJ (*Her et al., 2018; Morgan et al., 2015*). Tumor suppressors p53 and BRCA1 control the course of HR and NHEJ, but their effect on radioresistance remains the subject of intensive research (*Gorodetska et al., 2019; Moureau et al., 2016*).

The inability of cells to repair double-stranded gaps leads to genome destabilization and tumors. In some cases, double-stranded DNA breaks contribute to the induction of apoptosis. Some researchers attribute the resistance of many cells to radiation to the kinetics and repair efficiency of single- and double-stranded DNA breaks, which can also serve as an additional method of monitoring the radiotherapeutic response in the clinic (*Huang et al., 2020; Veleg-zhaninov et al., 2020*).

#### 4. Tumor metabolism alterations in irradiated cells

Cancer cells can obtain sufficient oxygen only when they are localized close to tumor blood vessels. On the other hand, cancer cells far from functional blood vessels can get the minimal oxygen level necessary for their survival but insufficient for active proliferation (Suwa *et al.*, 2021). Cancer cells in hypoxic regions produce ATP energy mainly via anaerobic glycolysis and generate lactate as a byproduct, leading to a unique microenvironment formation with a low pH. On the other hand, cancer cells farther away from blood vessels are deprived of oxygen and nutrients, and they die because of necrosis (Harada, 2011; Yeom *et al.*, 2012). Cancer cells adapt to hypoxic conditions by inducing the expression of hypoxia-inducible factor 1 (HIF-1), which causes the expression of various pro-angiogenic factors, such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). Besides, HIF-1 mediates the activation of the pentose phosphate pathway (PPP) (Meijer *et al.*, 2012). The PPP, whose initial metabolite glucose-6-phosphate, is supplied via glycolysis, generates pentose sugars and ribose-5-phosphate and generates NADPH as a byproduct (Patra *et al.*, 2014). Because NADPH is a coenzyme of an antioxidant enzyme glutathione reductase, which converts oxidized glutathione (GSSG) into reduced form (GSH), and ribose-5-phosphate is essential for nucleotide synthesis, so HIF-1-mediated activation of the PPP is associated with the radioresistance of hypoxic cancer cells (Harada, 2016; Nakashima *et al.*, 2017; Telarovic *et al.*, 2021).

Glucose transporter 1 (GLUT1) protein is involved in the glucose transport through the cellular membrane into the cell, and it is upregulated under hypoxic conditions (Chung *et al.*, 2009). GLUT1 overexpression was reported to be associated with radioresistance and poor prognosis in patients with oral squamous cell carcinoma and head and neck squamous cell carcinoma (De Schutter *et al.*, 2005; Kunkel *et al.*, 2007). Radioresistant tumor cells often have high levels of GLUT1, which was associated with oncogene activation, tumor suppressor inactivation, hypoxia stimulation, and the regulation of different signaling pathways, such as MAPK and PI3K/AKT (Fang *et al.*, 2015).

The elevated glycolysis rate facilitates the rejoining of radiation-induced DNA strand breaks by activating NHEJ and HR repair mechanisms, thus reducing the radiation-induced cytogenetic damage in cancer cells (Bhatt *et al.*, 2015).

#### 5. Tumor microenvironment and radioresistance of cancer cells

Malignant tumor tissues consist of cancer cells and a wide variety of other cells responsible for the inflammatory response, angiogenesis, immune response, and support of the tumor tissue architecture, forming a complex cellular society (Suwa *et al.*, 2021). Many immunosuppressive processes increase the risk of tumor recurrence and metastasis, and immune evasion has emerged as a severe obstacle in cancer treatment (Tang *et al.*, 2017). Changes in the cytokine levels, epithelial-mesenchymal transition (EMT)-related processes, and hypoxic conditions can promote radioresistance in tumor cells (Goffart *et al.*, 2017; Krisnawan *et al.*, 2020; Zhang *et al.*, 2016).

Recent studies have shown that tumor-associated macrophages (TAMs), which exist abundantly in tumor tissues, promote tumor growth and enhance radioresistance in various cancers (Brown *et al.*, 2017; Dehne *et al.*, 2017). TAMs induce the expression of cyclooxygenase-2 (COX-2) – an enzyme participating in the synthesis of prostaglandin E2 (PGE2) which has been reported to accelerate tumor growth and induce radioresistance in cancers (Cook *et al.*, 2016). In addition, it has been suggested that TAMs induce high expression of tumor necrosis factor- $\alpha$



(TNF- $\alpha$ ), promote angiogenesis, and facilitate tumor recurrence after radiotherapy (Meng *et al.*, 2010). Vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), secreted from M2-type macrophages, promote tumor regrowth after radiotherapy (Balkwill *et al.*, 2001). It has been reported that some factors secreted from cancer-associated fibroblasts (CAFs) induce radioresistance in cancer cells. For example, CAFs secrete epidermal growth factor (EGF), insulin-like growth factor 2 (IGF2), and fibroblast growth factor 4 (FGF4), which increase both the proliferation and the survival of cancer cells after irradiation (Chu *et al.*, 2014; Krisnawan *et al.*, 2020). Besides, stromal cell-derived factor 1 (SDF-1) and  $\beta$ -hydroxybutyrate secreted from CAFs induce autophagy in irradiated cancer cells and accelerate the regrowth of tumors (Wang *et al.*, 2017).

In addition, non-irradiated cells may receive apoptotic signals from neighboring irradiated cells through the bystander effect, inducing the non-irradiated cells to exhibit similar biological effects to those of the irradiated cells (Chevalier *et al.*, 2015).

## 6. Conclusions

Radioresistance of cancer cells can be provided by more than one mechanism. Radiotherapy may induce alterations in many molecules and signaling pathways involved in the tumor cell metabolism, and radiation-induced metabolic changes in cancer cells may affect the efficacy of radiotherapy. Cancer cells undergo a range of interphase and mitotic death after irradiation via direct and indirect effects of ionizing radiation. Although most radiation-induced DNA breaks in cancer cells are rapidly repaired, residual breaks or erroneous DNA repair lead to genetic instability, increased mutation rates, and chromosomal aberrations. An increased understanding of the molecular mechanisms of radiation-induced cell death will reveal novel opportunities for improving the overall anti-tumor efficacy of radiation therapy.

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