Section A-Research paper

XRCC1 gene polymorphism predisposition of HCC in cirrhotic patients with HCV infection

Lamiaa.M. Kamel[1], Rana.M.Ahmed[1],Ola.A.Hussein,[1]Amal.A.Jouda[2],Manal.M.El Gerby[1]
1: Clinical Pathology Department, Faculty of Medicine, Zagazig University, Egypt
2: Tropical Medicine Department, Faculty of Medicine, Zagazig University, Egypt
Email: drranashalaby1991@gmail.com

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Abstract

Both genetic and environmental factors affect liver pathogenesis contributing to carcinogenesis. Identifying those factors could guide understanding various pathways involved in hepatic carcinogenesis. This may improve screening policies for high risk patients. DNA repair mechanisms interact to conserve genome integrity and avoid carcinogenesis. Base excision repair (BER) constitutes the primary defense against lesions generated by ionizing radiation and strong alkylating agents, in addition to other DNA-damaging agents as viruses. XRCC 1 gene has been found to play a pivotal role in the base excision repair (BER) pathway. Mutations of XRCC 1 may increase the risk of cancer through impairing its interaction with other enzymatic proteins with consequent impairment of DNA repair activity.

Keywords: XRCC1 gene polymorphism, HCC, cirrhotic patients, HCV

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Hepatitis C virus (HCV) accounts for approximately 15%-20% cases of acute hepatitis. After acute infection, around 50% to 80% of HCV patients will develop chronic infection. Approximately, HCV infects 170 million individuals worldwide. Chronic hepatitis C (CHC) patients are at high risk to develop life-threatening complications, including cirrhosis in 20% of cases and hepatocellular carcinoma (HCC) at an incidence of 4%- 5% per year in cirrhotic patients. Epidemiological studies also indicate that HCV is associated with a number of extra hepatic manifestations including insulin resistance, type 2 diabetes mellitus, glomerulopathies, oral manifestations[1].

Cirrhosis is a precancerous condition that predisposes to the development of hepatocellular carcinoma (HCC). European cohort studies have reported that HCC-related mortality accounts for 54– 70% of deaths in patients with compensated cirrhosis, with an annual incidence ranging from 2% to 6%. This trend is increasing in both Europe and North America, and is due to a decline in mortality due to other liver-related causes in such patients[2].

Several risk factors for the occurrence of HCC have been identified . The identified features so far encompass the cause of cirrhosis, older age over 55–60 years and male gender, the severity of the underlying cirrhosis, increased basal levels of serum alpha-fetoprotein and the degree of inflammatory activity within the liver. The additional impact of overweight and diabetes mellitus has also been strongly implicated. The additional contribution of yet undefined biological factors obtained through analyses of serum or tissue samples from cirrhotic patients, represents a new exciting challenge in this field [3].

Risk factors of HCC:

1-Oxidative stress and detoxifying systems

Various SNPs modulate the activity of several anti- or pro-oxidant enzymes. Among these systems, glutathione S-transferases (GSTs) are a large family of detoxifying enzymes protecting against oxidative DNA damage. The influence of genetic traits involved in GSTM1 and GSTT1 activities has been extensively

studied in various cancers, with controversial results obtained in small-sample case–control studies for HCC [4].

Other pro- or antioxidant systems are modulated by genetic heterogeneity and act at different subcellular levels. Myeloperoxidase (MPO) is expressed in neutrophils and Kupffer cells, and leads to the formation of highly reactive hypochlorous acid (HOCl) and anion (OCl^-) . Manganese superoxide dismutase (SOD2) generates H_2O_2 in mitochondria. the implication of variants modulating the activity of these enzymes has been underlined, and suggested both similarities and differences in HCV- and alcohol-induced hepatocarcinogenesis [5].

2-Iron metabolism

The risk of HCC in patients with genetic haemochromatosis is well established, particularly in patients with overt cirrhosis . A prospective study enabled a link to be made between hepatic iron overload, HFE variations and development of HCC in alcoholic cirrhosis while neither of these two factors affected the risk of liver cancer in HCV-infected cirrhotic patients [6].

3-Inflammation, cytokine, and chemokine systems

Polymorphisms that affect the production of some pro-inflammatory molecules such as IL-1,IL-6 and TNF may constitute the most studied genetic background in the HCC setting, One of the first case–control studies in this field reported that the high IL-1 production-conferring alleles in two SNPs, in the promoter region of the IL-1β gene were associated with the presence of HCV-related HCC after adjustment for confounding factors, which included the presence of cirrhosis [7].

Other mediators of inflammation such as chemokines and their receptors are implicated in the promotion of tumour growth and the invasion in the liver . Genetic variations commonly occur in their regulatory regions, affecting chemokines gene transcription. A prospective study suggested that chemokine RANTES G-403A dimorphism influenced the occurrence of HCC in patients with alcoholic cirrhosis [8].

4-DNA synthesis and repair mechanisms

SNPs in the methylene tetrahydrofolate reductase (MTHFR) gene, which lead to alterations in folate metabolism, an essential component of DNA synthesis and methylation, seem to be associated with HCC development. In a large case–control study, which included HCV- and HBV-infected patients from two cohorts of distinct ethnicities (Asians and non-Asians). The authors were able to observe a joint protective effect of genotypic associations in two variants conferring a low MTHFR activity (namely rs1801133 and rs1801131) [9].

Genetic predisposition and HCC

SNPs correspond to a modification of a DNA sequence due to the change of a single nucleotide; they account for >90% of allelic disparities scattered throughout the human genome. Although the vast majority of these modifications are situated in non-coding regions, some can modify gene-product expression and function, which may affect biological pathways. If the genes are involved in liver carcinogenesis, these modifications may partly explain the genetic heritability thought to influence individual susceptibility to HCC [10].

The loss of p53 function, enabling damaged cells to escape the cell-cycle checkpoint control and become carcinogenic, plays a critical role in carcinogenesis, and places variants of the p53 gene as fair candidates in the modulation of HCC risk. MDM2 is an important regulator of p53 that represents a negative auto-regulatory feedback loop with p53 protein. Some other polymorphic traits affecting the promoter region of MDM2 may be associated with HCV-related HCC [10].

The epidermal growth factor (EGF) is implicated in malignant transformation and tumor progression, and seems to actively participate in liver carcinogenesis in animal models . They were able to report that patients bearing two copies varient polymorphism in EGF gene not only had higher EGF-serum levels but also displayed increased hepatic expression of this factor. Furthermore, the homozygous G/G genotype was over-represented in HCC patients in both cohorts and was independently associated with the presence of a liver tumour after adjustment for confounding factors [11].

MicroRNAs (miRNAs) are a class of short non-coding RNAs with post-transcriptional regulatory functions. The binding of miRNA to mRNA is critical in the regulation of mRNA level and protein expression.

Accumulating evidence has linked the dysregulated expression of miRNAs with HCC. Functional SNPs in miRNA genes can lead to changes in expression of mature miRNAs [12].

In a study of Huang et al., (2019) evaluated the significant association between the SNPs in the ERCC1 gene and partial AFP locus with the risk of developing HCC and clinicopathologic features of HCC .The 30 - UTR is one of the most important regulatory regions of the ERCC1 gene. Mutations in the 30 -UTR or coding region of ERCC1 gene may affect the coding of ERCC1. This might inhibit its binding to the XPF protein, and even affect the ability of nucleotide excision repair pathway, increase the genomic instability, and thereby cause various cancers and malignant diseases [12].

The AFP protein promotes tumor cell growth mutation in the rs737241 locus of the AFP coding region might affect mRNA synthesis, thereby, reducing the synthesis of the AFP protein, Weakening the AFP immune surveillance that helps cancer cells in escaping the host cells; thus, the body can effectively identify and remove tumor cells and tumor metastasis is reduced, leading to a certain degree of protection; it was reported that in an Indonesian population, the polymorphism at the rs4640638 locus was associated with reduced risk of liver cancer [13].

In a study of Vogel et al., (2001) analyzed polymorphisms of the UGT1A7 and UGT1A9 genes in HCC and demonstrated that polymorphisms of the UGT1A7 gene represent a novel risk factor for HCC. Three polymorphic sites leading to amino acid exchanges at codon 129 (N129K), codon 131 (R131K), and codon 208 (W208R) exhibit a highly significant association with HCC [14].

The identified association of UGT1A7 gene polymorphisms in HCC establishes a link between a low detoxification activity allele of a major drug and carcinogen detoxifying enzyme and a carcinoma inducible by environmental carcinogens. The role of carcinogens in HCC development has been shown epidemiologically and on a molecular biological level [15].

Expression of theUGT1A7 gene polymorphism in the target tissue of neoplastic transformation, i.e., the liver, is not required. UGT1A7 is expressed in the most proximal tissues, which establish primary contact to orally administered and inhaled (lung epithelium) xenobiotics and carcinogens. UGT1A7 may therefore exert a general effect of carcinogen glucuronidation and detoxification at the “point of entry” of chemical carcinogens. This is particularly relevant for tobacco smoke–borne mutagens that are capable of inducing neoplastic transformation at anatomically distant sites connected only via the blood circulation, examples of which include the bladder, the breast, and the pancreas, as well as in the liver [16].

XRCC1 gene

XRCC1 is a 69kDa scaffold protein that interacts with the nicked DNA and polymerase β through its N-terminal domain.

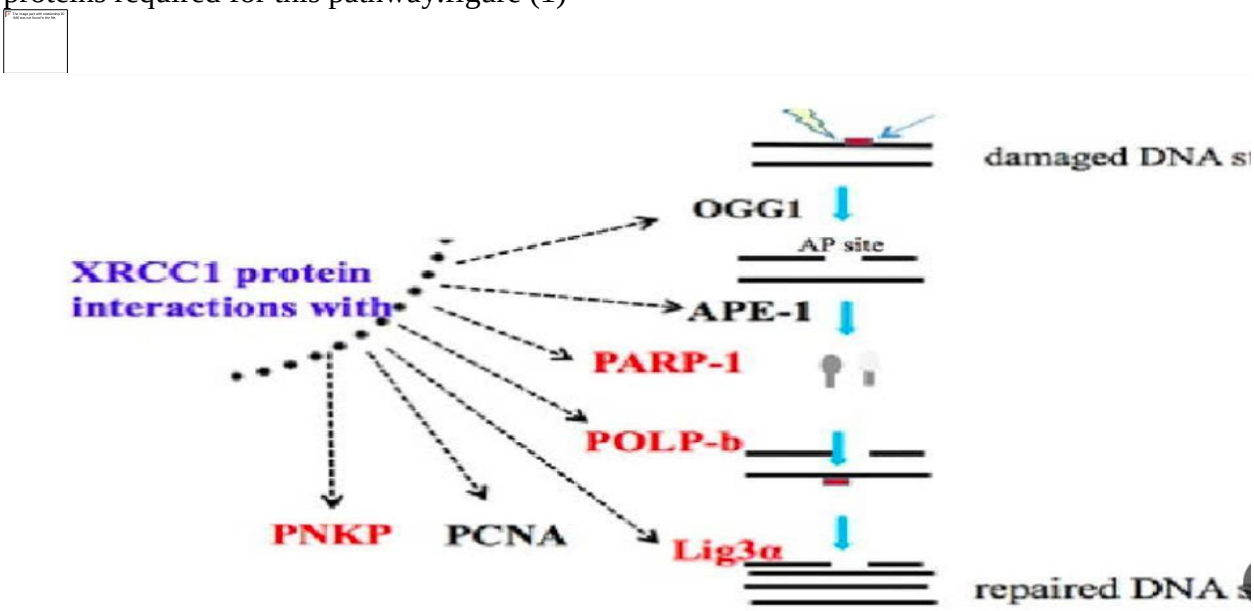
XRCC1 is involved in the efficient repair of DNA single-strand breaks formed by exposure to ionizing radiation and alkylating agents. This protein interacts with DNA ligase III, polymerase beta and poly (ADP-ribose) polymerase to participate in the base excision repair pathway. It may play a role in DNA processing during meiogenesis and recombination in germ cells. A rare microsatellite polymorphism in this gene is associated with cancer in patients of varying radiosensitivity [11].

The XRCC1 protein does not have enzymatic activity, but acts as a scaffolding protein that interacts with multiple repair enzymes. The scaffolding allows these repair enzymes to then carry out their enzymatic steps in repairing DNA. XRCC1 is involved in single-strand break repair, base excision repair and nucleotide excision repair.

XRCC1 protein has three globular domains connected by two linker segments of ~150 and 120 residues. The XRCC1 N-terminal domain binds to DNA polymerase beta, the C-terminal BRCT domain interacts with DNA ligase III alpha and the central domain contains a poly(ADP-ribose) binding motif. This central domain allows recruitment of XRCC1 to polymeric ADP-ribose that forms on PARP1 after PARP1 binds to single strand breaks. The first linker contains a nuclear localization sequence and also has a region that interacts with DNA repair protein REV1, and REV1 recruits translesion polymerases. The second linker interacts with polynucleotide kinase phosphatase (PNKP) (that processes DNA broken ends during base excision repair),

aprataxin (active in single-strand DNA repair and non-homologous end joining) and a third protein designated aprataxin- and PNKP-like factor [12].

XRCC1 has an essential role in microhomology-mediated end joining (MMEJ) repair of double strand breaks. MMEJ is a highly error-prone DNA repair pathway that results in deletion mutations. XRCC1 is one of 6 proteins required for this pathway.figure (1)



Over-expression in cancer

XRCC1 is over-expressed in non-small-cell lung carcinoma (NSCLC), and at an even higher level in metastatic lymph nodes of NSCLC [13].

Under-expression in cancer

Deficiency in XRCC1, due to being heterozygous for a mutated XRCC1 gene coding for a truncated XRCC1 protein, suppresses tumor growth in mice. Under three experimental conditions for inducing three types of cancer (colon cancer, melanoma or breast cancer), mice heterozygous for this XRCC1 mutation had substantially lower tumor volume or number than wild type mice undergoing the same carcinogenic treatments.

Both genetic and environmental factors affect liver pathogenesis contributing to carcinogenesis. Identifying those factors could guide understanding various pathways involved in hepatic carcinogenesis, this may improve screening policies for high risk patients. DNA repair mechanisms interact to conserve genome integrity and avoid carcinogenesis. Base excision repair (BER) constitutes the primary defense against esions generated by ionizing radiation and strong alkylating agents, in addition to other DNA-damaging agents as viruses. XRCC1 gene has been found to play a pivotal role in the base excision repair (BER) pathway. Mutations of XRCC1 may increase the risk of cancer through impairing its interaction with other enzymatic proteins with consequent impairment of DNA repair activity [14].

Previous studies showed significant association between HCC and different SNPs in XRCC1 gene. Xia et al., (2014)[15]noted that the genotypes and alleles distribution of XRCC1 variants c.910A>G and c.1686C>G were statistically associated with the risk of HCC .

Liu et al., reported that c.1804C>A genetic polymorphism of XRCC1 may influence the risk of HCC [15]. Kiran et al., [16] found that Arg194Trp and Arg280His genotypes showed an increased risk of HCC which was further enhanced when Arg280His genotype was found in association with Arg194Trp and Arg399Gln.

Previously c.1517G>C genetic variant of the XRCC1 gene also was reported to be significantly associated with pancreatic cancer in a study conducted by Zhao et al.,[17] They noted that The CC genotype was

significantly associated with an increased risk of pancreatic cancer. They reported that C allele may contribute to development of pancreatic cancer. Our study aimed to investigate the association between XRCC1 (c.1517G>C) polymorphism and the risk of HCC in Egyptian patients who are chronically infected with HCV. This genetic variant represents a non-synonymous G to C mutation in exon 14 of the XRCC1 gene, resulting in glycine (Gly) to alanine (Ala) amin o acid replace-ment (p.Gly506Ala) .

A higher frequency of XRCC1 (CC, GC) genotypes in patients with HCC (82.5%) in comparison to cirrhotic HCV patients (55%) as well as control group (40%) with higher percentage of C allele (70%) in HCC group. The multivariate analysis revealed that the presence of c.1517G>C SNP of XRCC1 gene was an independent risk factor for the development of HCC in chronic HCV patients with 3.7 fold increased risk of HCC development.

Furthermore, patients with CC, GC genotypes had significantly higher number and larger size of tumour foci and advanced Child Pugh grades. This was in agreement with Bi et al., [18] who studied c.1517G>C and c.1254C>T polymorphisms in XRCC1 gene among HCC Chinese Han population. They found that there was a statistically significant association between XRCC1 (CC, GC) genotypes and the risk of HCC. As in HCC group CC, GC and GG genotypes represented 15.21%, 47% and 37.79% respectively, with CC/GC genotypes versus GG genotype OR 1.63 increased risk of HCC; $p < 0.001$. They reported that the C-allele of c.1517G>C genetic variants may influence the susceptibility to HCC ($p< 0.001$). They also noted significant association between c.1254C>T polymorphism and HCC risk. However, there was no statistical significance between c.1517G>C CC homozygous genotype vs. GC and GG in HCC patients group regarding number of foci, focal size lesion or Child Pugh classification.

XRCC1 (c.1517G>C) polymorphism could be associated with increased risk of HCV- related HCC development in Egyptian population but the definite association between them needs to be validated in other large multicentre cohort studies [18].

Conclusion:

XRCC1 gene has been found to play a pivotal role in the base excision repair (BER) pathway. Mutations of XRCC1 may increase the risk of HCC through impairing its interaction with other enzymatic proteins with consequent impairment of DNA repair activity.

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