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POLYMORPHISM ASSOCIATED XRCC1 GENE WITH LATE TOXICITY AFTER RADIATION THERAPY IN BREAST CANCER PATIENTS IN IRAQ

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Abstract

Risk of normal tissue toxicity restricts the radiation dosage that can be safely administered in radiotherapy. All of the studies confirmed that the rs2682585 mutation of the XRCC1 base excision repair gene is associated with late radiation damage in normal tissues. Carrying the rare allele was linked to a considerably decreased incidence of skin toxicities in the combined analysis of discovery and replication cohorts. With the help of a staged design with replication, we found a variant allele in the XRCC1 base excision repair gene that, when combined with other variants, may be utilised to predict late toxicities after radiation in breast cancer patients.

Key words: XRCC1, breast cancer, radiation therapy

Introduction

Subsequent to radiation exposure, certain patients may encounter moderate to severe late adverse effects on healthy tissue (1,2). These effects can substantially impair the quality of life (3). In order to enhance the personalized treatment of cancer survivors and improve the well-being of high-risk patients, it is critical to identify biomarkers of radiosensitivity. This will enable the development of predictive tests that can be integrated with therapeutic and clinical attributes. Despite the development of numerous assays for predicting radiation toxicity, none have demonstrated therapeutic utility. Regarding the ability of genetic variants to predict sepsis in breast cancer patients, little is known. The overwhelming majority of research is impacted by tiny sample sizes and low replication rates (4, 5). XXX Consortium (6) was established in 2009 to facilitate collaboration, information exchange, and mutual education among specialists on this subject. Recent research has shown that because common variants are likely to have minor individual effects, it is crucial to analyze large groups of patients (7). Genome-wide association studies (GWAS) have identified novel candidate variations (9). These studies do not rely on a priori

assumptions regarding the potential relationships between genes. DNA damage and cellular demise are consequences of oxidative stress induced by radiotherapy, which is caused by the production of reactive oxygen species. Variations in genes implicated in DNA repair pathways and defense against reactive species have been postulated to impact the capacity of irradiated cells and tissues to recuperate from oxidative harm (10). In light of this, genetic polymorphisms may impact the likelihood that a cancer patient will develop radiation-induced long-term adverse effects, including fibrosis and skin toxicity. Throughout history, single nucleotide polymorphisms (SNPs) confined to the coding region of a putative gene have garnered the attention of scientists (11). On the contrary, it was the cancer risk GWAS that initially identified polymorphisms in noncoding regions, and subsequent studies have established their functional significance (12).

Materials and Methods

Samples collection

Patient samples with and without radiation therapy for breast cancer were collected from Al-Diwaniyah Hospital. The material was separated into two groups: one for DNA extraction and polymorphism, and another for other analyses.

Primers ARMS-PCR

In this investigation, we used the web tools NCBI-SNP data base and Primer1 ARMS-PCR primers design to create primers for a gene polymorphism (XRCC1, rs2682585). The (Scientific Researcher. Co. Ltd., Iraq) furnished us with these introductory materials.

Statistical methods

IBM's SPSS version 22 was used for all analyses requiring statistical significance.

Results

Detection of XRCC1 (rs2682585) Polymorphism

Allele-specific PCR was utilized to determine the frequency of the XRCC1 (rs2682585) polymorphism. At this SNP, three genotypes are possible: AA, GG, and GA. Wild type (TT) homozygosity was observed exclusively on the T allele, mutant type (GG) heterozygosity on the G allele, and heterozygous (G/A) alleles on both the A and G alleles. Heterozygotes (G/A) contain both alleles, as opposed to wild type homozygotes (GG), which contain only the T allele, and mutant homozygotes (GG), which contain only the G allele. A possessor of a particular allele, such as A or G.

Table 1. XRCC1 (rs2682585) POLY genotype frequency in patients with breast cancer with radiation therapy and without radiation

XRCC1 (rs2682585)	Patients with radiation n=50	Patients without radiation n=50	P1	P2	OR	95 % CI
GG	33 (66.00%)	22 (44.00%)	0.0024 S¥	0.0951 NS	1.562	0.9521-2.521
GA	19 (38.00%)	8 (16.00%)		0.0026 S¥	2.547	1.632-6.254
AA	5 (10.00%)	2 (4.00%)		Rec.	Rec.	Rec.

P1: overall comparison; P2: Individual genotype comparison versus reference; n: number of cases; ¥: Chi-square test; OR: odds ratio; CI: confidence interval; NS: non-significant.

The inverse association between the rs2682585 polymorphism in the essential excision repair gene XRCC1 and a decreased incidence of late normal tissue sepsis was confirmed in five cohorts of replication studies. A meta-analysis of data from seven patient groups revealed that individuals carrying the A minor allele of rs2682585 had a considerably reduced incidence of cutaneous toxicity. Additionally, those carrying the A allele had a substantially reduced likelihood of developing late toxicities. A correlation with breast fibrosis was demonstrated to be statistically significant.

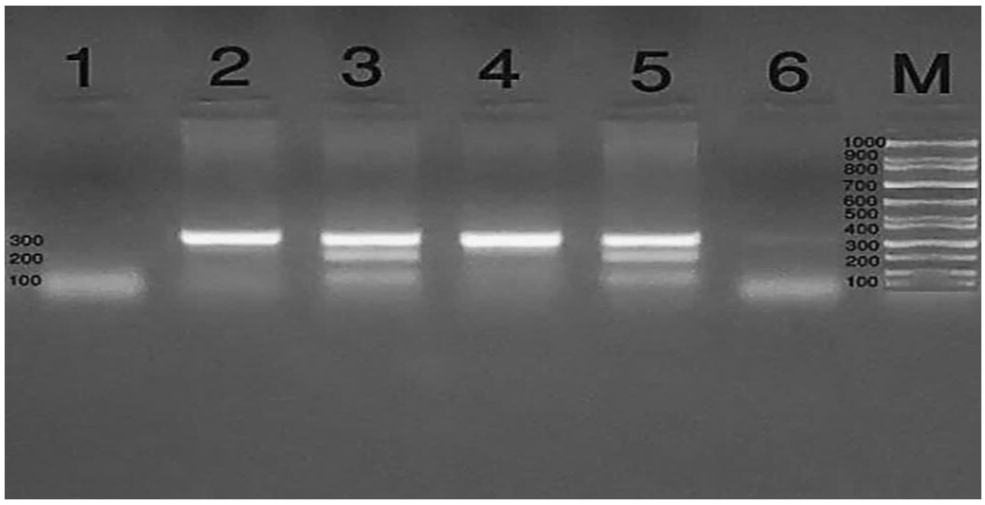

Figure 1. Agarose gel electrophoresis image that showed the Allele Specific PCR product analysis of gene polymorphism (XRCC1 (rs2682585) gene polymorphism in patient's samples.

Discussion

We employed a two-stage approach due to the limited efficacy and lack of replication of prior SNP studies. In breast cancer patients, the rs2682585 polymorphism in the basic excision repair (BER) gene XRCC1 was found to be significantly associated with a reduced risk of cutaneous toxicity and STAT scores. XRCC1 is an indispensable component of the BER mechanism. It is in contact with DNA polymerase (POLB) and ligase III (LIGIII), which are enzymes that are accountable for resealing the repair defect (21). The scaffold protein in question is hypothesized to coordinate the various repair processes (22).

This SNP may therefore alter the expression of the XRCC1 gene, potentially resulting in variations in protein levels. Minor modifications in the protein constituent induced by the SNP have the potential to undermine the chemical bonding and operational integrity of the complex. Additionally, a specific amino acid position in the PINLYP (LY6/PLAUR domain-containing inhibitor of phospholipase A2) locus is modified from His to Arg as a result of rs2682585. Nevertheless, as far as our understanding extends, rs2682585 does not possess any experimentally verified functional evidence at this time. In light of the absence of a significant association between rs2682585 in XRCC1 and breast fibrosis in any of the aggregated groups, this variant was not chosen for replication.

A similar association was observed in relation to epidermis toxicity; however, the available evidence was insufficient to establish definitive conclusions. Additionally, the lack of binding may initiate a multitude of processes that ultimately culminate in the onset of skin toxicity and fibrosis. Prior research has explored the correlation between XRCC1 single nucleotide polymorphisms (SNPs) and radiation toxicity in breast cancer patients, either in isolation or in conjunction with SNPs of other repair genes, due to the fact that base excision repair is implicated in the restoration of oxidative damage caused by radiation (5, 23-25).

Although mixed results have been observed (26). Pneumonitis and radiation-induced acute toxicity have been associated with the nonsynonymous single nucleotide polymorphisms rs25487 (Arg399Gln) in XRCC1 and rs1130409 (Asp148Glu) in APEX1 (27, 28).

Recent research (7) involving 637 prostate cancer patients and 976 breast cancer patients, the vast majority of whom were from the H trial, did not corroborate this intronic XRCC1 SNP in relation to late harm induced by radiation. Notwithstanding the implementation of photographic evaluation techniques, the follow-up period spanned a mere two years as opposed to the four years that were accounted for in our data. However, recent studies utilizing comet assay technology (29) have demonstrated that rs3213334 is associated with reduced basal DNA damage in lymphocytes of individuals with lung cancer, in comparison to controls without cancer.

In order to further reduce the probability of statistical bias resulting from heterogeneity, modifications were implemented to account for variations in patient characteristics and treatment parameters. Although there may be variations in treatment and allele frequency among distinct

ethnic groups, our findings regarding rs2682585 appear to be consistent. To be prepared for similar issues in the future, the XXX Consortium (33) is constructing large prospective cohorts using standardized data and biosample collection. Alternative treatment modalities, such as proton therapy or mastectomy, could be extended to patients who are at a greater risk of toxicity; this would significantly enhance their quality of life (10, 34). We have successfully determined, through the implementation of a sequential replication strategy, that a mutation in XRCC1 significantly decreased the likelihood of late toxicities in breast cancer patients who had received radiation therapy. For the development of a test with a sufficient effect size for clinical application in the prediction of late radiation toxicity, it is essential to identify additional variants.

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