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Inherent radiosensitivity and its impact on breast cancer chemo-radiotherapy

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► ***Review article***

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ABSTRACT

About 10% of apparently normal individuals are sensitive to clastogenic effects of physico-chemical agents. More than 45% of breast cancer patients' exhibit elevated radiosensitivity. Although the nature of inherent radiosensitivity is not fully understood, but insufficiency and impaired DNA repair mechanism might be prime cause of radiosensitivity. This is evident from genetically affected individuals such as ataxia telangiectasia, severe combined immunodeficiency, Xeroderma pigmentasum, Fanconi anemia who show sensitivity to ionizing radiation, ultraviolet light and alkylating agents. All these genetic diseases are caused due to impaired DNA damage repair mechanism. Radiation therapy (RT) is a common and effective way of treatment in several types of malignant tumors. Some cancer patients suffer from side effects of RT such as radiation induced early or late adverse responses in normal tissues within weeks, months, or even years post irradiation, due to intrinsic radiosensitivity. The RT efficiency limitation raises from ionizing radiation toxicity reactions in normal tissues. An appropriate protocol to prevent or treat these side effects, has not been developed yet. Molecular pathways involved in adverse responses to cancer treatment agents have not been well defined. Identification of molecular mechanisms may be promising to enhance the output of treatment technologies and overall survival of cancer patients. Several techniques such as microarray technology has been used to clarify molecular mechanisms involved in radiosensitivity by finding genes related to RT normal tissue responses. DNA repair, apoptosis, cell cycle, and growth factor associated genes are the most important candidates in this field.

Keywords: *Inherent radiosensitivity, breast cancer, radiotherapy, adverse effects, biomarkers.*

INTRODUCTION

Cancer survivors have an obvious risk for long term morbidity; which can exceeds several years from diagnosis [1]. One of the most serious life-threatening events after radiation therapy is developing a new second cancer or subsequent malignant neoplasms (SMNs), causing premature death after radiotherapy [2]. Radiation therapy (RT) is a common and effective way of treatment in several types of malignant tumors. About 70% of patients suffer from cancer are treated with radiation therapy [3]. Breast cancer (BC) is the most common and second leading cause of death among women worldwide [4].

RT is an effective tool in management of BC and has been used as a routine protocol after breast conserving surgery (BCS) for controlling local tumors and decreasing the risk of loco-regional recurrence [5]. Unfortunately early or late adverse side effects of this therapy in normal tissues are undeniable [6]. In other words normal adjacent tissues surrounding the

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malignant tumors are not safe from irradiation effects. Irradiation side effects can also be seen in distinct parts of body as a bystander effect [7]. Efforts have been made to develop new techniques of RT to minimize radiation dose affects in normal tissues. During or shortly after therapy, treatment of side effects such as mild erythema, ulceration, etc. occur in different part of the skin, which are reversible [8]. Late adverse outcome happens six months to several years after treatment, include subcutaneous fibrosis, atrophy, and vascular damage could be permanent [9]. RT response is not the same among different patients. Variety of factors are important in this phenomena including inflammatory interactions, oxidative stress, genetic background, variants in genes involved in the response to radiation-induced DNA damage, age and environmental conditions [10]. Turesson *et al*. (1996) [11] assessed ataxia telangiectasia patients treated under the same conditions and found dramatic variation in severity among them. They concluded that if extrinsic factors like irradiation dose are controlled, intrinsic factors related to individuals may account for ≥80% of clinical complication risk.

Ionizing radiation (IR) is a potent carcinogen and overreaction to it has been seen in rare chromosomal breakage syndrome for example ataxia-telangiectasia [12]. Now a days it is believed that this elevated sensitivity not only is recognized in this rare syndrome but also in many other cancer prone conditions. Induction of double strand breakage (DSB) in the genome is one of the most deleterious effects of IR which if not repaired accurately leads to genomic instability, chromosome aberrations and eventually may lead to mutagenesis and carcinogenesis [13]. To overcome these problems DNA damage response (DDR) is activated naturally in the cells in order to coordinate lesion detection, activation of repair machine and cell cycle checkpoints to ensure that these errors are removed properly [14].

In clinical radiotherapy, RT responses in patients may be with a broad range from latent to severe and sometimes lethal, thus, it is important to develop powerful diagnostic techniques to predict patients' responses to tumor therapy and also patients prone to radiation-related toxicity before RT [15]. Biomarkers are such potent tools but their capability for recurrences prediction after RT for BC is limited [16]. Radiosensitivity detection can be used in radiation protection of radiation workers, identification of radiosensitive cancer patients before RT in order to minimize side effects and overall survival increase, determination of outcomes from nuclear events and personalization of hyper-sensitivity to IR in astronauts who are exposed to cosmic rays [17]. Another potential benefits of such biomarkers could be early detection of cancer in individuals at high risk who doesn't show any external characteristic, which help to administer a better and more effective disease management for them.

What is radiosensitivity associated with?

Radiosensitivity means susceptibility of cells or tissues to damaging effects of IR. In fact radiotherapy has toxic effects not only in tumor cells but also in surrounding normal tissues. Some patients innately show higher sensitivity to radiation. Sensitivity can occur shortly after treatment or late from sixth to several years later. Acute sensitivity usually happens in tissues with fast proliferation such as skin, gastrointestinal tract and hematopoietic tissues, these effects are usually reversible [15]. Delayed sensitivity usually occurs in tissues and organs with slow proliferation such as kidneys, heart, and the nervous system, and may involve systemic dysregulations of the endocrine system. The mechanisms of higher tissue sensitivity to IR has been poorly understood. Although it's been proved that genetic variants are among the major factors which affect this feature [18]. IR induces various types of lesion such as DNA-protein cross-links, base and sugar alterations, DNA single-strand breaks (SSBs) and double-strand breaks (DSBs) eventually leading to chromosomal aberrations (CA) formation [19]. DSB is one of the most important deleterious effects of radiation which is supposed as a serious threat for genome integrity. DSBs can be induced naturally in cells by reactive oxygen

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species (ROS) produced during metabolic activities. ROS can directly attach to DNA and cause different lesions as well as DSBs. Programmed DSBs are also produced in certain types of cellular process like meiotic recombination and during lymphoid differentiation regulated by immunoglobulin (Ig) and T-cell receptor (TCR) genes [20]. They can also be produced accidentally in result of topoisomerase-mediated DNA cleavage [21]. If DSBs left unrepaired or have been misrepaired then may contribute to cell mortality, mutation and CA.

There are several conserved pathways to repair DBSs properly. It is unlike that a high steady-state level of unrepaired DSBs to exist in cells since they act as a signal for DDR that can repair the errors, stop the cell cycle or initiate apoptosis [22]. In other words when a damage occurs in the G1 or S phases of the cell cycle, transition through S phase will be blocked or happens slowly and if DSBs are generated in G2 phase of the cell cycle, entry to mitosis will be delayed. Cellular sensitivity to IR differs in each phase of the cell cycle. The highest degree of radiosensitivity belongs to G2/M phase after that G1 phase and the lowest degree is in near the ends of S phase [23]. Blocking of G2/M is the main goal for cell death induced by anti-cancer drugs and radiosensitizing agents. Activation of G2/M cell cycle checkpoints is needed for cell entry to M phase. These checkpoints ensure the cell progression accuracy [24]. Based on data have been achieved from yeast and mammalian somatic cells studies, it's been found that pathways involved in repair of the IR induced DSBs are the same as those found in repair of DSBs that occurs naturally. These pathways include homologous recombination repair (HRR) and single strand annealing (SSA) which is a variant of HRR and non-homologous end-joining (NHEJ) [25].

Homologous Recombination (HR)

Genetic material is exchanged equally between homologues chromosomes within prophase I of meiosis. This process is completely accurate and occurs between alleles located at identical positions of the involved parental chromosomes [22]. Due to precise reciprocal exchange, no genetic material gain or loss occurs during HR process. At the molecular level, HR is triggered by programmed generation of DNA DSBs in meiosis and DSB improvement by homologous sequences exchange on a non-sister chromatid [26]. As mentioned above the synthesis of an error free sequence as a precise copy of undamaged homologous chromosome and joint molecule generation (the Holliday junctions) are main features of HR pathway. Unfortunately these types of repair occur only in a small part of lesion improvement in mammalian cells [22].

Single-strand annealing (SSA)

When DSBs are generated between two adjacent repeated sequences which have high homology SSA, a variant of HR, can occur. It is triggered by massive 5', 3' resection of the DSB ends and repair is completed via deletion of non-homologous ends and ligation afterwards. This process causes the repeated sequences and interval DNA removal so this pathway is considered as an error prone way of DNA damage repair. It is worthwhile to note that unlike HR, strand invasion step will not happen in SSA. Both the extent of single-strand which is exposed and the length of the annealing homology, can range from a few bases (which is then called 'microhomologies') to hundreds of bases. In different studies it's been showed that yeast cells mutated in *RAD7* and *RAD76* (genes which act in SSA pathway) are not sensitive to IR concluding that this pathway has a small role in IR induced-DSBs [22]. With administering similar experiments on *ERCC7* and *ERCC0* mutant in mammals (orthologous of *Rad76* and *Rad7*, respectively) results were the same [27].

Non homologues end joining (NHEJ)

NHEJ is a process in which two ends of DNA DSBs will join together with no need to sequence homology between two ends or synapsis of the broken DNA with an intact partner DNA molecule. Indeed its activity naturally add higher immunoglobulin and T-cell receptors diversity during V(D)J recombination. It can repair DSBs without requiring intact homologous sequence so it occurs more

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frequently than HR. It's a non-conservative mechanism of DSBs improvement because several DSBs induced by IR cannot be directly joined, so some limited processing and/or polymerization has to happen before NHEJ. As a result, small sequence gain or loss are generated within the process [22] making NHEJ an error prone repair mechanism.

Both HR and NHEJ mechanisms are conserved evolutionary, but their role has not a same importance in different species. Lower eukaryotes like the yeasts uses HR for repair of DSB [28]. In mammals their importance differs in phases of cell cycle and that is related to its nature that HR and SSA require a sequence homology so they can take place only in late S and G2 phases when chromosomes have been duplicated; but NHEJ can happen any time in the cell cycle specifically G1 as it doesn't depend on sequence homology of sister chromatids.

One of the key events that happens during DSBs processing is fast phosphorylation of H2AX (called ɣ-H2AX), a highly conserved histone H2A variant in mammals [29]. This event leads to accumulation of proteins such as DNA-PK, Rad51, Nbs1, and BRCA1, which have repairing functions, at the site of DSBs so its absence cause demolished gathering of mentioned proteins at the site of lesion and makes cells more sensitive to IR [30]. Ionizing Radiation Induced Foci (IRIF) are produced usually after IR at the site of produced DSBs. They are dynamic unions which have thousands copies of factors which play important roles in DSB repair [14]. These proteins include phosphorylated 53BP1, MDC1, ATM, RAD51, MRN complex, RNF8/KIAA0646, RNF168, and BRCA1-A complex (BRCA1, BARD1, BRCC3/BRCC36, FAM175A/Abraxas, UIMC1/RAP80, MERIT40/ NBA1 and also BRE/BRCC45 [31,32]. Proteins involved in DSBs repair are often subjected to phosphorylation before being re-localized to IRIF [14]. An important part of IRIF formation is yielding ɣ-H2AX to act as a chromatin platform generated on a 2-Mb size chromatin domain involving DSBs and gather that factors related to DNA damage repair machine. Recent studies revealed that some ɣ-H2AX foci remain at the site of DSBs even after their repair has been finished [33]. The exact role of remained IRIF even after repair completion, is currently unknown but it's been suggested that they could possibly have a role in remaining chromatin alternations, late repair and mis-rejoining of DSB, apoptosis, activity of several kinases and phosphatases, and checkpoint signaling [34,35]. One possible role of remained IRIF could be yielding a suitable state of damaged cells for compromised communication with adjacent normal tissues so it can cause transferring of IR-induced-damage signals to surrounding tissues, called bystander effect, without directly being hit to IR [36].

It has been already established that innate radiosensitivity of cancer cells is affected by DSBs repair capacity [35]. Cells response to ionizing radiation in different ways such as activation of DNA repair, cell cycle checkpoints, and/or programmed cell death pathways such as apoptosis, inflammatory responses, etc. Several studies that assessed gene expression profile in peripheral blood lymphocytes or lymphoblastoid cell lines which were experimentally irradiated revealed that alternation of mentioned pathways can affect the normal tissue reaction or radiosensitivity to IR [37]. Efficiency of DSBs repair pathways seems to have an important role in radiosensitivity of normal tissues and radioresistance in cancer cells as well, since it's been found that cells of patients with rare chromosomal breakage syndromes are higher sensitive to IR and they are genetically mutated in genes related to DNA repair [38].

As mentioned earlier, IR could result in DNA damage indirectly through generation of ROS via radiolysis of water, which can cause damage to macromolecules such as proteins, carbohydrates and DNA. Cells can survive from ROS adverse effects by several pathways. Genetic variation in genes involved in these pathways can explain altered radiosensitivity in normal tissues surrounding tumor cells [39]. Few studies have confirmed correlation between polymorphisms in oxidative stress-related candidate genes and acute toxicity [40]. Some other reports support that SNPs in these genes can alter acute skin damages in BC patients [41] but further studies are needed to confirm these data.

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Who is considered as radiosensitive?

Even with special efforts in RT optimization for cancer therapy, some patients still suffer from its deleterious effects [42]. Finding a way to anticipate cellular responses to RT before using it for patient treatment may potentially have several benefits in disease management. Currently prediction of RT outcome is according to clinical features like tumor stage and grade. Known available predictive models for several types of tumors have been formed using different clinical parameters [43]. Considering different responses to RT in patients with the same clinical features, make these models more effective than other techniques using factors such as blood-based (e.g. protein), DNA-based (e.g. epigenetic modifications) or imaging (e.g. hypoxia-imaging) biomarkers [44]. RT causes activation of several signaling pathways in the tumors and surrounding normal cells such as DNA repair machinery, cell cycle controls, apoptosis, inflammation. Several important genes are acting in these pathway that their functional efficiency can potentially alter radiosensitivity.

Rare chromosomal breakage syndrome such as Ataxia telangectiasis [45,46], Nigmegan breakage syndrome [45,47], severe combined immunodeficiency (SCID) [12] and Fanconi anemia [12,45,48,49] are known clinical and cancer prone conditions related to radiation induced response that exhibit hypersensitivity to carcinogenic agents like IR. These syndromes have evolved the first interest in the human radiosensitivity since each of them are resulted from an inherited mutation in DNA repair genes and affected patients show hypersensitivity to different agents. An elevated susceptibility to DNA-damaging agents has also been established in Fanconi's anemia syndrome patients [50], although its response to IR is a controversial issue yet [51]. After performing several experiments in AT patients a great interest has been made for using ATM as a potential predictive marker of a radiosensitivity. Although the ATM mutation occurrence is very low; but several studies have shown that radiotherapy complications may be a consequence of defect in other genes rather than ATM [52]. It is interesting to note that there are several other inherited conditions that show hyper susceptibility to IR such as Bloom's syndrome [45], combined variable immunodeficiency (CVID) [12], breast cancer patients [53-56] and also RIDDLE syndrome [57-59] with several same clinical characteristics of AT are amongst them. At the molecular level, cells from the RIDDLE syndrome patient have bi -allelic mutations in the gene encoding RNF168 that has main role in recruitment of two key components of the DNA-damage response, *53BP1* and *BRCA1* [32]. In irradiated cells, RIDDLIN localizes rapidly at the site of DSBs along with other components of the DNA-damage response, including $_{\gamma}$H2AX, MDC1, NBS1, BRCA1 and 53BP1 [59]. There are other clinical conditions such as DNA ligase IV deficiency [45,60], Li-Fraumeni syndrome [12,61], Mre11 deficiency (AT-like disease) [62], Ruthmond syndrome [63,64] and X-linked agammaglubulinemia syndrome [12, 65]. Table 1 lists the main known disorders exhibiting radiosensitivity with their specifications. As most radiosensitive conditions are deficient in DNA repair processes, the involvement of main repair pathways in radiosensitivity is briefly described.

Involvement of homologous recombination repair (HRR)

HR is the mostly used mechanism to improve DNA lesions in *Saccharomyces cerevisiae*. Several genes known as the *RAD18* epistasis group, act in this pathway such as (*RAD16, RAD51, RAD52, RAD54, RAD54B, RAD55, RAD57, RAD59, MRE11* and *XRS2*). Products of some of mentioned genes form a multi-protein complex and work together. One such complex is RAD50/ XRS2/MRE11 that has activities not only in HR, but also in NHEJ pathways [22]. The orthologous of all '*RAD52* group' genes have been also identified in mammalian cells [66] .The primary sequence of most of these genes, such as *RAD10*, is conserved evolutionary from yeast to mammals and reflects their functional importance. More precise studies on mammalian cells showed that two additional proteins are also essential for HR activities in these organisms called *BRCA7* and *BRCA8* (familial

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breast cancer genes) [67]. Experimental data has revealed that both of them act directly or indirectly with RAD51 protein and interact with several HR factors in IRIF [68]. Another relevant study has found that the Fanconi anemia (FA) proteins, modulates HR regulation by interacting with BRCA1and BRCA2 [69].

Table 1. Disorders exhibiting radiosensitivity.

Disease	frequency	symptoms	Genes involved and location	DNA Repair defects	Type of damage	Sensitive to	Reference number
ADA Severe combined immunodeficiency (SCID)	rare	Immune-deficiency	*ADA*, 20q13.11	DSB repair deficient	Chromosomal breaks and rearrangements	Ionizing radiation	12
Ataxia Telangiectasia (AT)	1:40000	Tumor susceptibility, immunodeficiency	*ATM*, 11q22.3-23.1	Check point failure	Chromosomal breaks and rearrangements	Ionizing radiation	45, 46
Bloom's syndrome	1: 500000	Immunodeficiency Cancer susceptibility	*BLM*	DNA repair and replication defects	Increased SCE, quadriradials	DNA damaging agents	45
Breast cancer Breast/ Ovarian cancer	12:100	DNA- damage sensitivity, genomic instability	*BRCA2*, 13q12.3 *BRCA1*, 17q21	Impaired DSB repair	Mutations in involved genes	Ionizing radiation	53, 54, 56
Common variable immunodeficiency	1:25000-1:50000	Immune deficient Susceptible to some cancers	*TNFRSF13B* Unknown	disrupt B cell function	Mutations in involved gene	Ionizing radiation?	12
DNA Ligase IV Deficiency	unknown	microcephaly, growth retarda-tion, developmen-tal delay immunodeficien-cies	*LIG4*	Disruption of Nonhomologous end-joining (NHEJ) repair mechanism	Mutation in LIG4	Photosensitivity, Chemosensitivity, radiosensitivity	45, 60
Fanconi anemia (FA)	1-5:1000000	Susceptibility to leukemia	*FANCA*, 16q24.3 *FANCB*, ? *FANCC*, 9q22.3 *FANCD*, 3p26-p22 *FANCG*, 9p13	impaired response damage to DNA	Chromosomal breaks, multi-radial chromosomes	DNA Crosslinking agents, Ionizing radiation (Controversial)	12, 45, 48, 49, 51
Li-fraumeni syndrome	Rare	Cancer susceptibility	LFS1: *TP53*,Chromosome 17 *LFS2*: CHK2, 22q12.1	Uncontrolled cell cycle	Mutations in involved genes	DNA damaging agents	12, 61
Mre11 deficiency (AT like disease)	unknown	DNA- damage sensitivity, genomic instability	*MRE11*, 11q21	Impaired DSB repair	MRE11 deficiency	Ionizing radiation?	62
,Nigmegan breakage syndrome (NBS)	rare	Immunodeficiency radiosensitivity	*NBS1*, 8q21	DSB repair deficient	Chromosomal breaks and rearrangements (7p13, 7p35, 14q11,14q32)	Ionizing radiation	45, 47
RIDDLE syndrome	Rare, 4 cases upto 2017	Radiosensitivity, Immunodeficiency .	*RNF168* (3q29)	53BP1-mediated DNA damage signaling	Increased levels of chromosomal breaks	Ionizing radiation	57, 58, 59
Rothmund Thomson syndrome	unknown	Cancer susceptibility	*RECQL4*	Impaired replication	Mutations in involved gene, chromosomal radiosensitivity	Ionizing radiation	63, 64
Werner's syndrome	1: 200000 USA 1:20000 to 1:40000 Japan	Cancer susceptibility, premature aging	*WRN*	DNA helicase , exonuclease	Increased levels of chromosomal breaks	DNA damaging agents	45
X-linked agammaglobuline mia	1: 100000 new born male	Immune deficient	*BTK*, Xq21.3	Impaired B-cell development	Mutations in involved gene	Ionizing radiation	12, 65

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Experimental data in yeast shows that cells mutated in *RAD18* group genes, are more susceptible to IR as there will be serious problem in recombination activities during cell division. They are not sensitive to UV [70]. Although complete loss of function in most of these genes such as *BRCA7* or *BRCA8* in mouse models, causes embryonic mortality that shows the importance of their function in repairing DNA errors in early stages of embryonic development. Knockouts mice of *RAD10* and embryonic stem (ES) cells with deficiency in *RAD54* activities and neonatal mice have an elevated sensitivity to DSB inducing carcinogens [71].

Involvement of non-homologous end-joining (NHEJ)

In mammals there are two important multi-protein complexes that play essential roles in NHEJ pathway; 1) DNA-dependent protein kinase (DNA-PK) which is constructed via Ku70 and Ku80 (also called as KU86) proteins accumulation [22]. This complex joins to the ends of DNA molecules, then Ku complex will bind to DSBs ends that causes activation of catalytic subunit, DNA-PKcs, and 2) DNA ligase IV and XRCC4 which perform catalytic ligation in this pathway. Genes called *XRCC2* and *XRCC1* encode subunits of Ku70/Ku86 heterodimer [72]. *XRCC7* gene encodes DNA-PKcs proteins, this gene is a member of phosphatidyl innositol kinases (PIKs) family which has an important role in modulation of telomere length, cell cycle control, and DSBs repair [22]. In functional studies of knockout mouse models it has been found that dysfunction of these genes causes higher sensitivity of the cells to IR and other carcinogens probably due to DSB repair impairments [25]. In yeast, all of the mammalian NHEJ factors have an orthologous except the DNA-PKcs. Artemis is another protein involved in NHEJ pathway and have different activities like V(D)J recombination and also it can form a complex with DNA-PKcs. Its dysfunction has been seen in a class of SCID patients [22]. Although other functional studies showed that it has an essential role in cell cycle blockage after IR or UV treatment in the cells as interacts with important cell cycle checkpoints [22].

Several studies have found correlations among genetic variations and different response to IR in different cells [73]. Mutation of *BRCA7* and *BRCA8* genes are related to hereditary breast /ovarian cancer which have important roles in HR pathway, control of genome stability and cell cycle [74]. Murine embryos that have *BRCA1*-null mutation are developmentally retarded and are susceptible to IR, same conditions have been seen in *rad17* knockout mice, reflecting a defect in DDR [75]. Similar results has been seen in *BRCA8*-null embryos in murine and fibroblasts of mouse embryos with null alleles in *BRCA8* which are hypersensitive to IR as well [76].

Other possible markers of radiosensitivity

Variation in gene expression has also important effect on cellular radiosensitivity. In a study it was found that gene profiling could successfully distinguish subgroups of patients with different radiosensitivity after RT [77]. It had been clear those genes were involved in DNA DDR pathways, cell cycle control, proliferation, apoptosis and DNA repair [78]. It gives an additional tool for better subdividing patients with and without late toxicities of pelvic radiotherapy by investigation of functionally or structurally associated gene groups [77]. Additional studies have shown correlations between radiosensitivity to RT and a range of cellular and gene expression endpoints [79].

In patients showing radiosensitivity expression level of *p19* is usually increased even without IR treatment and it can also continue at a higher level at 6 days after *in vitro* irradiation. It is completely consistent with higher susceptibility to undergo permanent blockage of cell cycle which causes premature differentiation or senescence. It has also been shown that cells with severe radiosensitivity show an early strong elevated levels of *p19* that reflects a powerful reaction of temporary cell cycle blockage and DNA repair. A few but significant enhancement in the number of residual DSBs and higher levels of *p19*-positive cells has been found in fibroblasts of RT-sensitive than RT resistant patients in both

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conditions including no irradiation and 2 h and 6 d after *in vitro* irradiation [80].

Another consequences of IR is producing DNA base damage which the base excision repair pathway (BER) has the responsibility to repair it. *XRCC7* and *PARP7* are among the important factors playing role in BER pathway that build a platform for gathering other proteins involved in DNA repair complex and catalyzes the poly ADP-ribosylation of target proteins in DDR [81] respectively. It has been suggested that there are several polymorphisms which can have a possible role in radiosensitivity of normal cells in response to RT [82,83].

MiRNAs are small regulatory non-coding RNA molecules which can have roles in radiosensitivity of normal tissues by affecting pathways involved in IR responses such as changes in signaling pathway, DDR, cell differentiation, cell cycle blockage, alteration of gene expression patterns, mutations of important genes, genomic instability and initiation of carcinogenesis. Extra data suggest a key role of miRNAs in radiosensitivity [16]. Their importance has been evaluated in several studies which shows they could be potentially fine prognostic markers. For instance high expression of miR-21 [84] and miR-155 [85] have been correlated with radio resistance in BC, and the result has been opposite for miR-302 [86], miR-200c [87], and miR-31[88].

Analysis of cellular response to *in vitro* IR in BC revealed miR-139-5p and miR-1274a are associated with radiosensitivity, and radioresistance respectively [89]. After anticipation of their possible targets it has been shown that most of them have function in DDR pathways for example *RAD54L*, *POLQ*, *TOP2A*, *RAG1*, *PLK2*, and *SKP2*. Based on such observations it can be suggested that these genes might be potential biomarkers of early or late cellular response to RT [16].

Radiosensitivity is a multifactorial feature that could be influenced by a variety of factors such as irradiation dose and environmental conditions as well as genetic characteristics of individuals that should be considered for more accurate achievement of RT in personalized treatment of cancer patients.

Radiosensitivity and breast cancer

Breast cancer is a common type of malignancy occurring in women. One of the most common indications for RT in western countries is adjuvant treatment of BC because of the high prevalence of BC and the multiple indications for RT in this disease.

Breast-conserving surgery (BCS) followed by whole breast RT has same result comparing to mastectomy and combination of RT with BCS is in priority with respect to local control and survival. Currently standard post-BCS fractionation is performed 5–6 weeks of daily treatments of 1.8–2 Gy/d [90].

Ionizing radiation used in RT is a known carcinogen and can generate different DNA lesions such as DSBs in tumor cells and normal adjacent tissues. Breast cancer radiosensitivity refers to inherent sensitivity of cells or tissues to IR, which is multifactorial features related to several factors among them genetic factors have dramatic role.

Studies have revealed that genomic instability occurs in hereditary BC and some other hereditary cancers [56] .Data suggest that some BC patients have a significant increased chromosomal radiosensitivity (CRS) [54,91,92] and CRS in lymphocytes of patients could be a potential marker for low penetrance genes related to breast cancer development. It is estimated that almost 10% of normal individual and 40% of unselected BC patients have increased radiosensitivity [92]. A sub group of these populations are AT heterozygotes which can make a correlation between high radio sensitivity and predisposition to cancer [93] and BC patients with known mutation in *BRCA7* or *BRCA2* high penetrance genes or those with positive family history have increased CRS than healthy population [94]. These genes have role in repair of lesions induced by IR and their mutation create a strong predisposition to BC. Another high penetrance gene that increases cancer risk is *TP19* and is associated with the cancer-prone Li-Fraumeni syndrome [61]. These three high penetrance genes are involved in small part of all BC cases. For example *BRCA7*

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and *BRAC8* mutation form 15% of familial and about for 5% of sporadic BC cases. With respect to frequency of CRS in about 40% of all BC patients it can be concluded that other DNA damage response genes with low penetrance may alter breast cancer susceptibility and radiosensitivity in these patients [93].

One of such genes is *ATM* which its heterozygote mutant can elevate BC predisposition and radiosensitivity in some cases although the frequency of ATM mutations among patients with breast cancer may be considerably lower than early estimates. Polymorphic alternations in *BRIP7, BARD7, PALb2 NBS1, CYP17, NAT2, CYP1A1, FGFR2, GSTM1, GSTP1* and several other genes have been already studied and showed that these genes can increase both familial and sporadic BC risk and induce characteristics like normal tissue toxicities to IR [95]. Most of these genes have function in DNA repair system and their mutation create higher levels of CA. For instance variation in genes like *XRCC9* and *RAD17* increase the risk of radiosensitivity [96].

Based on available data we it can be suggested that by performing suitable functional tests evaluating DNA repair capacity, it can be possible to make better decision for BC treatment. Results of a global gene expression study in lymphocytes of breast and cervical cancer patients indicated that 157 different genes had significantly different expression when using IR. Most of them play role in cell cycle control and apoptosis in response to radiation. Interestingly 67 of these genes were able to successfully divide different patients to normal reacted vs hyper-sensitive to IR. These studies were performed on peripheral blood lymphocytes of individuals so investigation of expression in different tissues would be required to produce more accurate gene signature [97].

A distinct group of BC patients have loco regional recurrence (LRR). A study showed that HER2+ tumors have an elevated sensitivity to RT [98], although another research revealed that LRR is considerably higher in triple negative BC cases, although LRR events had a low frequency [99]. There are no powerful molecular techniques to make difference among patients with high and low LRR. Furthermore, poor information is available with respect to the possible negative results of RT that may be consequence of genetic and epigenetic alternations as well as alternation of gene-expression patterns in BC [100].

A group of scientists investigated radiation response in lymphocytes of patients with advanced BC conditions which were treated *ex-vivo* with high radiotherapy doses. In consistence with previous discussion, Lymphocytes from patients with low DNA damage and high apoptosis capacities showed low incidence of radiation adverse response. A research was organized on certain types of cell lines like Bca10 (sporadic breast cancer) and Bca11 (familial breast cancer) to assess DNA repair capacity. It has revealed that NHEJ and an error-prone direct form of HR (SSA) pathways were at a high level in Bca11 cell lines. Additionally, SSA repair mechanisms was also high in Bca10 but less than what was seen in Bca11 [15].

Patel *et al.* analyzed DNA repair capacity in BC patients via performing G2 assay and counting the number of chromatid aberrations in several time intervals [101]. In consistent with other studies which showed that in cancer prone cases DNA repair capacity is dramatically defective [102]. Further researches also indicated that genome of individuals with cancer susceptibility and BC patients generate more DSBs and other lesions and elevated radiosensitivity because of defective DNA repair mechanisms rather unlike healthy individuals [102,103]. It supports the idea that cells with elevated chromatid radiosensitivity have deficiency in DNA repair. It can be suggested that that radiosensitivity could be a potential predisposing condition to BC through mutations in low penetrance genes [53] that could play a role in DDR mechanisms. It is worth to mention that triple negative BC patients showed no radiosensitivity when assessed with the Go micronuclei assay [104]. It is worth to mention that, even if the intrinsic radiosensitivity could be identified precisely, it is not certain that a research can make the relation between

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radiosensitivity and adverse responses. There have been some studies with disappointing results [105].

Impact of radiosensitivity on treatment of breast cancer patients

Some cancer patients suffer from side effects of RT and thus develop radiation induced early or late adverse responses in their normal tissues within weeks, months, or years, because of intrinsic radiosensitivity [106]. The toxicity reactions of normal tissues to ionizing radiation brings limitation in efficiency of RT. Unfortunately an appropriate protocol to prevent or treat these side effects, yet has not been developed. Therefore radiosensitivity of normal cells is supposed to be a serious problem in management of cancer therapy for instance in breast cancer RT [3]. As discussed earlier, radiosensitivity is caused by extrinsic (i.e. radiation dose), and intrinsic factors (like genetic factors) which the second accounts for almost 80% of normal tissue responses. Currently, our knowledge of molecular pathways involved in related adverse responses to cancer treatment agents are fairly poor. Hence, by identification of these molecular mechanisms it'll be promising to enhance the output of treatment technologies and then increase overall survival of cancer patients. Several techniques has been used to achieve this goal, for example microarray tests administration to clarify molecular mechanisms related to radiosensitivity [3,97]. These experiments try to identify genes and their expression levels which may be related to normal tissue responses to RT. Among them, DNA repair, apoptosis, cell cycle, and growth factor associated genes were tested in these researches [3].

Sensitivity to cancer treatment therapy can also be problematic when using chemotherapy. In a study it was revealed that lymphocytes with heterozygous mutation in *BRCA7* gene had a hypersensitivity to chemical agents used in therapy such as CDDP, BCNU, and CP that creates alkylation and/or cross-linking of DNA [69]; it can may suggest a role in nucleotide excision repair and mismatch repair pathways for mentioned genes [107]. With respect to recent data showing that carriers of *BRCA7* and *BRCA8* mutations have susceptibility to show hyper radiosensitivity, clinical concerns have been made about RT and screening mammography in this group of populations [56].

Radiosensitivity as a screening test for susceptible breast cancer patients

Developing breast cancer in individual without family background is highly depend on alternation in genes with low penetrance rather than high penetrance but rarely mutated genes like *BRCA7* and *BRCA8*, which have high frequency in general population. One of them is *ATM* gene which is known in a rare chromosomal breakage syndrome called Ataxia telangiectasia [108].

As discussed earlier, one of the most destroying effects of IR is DSBs that if they are not repaired appropriately or remained unrepaired could produce chromosomal aberrations. These CA can elevate the risk of cancer formation in the breast epithelium based on the facts that important cancer predisposition genes like *BRCA7* and *BRCA8*, *ATM* and *TP53* have essential roles in DNA repair mechanisms and also chromosome stability [108]. It was mentioned previously that two main mechanisms to improve the DSBs in the cells are HR and NHEJ. After destruction of each mechanism, oncogenic chromosomal rearrangements were identified in studied animal models [109]. It was found in related researches, mutation of genes involved in NHEJ pathway caused both breast cancer predisposition and chromosomal radiosensitivity increasing [15].

Although carcinogenesis is a complex biological phenomena associated with genome instability [7] but the correlation of CA and carcinogenesis has been proved [110]. Some chromosomal rearrangements play role in tumor initiation and it has been found the number of chromosomal abnormalities are elevated dramatically before medical manifestation of cancer [111]. DSBs produced by chemical or physical carcinogens directly or indirectly, can end up with CA in exposed cells

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and in some extent in normal adjacent tissues. Two main factors for increasing development of tumors can be defective DNA repair capacity and genome instability due to elevated CA [112] and the importance of these two factors as mentioned before has been well proved in chromosomal breakage syndromes, which show inherited chromosomal instability, susceptibility to IR and higher risk of cancer development [113]. Independent studies have revealed that significantly defective DNA repair capability increase susceptibility to inherent and non- inherent forms of breast cancer [102]. Chromosomal instability has been identified in various hereditary cancers including hereditary breast cancer as well [94]. We mentioned before that about 10% of normal population and 40% of BC patients have increased susceptibility to IR i.e. in AT carriers this make a relation between elevated radiosensitivity with susceptibility to tumor incidence [93]. Further researches proved that alternations in DNA repair mechanisms in the general population can possibly have impact on cancer predisposition [114].

Several parameters are known to have impact on tumor response to IR, including total dose, fractionation, and tumor potential doubling time, hypoxia and innate radiosensitivity. It was clarified before that alternation in DNA repair capacity and genome instability not only can increase susceptibility to cancer development but also enhance radiosensitivity which means reaction of normal tissues to IR along with tumor cells. With respect to these information it can be concluded that biomarkers which predict radiosensitivity in addition to identification of hypersensitive patients to IR before administration of RT, could be possibly used for early detection of breast cancer in population at risk as well. For example by using such biomarkers in close relatives of invasive breast cancer patients, we can identify individuals at risk before any clinical manifestation.

An example of such radiosensitivity biomarkers application is explained here. We discussed before that *ATM* gene (mutated in AT patients) could be a potential biomarker in radiosensitivity and also is a low penetrance cancer predisposing factor in breast cancer. To evaluate importance of *ATM* and *cyclin D7* expression (genes involved in DNA repair and cell cycle control) in sporadic breast cancer, and study tested the potential relation among their RNA expression amounts in ductal carcinoma and surrounding normal tissues against normal breast tissues in a group of BC patients. It was found that cyclin D1 expression was elevated significantly in 51.4% of cases, although ATM had down-regulation in 55% of BC patients in comparison with both normal samples. On the whole they conclude that these changes in *ATM* and *cyclin D7* expression may be predisposing factors in breast carcinomas initiation and/ or progression [115].

How to measure radiosensitivity?

Clonogenic assays or colony forming assays is a method to investigate some final outcomes of DNA damage response in cells such as reproductive cell death, apoptosis, accelerated differentiation, and senescence [116]. However this method is applicable only for anchorage dependent cells capable for colony formation. Other tests such as Pulsed field gel electrophoresis (PFGE) is developed to assess un-repaired DSBs. This approach has not been used commonly in clinical setting, since it is a relatively hard and also time consuming method [117]. DNA damages like single stranded and double stranded breakages is also measured by the use of comet assay. In this method cells are located in a thin layer of agarose gel, using appropriate solvent, DNA will be extracted from surrounding proteins but still joint to the nuclear membrane. DNA migration through electrophoresis process appears as comet-like statues under fluorescence microscopy [102,103]. It is a fast and cost effective experimental method. Suitable software's have been developed to analyze the results. Briefly length of comet tail is associated with DNA damage extent [118,119].

For investigation of DDR capacity in the cells after exposure to IR, H2AX assay is developed. H2AX foci can be quantified by microscopic analysis of induced DSBs after immune-staining for identifying, flowcytometry and Western blotting tests [29,120]. Non-invasive tests on blood

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leukocytes of patients or their non-affected relatives are available for *in vitro* radiosensitivity testing. Cytogenetic assays are among the most common approaches used in radiation exposure of cells including G2 chromosomal radiosensitivity [46,53,92] and the G0 micronucleus induction assay [53,54,121]. In G2 assay the number of chromatid aberrations is measured within peripheral blood lymphocytes or other types of cells in the G2 phase of the cell cycle which are exposed to IR. It might also reveal correlation between radiosensitivity and genetic susceptibility to cancer as this condition usually leads a higher chromosomal aberration and a hyper sensitivity to IR as well. G0 micronucleus assay measures small extracellular bodies called MNs which have been formed of chromosome lagging during anaphase or partial breaks in chromosome and in the first interphase after cell division these structures can be identified and scored [121]. The amount of MN in lymphocytes is considered as a biomarker of chromosomal damage and genome instability. These cells can be detected as bi-nucleated cells via cytoplasmic division inhibitor cytochalasin B during cell culture. This method is named as the cytokinesis-blocked micronucleus (CBMN) assay [121].

Other sophisticated cytogenetic tests can also be used such as premature chromosome condensation (PCC) [122], Fluorescent *in situ* hybridization (FISH) [123-126] have also been used to measure individual radiosensitivity. Cytogenetic markers need cell cycling to measure chromosomal damage, are time-consuming, and are of limited sensitivity at doses Below 1 Gy which are considered as limitation of cytogenetic techniques.

Radiation induced apoptosis is another method to measure radiosensitivity. This method known as RILA assay (radiation induced apoptosis in lymphocytes) is under extensive investigation as a suitable method for radiation induced late toxicity in cancer patients [127-129]. Molecular method such as assessment of genetic or epigenetic modification via candidate's gene approaches or whole genome methods can also be performed in radiosensitivity detection. Several studies have administered gene expression analysis in blood to discriminate radiosensitive or resistant cells successfully and the results were sufficiently powerful in this point of view [129,130]. Therefore gene expression approaches might be turned into clinically useful techniques; although, additional experiments are necessary to establish them. A list of available methods for quantification of personalized based radiosensitivity is provided in table 2.

Table 2. Available assays for radiosensitivity assessment.

Method	End point	Reference number
Colonogenic assay	Cell survival observed as colonies following certain doses of ionizing radiation, e.g. 2 Gy (SF2)	116
G0 micronucleus assay	Observation of micronucleus formed due to acentric fragments or lagging chromosome in binucleate cytokinesis blocked cells	53,54,121
G2 assay	Chromosomal aberrations formed in G2 phase of the cell cycle seen as chromatid breaks or exchanges	46,53,92
Fluorescent *in situ* hybridization	Observation of chromosomal aberrations	123, 124, 125, 126
Pulsed field gel electrophoresis	DNA breaks	117
Comet assay	DNA damage and repair	102,118,119
γH2AX	Residual DSB observed as foci	29,120
Radiation induced apoptosis in lymphocytes (RILA) assay	Apoptosis	127,128,129
Molecular methods	Gene polymorphism, SNPs, gene expression	129,130
Premature chromosome condensation	Observation of chromosomal aberrations in inter-phase cells	122

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CONCLUSION

Elevated inherent radiosensitivity is a major cause of adverse side effects of radiotherapy and chemotherapy of cancer patients. Although the underlying nature of radiosensitivity is not clearly known yet; insufficiency and impaired repair mechanisms of DNA damage may be of prime cause. The biological importance of genomic instability and DNA repair mechanisms in cancer development are well illustrated by several heritable genetic disorders known as chromosomal instability syndromes. These syndromes are characterized by various defects in DNA repair, predisposition to various forms of malignancies and increased radiosensitivity. It has been suggested that individuals who are genetically susceptible to cancer, manifest the impaired DNA damage identification and repair by exhibiting increased DNA radiosensitivity. However, although possible associations between genetic markers and radiosensitivity has been found, strong association between a specific marker or even markers has not yet been established; probably due to inadequate knowledge of the molecular pathology of adverse reactions induced by radiotherapy. In terms of carcinogenesis, radiosensitivity might potentiate effects of ionizing radiation and increase the frequency of radiation induced cancer. On the other hand it might also potentiate the destroying effects of radiation when used for treatment of tumors, although induced bystander effects cannot be neglected. There are methods allowing radiosensitivity assessment of cancer patients and susceptible individuals. Although cytogenetic methods have been shown appropriate, RILA assay seems a suitable method for radiation induced late toxicity assessment in cancer patients. Molecular method such as assessment of genetic or epigenetic modification via candidate's gene approaches or whole genome methods have also been shown powerful approaches for radiosensitivity detection. Therefore gene expression approaches might be turned into clinically useful techniques for radiosensitivity assessment in future.

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