Impaired base excision repair is related to the pathogenesis of non-alcoholic fatty liver disease

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ABSTRACT

Non-alcoholic fatty disease (NAFLD) is a liver disorder that affects up to 30% of the population, mainly in Western countries. It is estimated that up to 75% of NAFLD patients will develop a more aggressive form of the disease, non-alcoholic steatohepatitis (NASH). NAFLD can lead to fibrosis and liver failure; however, it is difficult to diagnose NAFLD due to its non-specific symptoms. Unfortunately, there is no treatment available for this disease. The risk factors of NAFLD are obesity and insulin resistance (IR). The molecular factors that seem to play an important role in the pathogenesis of NAFLD are oxidative stress as well as impaired DNA damage repair processes; a great body of evidence confirms an association with the base excision repair (BER) pathway. The activity of BER is decreased in patients with NAFLD and in animal models of this disease. In order to better understand the underlying basis of the disease, knowledge should be broadened in the area of DNA repair in NAFLD.

KEYWORDS: DNA repair, non-alcoholic fatty liver, base excision repair

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a condition that affects up to 30% of the population around the world, most prevalently in Western countries. It is estimated that up to 75% of people suffering from simple fatty liver disease will develop a more aggressive form of the disease, non-alcoholic steatohepatitis (NASH), which can lead to a cirrhosis, primary hepatocarcinoma or liver failure. However, it is difficult to diagnose the NAFLD due to the non-specific symptoms of the disease. NASH is characterised by the occurrence of inflammation and fibrosis in the liver (Abd El-Kader and El-Den Ashmawy 2015; Paschos and Paletas 2009).

Little is known about the molecular mechanisms involved in the pathogenesis of NAFLD. However, the current literature shows that NAFLD is associated with increased production of reactive oxygen species (ROS) and with elevated levels of 8-oxo-2'-deoxyguanosine (8-oxo-dG), which is characteristic of DNA damage caused by increased

oxidative stress. Mitochondria are essential organelles for cell survival, mainly due to their function as energy producers through the electron transport chain (ETC). On the other hand, ETC generates ROS and may contribute to increased oxidative stress. Oxidative stress and mitochondrial DNA (mtDNA) damage are linked and affect each other. ROS produced due to the electron transport chain may target mtDNA. In effect, damaged mtDNA needs to be repaired, mainly through the base excision repair (BER) pathway in mitochondria (Masarone *et al*. 2018; Yang *et al*. 2019).

A growing number of reports, which have been presented in the review, indicate that oxidative stress as well as impaired processes responsible for repairing DNA damage can play important in the development of NAFLD. The main factor that links DNA repair and NAFLD is an increased level of oxidative stress followed by an elevated level of oxidative damage in patients. In cases of faulty DNA repair, these lesions cannot be repaired (Nagahashi *et al*. 2016). The most important aspect of further research is the absence of a treatment that could prevent liver failure and eventually the death of the patient.

The pathophysiology of non-alcoholic fatty liver disease

NAFLD is a pathological condition in which fats build up in the liver; it can progress to a more aggressive form of the disorder, NASH. It can lead to cirrhosis and may develop into primary liver cancer (Pinter *et al*. 2016). Unfortunately, the disease has non-specific symptoms like a feeling of discomfort in the upper right side of the abdomen; often, there are no symptoms until considerable liver cell damage occurs. One of the risk factors is obesity; the prevalence in obese population is in the range of 57–74% (Sharma *et al*. 2015). Moreover, up to 75% of people suffering from NAFLD develop NASH (Chitturi *et al*. 2018). As there are no approved medicines to treat NAFLD (Wong and Singal 2019), doctors recommend vitamin E supplementation and losing weight as well as medicines for type 2 diabetes such as pioglitazone (Bril *et al*. 2018).

NAFLD is a hepatic disorder characterised by triglyceride (TG) accumulation in hepatocytes. It is caused by an imbalance between lipogenesis or fatty acid uptake and fatty acid removal, e.g. via mitochondrial fatty acid oxidation (Caligiuri *et al*. 2016). In the case of NASH, hepatic inflammation, hepatocellular ballooning and often fibrosis can occur (Cohen *et al*. 2011). The hypothesis explaining the pathogenesis of NAFLD assumes that (i) hepatic steatosis is a result of insulin resistance (IR), and (ii) the progression to NASH is associated with oxidative stress, lipid peroxidation, cytokine production or mitochondrial dysfunction (Day and James 1998).

Particularly in obese patients with NAFLD, there is an overload of TG in adipose tissue, which is an energy storage organ and is involved in the secretion of hormones, cytokines and chemokines (Kershaw and Flier 2004). Excess TG is converted into free fatty acids (FFA) that can enter the liver, which can result in peripheral IR (Boden 1997). Insulin, a lipolysis-inhibiting hormone, controls the release of FFA into the liver. Thus, IR leads to intense lipolysis of adipose tissue and, consequently, to an increased influx of FFA into the liver. Additionally, FFA may serve as ligands for Toll-like receptor 4, thus inducing cytokine production and eventually inflammation, which play important roles in NAFLD development (Shi *et al*. 2006). IR is an effect of an

increased level of cytokines, such as interleukin (IL)-6, IL-8 and tumour necrosis factor (TNF)-α; high cytokine levels are correlated with a greater content of adipose tissue. NAFLD is more associated with visceral adipose tissue than with subcutaneous adipose tissue. (Parker 2018). Because of an excess of visceral adipose tissue, there is a dysregulation in a production of chemokines responsible for fatty acid oxidation (Rotter *et al*. 2003; Skurk *et al*. 2007). However, the liver can eliminate TG through secretion as very low-density lipoprotein (VLDL) or by performing a fatty acid oxidation. Both reduced oxidation and increased TG levels have a place in NAFLD pathogenesis (Koo 2013).

The fat accumulation in the liver may trigger cellular injury and death because of fatty acid intermediates (Lee *et al*. 1994; Tomita *et al*. 2014). Palmitic acid, a type of FFA, is able to cause inflammatory activation in endothelial cells (Maloney *et al*. 2009). Free cholesterol accumulation also induces lipotoxicity (Tomita *et al*. 2014). However, fatty acid oxidation can directly lead to the production of ROS (Neuschwander-Tetri 2010). Oxidative stress induces hepatocellular damage through mechanisms such as lipid peroxidation, which is responsible for activation of cell necrosis and the intrinsic pathway of apoptosis. In effect, it can trigger fibrosis (Koek *et al*. 2011). Palmitic acid can also activate the c-Jun N-terminal kinase (JNK) and the NF-κB proinflammatory pathways and may lead to mitochondrial dysfunction (Maloney *et al*. 2009). Additionally, free cholesterol induce the JNK pathway, thus generating a higher amount of ROS (Caballero *et al*. 2009). TG accumulation and steatosis can occur without liver damage, as shown by studies on genetic defects in diacylglycerol acyltransferase (DGAT), which catalyses the final step in TG synthesis, and in microsomal transfer protein (MTP), which influences VLDL synthesis (Liao *et al*. 2003; Monetti *et al*. 2007).

In pathophysiology of NAFLD also involves Kupffer cells, i.e. resident hepatic macrophages, which play a role in the activation of the macrophage M1 phenotype and altered activation of M2 macrophages (Stienstra *et al*. 2010). Toxic lipids accumulate in hepatocytes and may be phagocytised by Kupffer cells, which may indirectly lead to inflammation. Kupffer cells are involved in inflammation via inflammatory and apoptotic pathways mediated by NF-κB, which induce further release of proinflammatory cytokines (Seki *et al*. 2007).

Base excision repair pathway

Mitochondria are crucial in the pathogenesis of NAFLD. This statement has been confirmed by the relationship between NAFLD and the metabolic syndrome, which is tightly associated with mitochondrial dysfunction and oxidative stress (Kim *et al*. 2018; Mabalirajan and Ghosh 2013). The most important DNA repair pathway in mitochondria is BER, because it eliminates oxidative lesions (Alexeyev *et al*. 2013). BER recognises forms of oxidative, deamination, alkylation and abasic sites. which are not significant alterations to the DNA helix shape. It is divided into four steps (Figure 1): (i) recognition of the DNA damage, (ii) excision, (iii) synthesis and (iv) ligation of DNA. The first two steps are executed by DNA glycosylases, e.g. OGG1, MYH, NEIL1 and AP endonuclease (APE1) (Kim and Wilson 2012). DNA glycosylases are able to recognise and excise damaged bases, while the endonuclease cleaves the phosphodiester bonds. During synthesis, polymerase (POLG in mitochondria) inserts the correct nucleotide in the
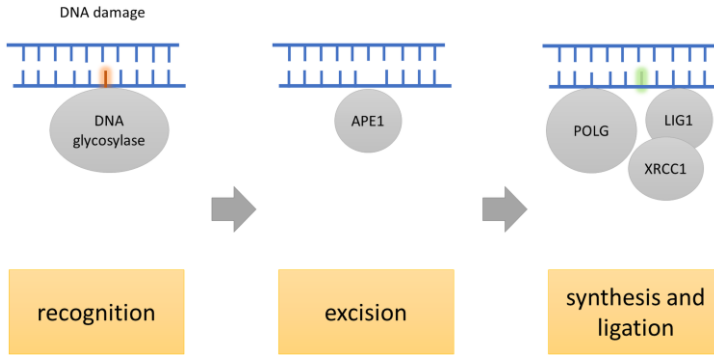
Figure 1. The scheme of base excision repair.

generated gap. The final step of BER in mitochondria, i.e. ligation, is performed by the complex of X-ray repair cross-complementing protein 1 (XRCC1) and DNA ligase III (LIG3) or ligase 1 (LIG1) (Chatterjee and Walker 2017; Kim and Wilson 2012). In the BER machinery, an important role is also played by structure-specific nucleases that remove 5' overhanging flaps and process the 5' ends of Okazaki fragments in lagging strand DNA synthesis. This nuclease is encoded by flap structure-specific endonuclease 1 (FEN1) (Kim and Wilson 2012).

The relationship between non-alcoholic fatty liver disease and base excision repair

There is still little known about the relationship between NAFLD and DNA repair systems. However, the results of recent studies allow us to assume that fatty liver disease has a tight link with the BER pathway. These studies were conducted not only on NAFLD patients, but also on animal models mimicking human NAFLD, i.e. the high fat diet (HFD), methionine-choline-deficient diet (MCD) and fructose-rich diet models. The findings confirm that MCD upregulates gene expression of BER enzymes, i.e. DNA glycosylase and APE1, in mice and induces hepatic steatosis, confirming the link between

diet and steatosis (Takumi *et al*. 2015). Furthermore, both MCD and HFD not only increase ROS production and oxidative damage DNA, but also reduce DNA repair by decreasing *MYH* expression (Gao *et al*. 2004). In addition, genetically modified *OGG1* knockout mice fed an HFD had impaired glucose tolerance and a higher plasma insulin level, as well as downregulation of carnitine palmitoyl transferase-1, important in fatty acid oxidation and associated with the development of NAFLD (Sampath *et al*. 2012).

Moreover, there are studies that confirm the appearance of single-nucleotide polymorphisms (SNPs), which can contribute to impaired BER in mitochondria (Czarny *et al*. 2018; Ibarrola-Villava *et al*. 2011; Lillenes *et al*. 2017; Popanda *et al*. 2013). The SNPs are broadly present in other diseases, mainly in neurodegenerative disorders and carcinomas. However, they should be evaluated in the context of NAFLD. The SNPs mentioned above have been found in such genes as *OGG1, MYH, POLG, POLB* (polymerase β) *NEIL1, APE1, FEN1, LIG1, LIG3* and *XRCC1.*

In terms of therapeutic approaches to NAFLD, interesting results have been obtained in a study that used pioglitazone as treatment method. This drug is

sometimes given to treat this disease, and has been beneficial in NASH patients. An HFD induced hepatic steatosis, but this effect was reversed by adding pioglitazone. Furthermore, treatment increased the expression level of *OGG1* and *MYH*, which could indicate that the improvement in the health of patients with steatosis may be related to an impact on DNA repair systems (Hsiao *et al*. 2008). Interestingly, in the liver of rats fed a high-fructose diet, an increase in mtDNA damage was found. The diet also caused a decrease in the expression level of DNA polymerase gamma and reduced mtDNA copy number (Cioffi *et al*. 2017).

There are also some research findings that support an association between NAFLD and other DNA repair mechanisms. MCD-fed wild type mice were compared to MCD-fed growth arrest and DNA damage-inducible gene (*Gadd45a*) knockout mice. This gene plays a role in DNA survival and repair, in both the BER and nucleotide excision repair (NER) pathways. Knockout mice had significantly more severe hepatitis and fibrosis, elevated expression levels of pro-inflammatory proteins as well as decreased TG levels in comparison to wild type mice (Tanaka *et al*. 2017). Obese patients with steatotic livers have elevated oxidative stress in the liver and, at the same time, a significant decrease in NER activity (Schults *et al*. 2012).

Summary

Accordingly to the latest literature, a growing body of evidence suggests that mitochondrial dysfunction may play an important role in NAFLD, which can be triggered by impaired mitochondrial genome stability. Increased ROS production and elevated oxidative stress in mitochondria contribute to the development and progression of NAFLD. Many reports have shown that an

important factor in the pathogenesis of fatty liver disease is impaired DNA repair systems. Furthermore, the expression of genes involved in DNA repair is increased upon treatment of this disease. This review suggests the need for further research into the molecular processes underlying NAFLD, especially in context of DNA damage and repair. This could contribute to the development of an appropriate treatment for this disease.

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