# VII.1. DETECTION AND CHARACTERIZATION OF POLYMORPHISMS IN XRCC DNA REPAIR GENES IN HUMAN POPULATION

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Abstract
Human population is continuously exposed to low levels of ionizing radiation. The main contribution of the exposure is due to medical application. Nevertheless, most of the damage induced is repaired shortly after exposure by cellular repair systems.
The review is focused on the development and application of methods to estimate the character of polymorphisms in repair genes (XRCC1, APE1), involved in single strand breaks repair which is corresponding mainly to the repair of X-ray induced DNA damage.
Since, DSB are major factor for chromosomal aberrations formation, the assays described in this review might be useful for the assessment of the radiation risk for human population.

Key words: ionizing radiation, radiation repair, XRCC polymorphisms, DSB

Humans are exposed to low doses of radiation by natural radiation background during entire life. Understanding the long-term health effects of low and high doses of ionizing radiation on living organisms requires identification of critical radiation-induced DNA lesions, measurement of their repairability, and determination of the consequences of misrepaired or unrepaired persistent lesions.

Ionizing radiation can induce a wide range of DNA lesions – chromosomal aberrations or point mutations.
Structural chromosomal aberrations can by themselves, lead to changes in oncogenes - activation of proto-oncogenes, or elimination of tumor suppressor genes and affect tumorogenesis in this way. It is well clear that, the latest can lead to instability of the genome and development of malignancy.

Medical irradiation is the main source of human technogenic exposure. Ionising radiation (IR) is an important tool in cancer therapy. Its application however is limited by acute and late side effects in the irradiated normal tissue. Extreme side effects of radiotherapy, including an enhanced cancer risk after radiation, were observed in patients with inherited disorders such as ataxia telangiectasia (AT) and the Nijmegen breakage syndrome (NBS). In these disorders, the increased reaction to radiation could be demonstrated on the cellular level, indicating that genetic predisposition might modulate radiation effects in normal tissue.

Markers predicting radiosensitivity of normal tissue could help to improve radiotherapy of carcinoma patients. Mainly three biologic endpoints have been used to determine radiosensitivity in vitro. After in vitro irradiation of cells, clonogenic survival (1), chromosomal aberrations (2), and repair of radiation-induced DNA damage (e.g., single- and double-strand breaks) (3) have been measured and the data obtained have been compared with different types of clinical side effects. Data from assays based on clonogenic survival and chromosomal aberrations showed, at least in several studies, a good correlation of in vitro data with late radiation effects, but these assays are time-consuming and not very suitable for

the characterization of a large number of patients. In addition, the nature of the genetic defects associated with radiosensitivity (i.e., mutations in the ATM and NBS gene) point out that reduced cellular DNA repair capabilities may play a major role in causing radiosensitivity.

It is well documented that elevated spontaneous levels of chromosomal aberrations and enhanced sensitivity to the induction of chromosomal aberrations by ionizing radiation is a common feature of many heritable diseases with cancer predisposition like Ataxia Telangiectasia (AT), Xeroderma Pigmentosum (XP), Fancony Anemia (FA).
Individual sensitivity is widely investigated on chromosomal level as a chromosomal sensitivity to ionizing radiation and is detected in cancer prone groups. There are several different mechanisms leading to chromosomal radiosensitivity including defects in DNA repair, cell cycle checkpoint control, alterations in the structure of chromatin and elimination of the damage via apoptosis.
In the present report we present two genes, which are thought to be involved in DNA repair and the appropriate methodological approach to apply them as a screening biomarkers of radiosensitivity. Screening for mutations / polymorphism in the XRCC1 and APE1 genes will be conferred with a high risk subjects - patients and controls with high radiosensitivity proved on chromosomal level.

On the cellular level, IR-induced various types of DNA damage. Repair of radiation-induced DNA damage plays a critical role for both the susceptibility of patients to side effects after radiotherapy and their subsequent cancer risk. Thus, the ability of normal cells to repair IR-induced DNA damage was suggested by many studies to be an essential host factor to prevent side effects in normal tissue. Nevertheless, none of the experimental markers for DNA damage and repair evaluated up to now proved to be conclusively predictive for adverse reactions to irradiation in normal tissue.

Single-strand breaks (SSBs) in DNA arise from exposure to DNA-damaging agents, from endogenous free radicals, and as intermediates in the base excision repair (BER) pathway. If not repaired, these breaks can accumulate and be converted into the potentially lethal double-strand breaks (DSBs). BER involves steps that include removal of a damaged base by a damage specific DNA glycosylase; phosphodiester backbone cleavage by AP endonuclease (APE1) to yield single-strand break DNA with a 5'-deoxyribose phosphate (dRP) group; DNA synthesis and dRB excision by β-Pol; and ligation by XRCC1/DNA ligase III.

Ionizing radiation also induces closely spaced lesions, including DSBs —two or more SSBs on opposing strands within about 10–20 bp. Double strand DNA breaks are usually caused by ionizing radiation and radiomimetic drugs, but can also occur under normal physiological conditions. The main repair mechanism for double strand breaks in higher eukaryotes is nonhomologous DNA end joining (NHEJ), which modifies and ligates the two DNA ends without the help of extensive base-pairing interactions for alignment. Defects in double strand break repair are associated with radiosensitivity, predisposition to cancer and immunodeficiency syndromes.

Genetic variability in DNA repair may contribute to ionizing radiation sensitivity and cancer susceptibility. Several studies have identified many single-nucleotide polymorphisms (SNPs) in genes involved in nucleotide excision repair (NER), BER, double-strand break repair/homologous recombination repair (HRR) and cell-cycle checkpoint (4). These DNA repair genetic variants could be classified as cancer susceptibility genes, particularly if SNPs have functional significance.

Our goal is to measure the XRCC1-Arg399Gln and APE1-Asp148Glu polymorphisms as markers for radiosensitivity and to validate these biomarkers in epidemiological/clinical studies.

XRCC1 is a single-strand break DNA repair protein which is found in eukaryotic species ranging from insects to human and is composed of an N-terminal domain, a central, breast cancer susceptibility protein-1 C-terminal (BRCT) domain (BRCT-I), and a C-terminal BRCT domain (BRCT-II). XRCC1 has no known enzymatic activity but provides multiple functions in DNA BER that include assembly of DNA repair complexes containing DNA polymerase β (β-Pol), poly-(ADP-ribose) polymerase (PARP) and DNA ligase III. It is thought that by binding β-Pol and damaged DNA, XRCC1 may protect the template strand at the site of DNA damage, such that short patch repair can proceed efficiently.

XRCC1 forms a heterodimer with DNA ligase III in cells via a C-terminal BRCT-II domain with a C-terminal BRCT domain in ligase III and stabilizes ligase III. BRCT-II of XRCC1 has also been reported to have DNA binding activity. Mutations in the BRCT-II domain of XRCC1 render cells defective in single-strand break repair in G0 and G1, but the same mutations do not affect XRCC1-dependent repair in S phase.

The multifunctional mammalian APE1 is responsible for the repair of AP sites in DNA. The enzyme also functions as a redox factor facilitating the DNA-binding capability of AP-1, and numerous other transcription factors. The major mammalian APE1 plays a central role in BER in two distinct ways (5). First, APE1 initiates repair of AP sites in DNA produced either spontaneously or after removal of uracil and alkylated bases in DNA by monofunctional DNA glycosylases. Second, APE1 can act as a 3'-phosphoesterase to initiate repair of strand breaks, either directly induced by reactive oxygen species or indirectly through the AP lyase reaction of DNA damage-specific glycosylases.

The possibility to identify individuals with enhanced radiosensitivity could be of main importance to select a population at increased risk of cancer development. This will be of great value for proper profilaxis and control of selected group subjects. DNA analysis of radiosensitivity may account for identification of these individuals.

**References**


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