Invited Commentary Open Access

Archives Of Breast Cancer

DOI: 10.19187/abc.201634102-105

Breast Cancer and Paradigm of Genomic Instability

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Genome instability could be defined as an elevated tendency for the genome to acquire genetic alterations; ranging from changes to the nucleotide sequence to chromosomal gain, loss or rearrangements. Accumulating evidence indicates that cell transformation is associated with genome instability leading to an imbalance between the mechanisms of cell-cycle control and mutation rates within the genes. Genomic instability is broadly classified into microsatellite instability (MIN) associated with mutated phenotype, and chromosome instability (CIN) expressed as gross chromosomal abnormalities. The development of cancers can be mediated through DNA repair mechanisms, genetic (or epigenetic) alterations in oncogenes and tumor suppressor genes that regulate cellular processes such as cell-proliferation, differentiation, death and genome stability. Genomic instability is often associated with cancer and can be indicative of a poor prognosis for some types of cancer.[1] But, we still do not know clearly whether genomic instability is a consequence of tumor progression or an active process in tumor evolution. However, many new findings have highlighted certain DNA repair pathways and cell cycle control processes that have important consequences for genomic stability and tumor cell biology.

There are many different man-made and environmental agents that may cause genomic instability. Human is under constant exposure to toxic natural or synthethic chemical substances, air pollutions, various sources of non-ionizing radiations (microwaves, radiowaves, mobile, etc.) and natural or man-made ionizing radiation mainly used for medical (imaging and therapy) or industrial purposes. All these physico-chemical agents are mostly potent inducers of oxidative stress and reactive oxygen species (ROS). ROS are a group of highly reactive molecules implicated in the oxidative damage of biological structures; consequently give rise to various types of DNA lesions, including various types of base damage as well as DNA-DNA and DNA-protein cross links, single-strand breaks and double-strand breaks (DSBs). The formation of ROS produces not only DNA strand breakages, but also might act as a signaling event leading to the release of cytokines or epigenetic changes, or trigger DNA repair machinery. Several DNA damage processing and repair pathways constitute a guard system that protects cells against genetic instability and tumorigenesis; however, the unrepaired or misrepaired lesions may give rise to gene mutations and chromosomal aberrations (CA).[2] Although double-strand breaks are considered as serious DNA damage, they may be repaired very effectively by either one of the two different repair mechanisms namely, homologous recombinational repair (HRR) and non-homologous end joining (NHEJ).[3] HRR, an error free pathway, is able to restore the original sequence of DNA DSB leading to a lower risk of generation of deletions and insertions at the site of DSB. NHEJ, an error prone pathway, is subject to a high risk of generation of *de novo* mutations at the sites of DSBs. Thus, a direct consequence of the NHEJ repair machinery is susceptibility to mutagenesis.[4] The biological importance of genomic instability and DNA repair mechanisms in cancer development are particularly well illustrated by several heritable genetic disorders known as chromosome breakage or chromosomal instability syndromes. These chromosome breakage syndromes such as ataxia-telangiectasia and Nijmegen breakage syndrome are characterized by various defects in DNA repair, predisposition to

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various forms of malignancies and increased radiosensitivity. Therefore, individuals who are genetically susceptible to cancer manifest the impaired DNA damage identification and repair by exhibiting increased DNA radiosensitivity.[1,5]

Breast cancer is a common type of malignancy occurring in women in developed countries that ranks as the fifth cause of death from all cancers.[6] A worldwide increase has been estimated to around 16,500 yearly new cases of this neoplasia by 2020.[7] About 15% of breast cancer is familial and the rest (85%) is sporadic which express as different subtypes. Current approaches fail to provide a single molecular marker for breast cancer detection and prediction of treatment response and prognosis. The gene expression signatures that define specific prognostic subtypes in other breast cancer datasets, such as luminal A and B, basal, normal-like, and ERBB2+, and prognostic signatures including MammaPrint® and Oncotype DX, predicted genomic instability in breast cancer tissue samples. Gene expression profiling of breast cancer specimens have shown considerable difference in their degree of genomic instability and identified a set of 12 genes that defines the two sub groups luminal A and B.[8] There is no doubt that these approaches are expensive for screening purposes and genome instability defined as a high number of chromosomal breakpoints, is suggested as a stronge prognostic marker for early stage luminal breast carcinoma.[9]

Radiation therapy (RT) is an efficient treatment for cancer. About 50% of patients with malignant breast tumors receive RT and most patients seem tolerate it, but some suffer severe adverse effects induced by the therapy. This variability of response may be caused by several factors, such as age, life style, oxidative stress, genetic predisposition and various genes involved in the response to radiation-induced DNA damage.[10] Therefore, it is important to develop and implement new diagnostic techniques for predicting responses to cancer treatment and for identifying patients susceptible to radiation-related toxicity. 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, inherent radiosensitivity of normal cells is supposed to be a serious problem in management of many cancers including breast cancer RT.[11] Radiosensitivity is caused by extrinsic (radiation dose), and intrinsic factors (genetic factors) which the second account for almost 80% of normal tissue responses. At present, our knowledge of molecular pathways involved in relation to adverse responses to cancer treatment agents is fairly poor. Hence, by identification of these molecular mechanisms it'll be possible 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.[11] Variation of inherent radiosensitivity between individuals has also been linked to polymorphisms in single nucleotides. Single nucleotide polymorphisms (SNPs) make up to 90% of the naturally occurring sequence variation in the human genome and SNPs in genes related to the biological response to ionizing radiation. A substantial effort has been made to discover genetic markers, primarily SNPs, associated with variation in the intrinsic radiosensitivity of individuals and adverse responses to RT.[12] Genome wide screen based studies identified microsatellite markers associated with acute adverse effects following radiotherapy in cancer patients. 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 ionizing radiation. It has also been suggested that several polymorphisms might have a possible role in radiosensitivity of normal cells in response to RT.[13] MicroRNAs, small regulatory non-coding RNA molecules, might have a role in radiosensitivity of normal tissues through pathways involved in IR responses such as changes in signaling pathway, DNA damage repair, cell differentiation, cell cycle arrest, alternation of gene expression patterns, mutations of important genes, genomic instability and initiation of carcinogenesis. MiRNAs may also have a key role in radiosensitivity. Their importance has been evaluated in several studies which show they could be potentially fine prognostic markers.[14]

It is shown a significant elevated chromosomal radiosensitivity (CRS) in some BC patients.[15, 16] CRS of lymphocytes of these patients could be a potential marker for low penetrance genes related to breast cancer development. It is estimated that almost 10% of normal individuals and over 40% of unselected BC patients exhibit increased inherent radiosensitivty.[17] A sub group of these populations are AT heterozygotes which can make a correlation between high radio sensitivity and predisposition to cancer.[18] And BC patients with known mutation in BRCA1 or BRCA2 high penetrance genes or those with positive family history have an increased CRS than healthy population.[19]

Our knowledge of mechanisms leading to higher radiosensitivity of normal tissues is fairly poor until now, but it's been estimated that 70% of this feature is a result of genome instability and defective repair of radiation induced DSB.[20] Ionizing Radiation Induced Foci (IRIF) are produced usually after IR at the site of produced DSBs. γ-H2AX is an important part of IRIF formation which act as a chromatin platform

generated on a 2-Mb size chromatin domain involving DSBs and gather related factors to DNA damage repair machine. Recent studies revealed that some γ-H2AX foci remain at the site of DSBs even after their repair has been finished.[21] The exact role of remained IRIF even after completion of repair 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.[21] Impaired repair of DNA damage in lymphocytes of breast cancer patients was previously shown by the comet assay and G2 chromosomal aberration studies.[22, 23] It is therefore possible that genomes of individuals with cancer susceptibility as well as BC patients generate more DSBs and elevated radiosensitivity because of defective DNA repair machinery. This idea is supported by the fact that cells with elevated chromatid radiosensitivity have deficiency in DNA repair.[24] It can be suggested that radiosensitivity could be a potential predisposing condition to BC through mutations in low penetrance genes that could play a role in DNA damage repair mechanisms.

Accumulating evidence indicates that the unrepaired DSBs as the major lesion in the cellular, chromosomal, mutagenic and oncogenic effects of ionizing radiation. Radiation-induced instability endpoints have been shown to be manifested as chromosomal alterations, micronuclei, cell transformation, gene amplification, apoptosis, and sister chromatid exchange, etc. Oncogenic transformation has been demonstrated in many studies to date to be an integral stage in carcinogenesis. Gene amplification might also play an important role in oncogenic transformation. Studies on the organization of the amplified DNA in tumor cells have suggested that a single DNA DSB can trigger a cascade of events leading to amplification of a gene in the genome.[25]

In essence, detection of genetic alterations in genes associated with breast cancer, particularly those related to DSB repair, may be used for the diagnosis for breast cancer patients. Current approaches based on genomic methodologies for mutation detection are expensive and not suitable for screening individuals under risk for increased DSB events. Almost 40% of breast cancer patients exhibit elevated chromosomal radiosensitivity, hence showing adverse complications due to radiotherapy or chemotherapy. Therefore, evaluation of DSB repair or expression of unrepaired DSB as chromosomal aberrations and micronuclei might be a useful tool for assessing breast cancer risk and predicting the response and complications associated with conventional radiotherapy and even chemotherapy. These methods can also be used for screening of breast cancer predisposition. Methods for studying DSB repair deficiency in peripheral blood lymphocytes such as γ-H2AX, comet assay, G2 chromosomal aberration assay and micronucleus assay are less expensive and suitable for screening subjects at high risk for breast cancer, to reduce adverse events and to offer individualized therapies. These methods will also be relevant for preventing unnecessary radiation exposure, for screening of patients who will not benefit from radiotherapy, and for adjusting radiotherapy regimes in patients requiring RT, in order to avoid adverse side effects associated with generation of DSB in tissues ameliorating prognosis of patients.

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