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Invited critical review

MicroRNA responses to environmental liver carcinogens: Biological and clinical significance

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ABSTRACT

A large number of biological, chemical, and dietary factors have been implicated in the development of liver cancer. These involve complex and protracted interactions between genetic, epigenetic, and environmental factors. The survival rate for patients diagnosed with late-stage liver cancer is currently low due to the aggressive nature of the disease and resistance to therapy. An increasing body of evidence has offered support for the crucial role of non-coding microRNA (miRNA) in directing hepatic responses to environmental risk factors for liver cancer. In this review we focus on miRNA responses to environmental liver cancer risk factors and their potential biological and clinical significance.

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1. Introduction

Primary liver cancers are those that originate in the hepatic tissue itself, rather than arising in non-hepatic tissues followed by metastasis to the organ. Liver cancers are currently the sixth most frequent cancer worldwide with more than 600 000 new cases every year [1]. In adults the most common forms of liver cancers are hepatocellular carcinomas (HCC) accounting for 85% of primary liver cancers and cholangiocarcinomas (cancers of bile ducts) which represent 10% of liver cancers. Rarer liver cancers include hepatoblastomas (liver cancers

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found in infants and children), hemangioendotheliomas (cancers of the blood vessels), tumors displaying mixed hepatocyte/cholangiocyte phenotype, as well as a number of other cancer forms [2].

It is established that biological, physical, dietary, and chemical agents are implicated in the development of human liver cancers. These environmental factors include infection with hepatitis viruses, excessive alcohol intake, exposure to environmental hepatotoxicants, smoking, use of oral contraceptives, and diets that promote obesity and metabolic syndrome [1,2]. The susceptibility of the liver to environmental risk factors is due to the central metabolic and detoxifying role of the organ. The liver is directly exposed to high levels of orally ingested biological and chemical factors which pass to it directly from the gastrointestinal tract. Hepatocytes are also the major detoxification cells of mammalian organisms. The metabolic and biochemical modification of exogenous compounds by hepatocytes can inadvertently lead to their transformation to a more harmful state where they can directly attack the liver [3]. The liver also plays a key role in the metabolism and storage of carbohydrates, proteins, and lipids and is consequently vulnerable to unhealthy diets and systemic metabolic disorders [4]. A multitude of mechanisms by which environmental carcinogens can increase the incidence of cancers have been proposed [5,6]. One important division is between carcinogens that damage DNA directly (genotoxic agents) and those that act by different mechanisms (non-genotoxic agents). Genotoxic agents are most frequently electrophiles which increase the incidence of cancer by causing mutations affecting oncogenes or tumor-suppressor genes. Non-genotoxic hepatic carcinogenic agents have been proposed to act by diverse mechanisms that include the disruption of normal cell cycle and apoptotic regulation, induction of oxidative stress, chronic disruption of the immune system, activation of CYP450 enzymes, and disruption of normal metabolism [7–9].

A worrying trend over the past decades is the increased incidence of liver cancer worldwide [1]. In the developing world this increase is associated with higher levels of chronic hepatitis infection and with contamination of the human food supply by hepatocarcinogens. In the developed world the increased incidence of liver cancer is associated with the epidemic of obesity and metabolic disorders in these countries. Unfortunately liver cancers, and HCC in particular, are aggressive diseases with low survival rates [10]. A primary reason for this is the relatively late detection of liver tumors which usually develop with no early signs or symptoms. When liver cancers are detected they are often already metastatic and refractory to chemotherapy. Treatment of liver cancer patients is also commonly rendered more difficult by the presence of cirrhosis and liver damage. There is therefore an urgent need for the development of novel methodologies for the earlier detection and the more efficient treatment of liver cancer.

As discussed previously environmental risk factors are the main drivers of liver cancers in human populations. An accumulating number of studies have examined miRNA responses to environmental liver cancer risk factors. In this review we will discuss the miRNA responses to environmental liver carcinogens and their potential biological and clinical significance.

2. miRNA biogenesis

The microRNA (miRNA) are a family of small 21–25 nucleotide long, evolutionary conserved non-coding RNA genes that post-transcriptionally regulate the expression of their target genes. The human genome has been estimated to contain between 1000 and 2000 distinct miRNA genes [11]. The biogenesis of mature miRNA is a multi-step process that involves several enzymes and that is under tight spatial and temporal control (Fig. 1). The canonical miRNA biogenesis starts with the transcription of a long primary miRNA (pri-miRNA), that can be thousands of kilobases in length, through the action of DNA polymerase II or III. The characteristic step of miRNA biogenesis is the folding of the pri-miRNA onto itself to form a hairpin structure. The pri-miRNA will subsequently undergo two cleavage steps from RNAse III type enzymes in order to release the mature miRNA. The first cleavage step involves the DiGeorge Syndrome Critical Region 8 (DGCR8) and Drosha microprocessor complex and occurs within the nucleus. This cleavage step results in the production of a 60–80 nucleotide long pre-miRNA which is subsequently transported to the cytoplasm. There, the second cleavage step occurs by the DICER enzyme which cuts the pre-miRNA at 21–24 nucleotides from one end to form a miRNA-miRNA* duplex. The strands are subsequently dissociated and single strands associate with the RNA activated silencing complex (RISC). For a subset of miRNA termed mirtrons, generation of pre-miRNAs occurs directly from the introns by activity of the spliceosome and debranching enzymes. The pre-miRNAs generated by this non-canonical pathway are then exported to the cytoplasm and processed to mature miRNAs as in the canonical pathway [12]. Currently only a small number of mammalian mirtrons have been identified.

Once associated with the RISC the mature miRNA can act as a guiding strand directing the silencing of its mRNA target based on incomplete base-pair matching between the two RNA strands, generally within the 3′UTR region of the mRNA. Manipulation of the levels of miRNAs have been demonstrated experimentally to affect the expression of hundreds or thousands of genes at mRNA [13] and protein level [14,15]. Moreover, experimental data and *in silico* prediction concur that the majority of mammalian genes are subjected to miRNA regulation [16]. Nevertheless the mechanisms by which miRNA mediate the repression of their target mRNA remain a topic of debate, probably involving both translational repression and mRNA destabilization [17].

3. miRNA in liver cancer

Similar to what is observed for other organs miRNAs are abundant in the liver and are important for the proper development and function of the organ [16]. Strong evidence also implicates miRNA dysregulation to the initiation and progression of liver tumorigenesis. Numerous studies have reported altered miRNA expression in liver tumors compared to normal tissue [18–21]. The requirement for normal miRNA signalling to prevent hepatic tumors was demonstrated in a conditional knockout mouse model in which *dicer1* was deleted specifically in hepatocytes three weeks after birth. Hepatocytes in these animals lacked mature miRNAs. At one year of age two-thirds of these mice developed HCC, clonally derived from *dicer1* knockout hepatocytes [22]. Mice with genetic modifications affecting single miRNA have also offered evidence supporting the hepatocarcinogenic role of miRNA. Knockout mice for the miR-122 gene developed hepatosteatosis, hepatitis, and liver cancers. Loss of miR-122 was associated with increased activity of oncogenic pathways and of inflammation in the liver [23]. A second group generated genetically modified mice that over-expressed miR-221. Half of the male mice over-expressing miR-221 developed liver cancers, while there was a strong acceleration in the development of liver cancers in animals treated with the genotoxic hepatocarcinogen diethylnitrosamine. Within the liver tumors formed there was a further increase in the levels of miR-221 and a decrease in the expression of a number of tumor-suppressors that are regulated by the miRNA [24].

Altered expression of a number of specific miRNAs has been causally implicated to liver cancer initiation and progression. The miRNA with altered expression include both upregulated and downregulated miRNA. The miRNA implicated in liver cancer have been shown to act by a large variety of mechanisms. These include the activation of oncogenic pathways or the repression of tumor-suppressors resulting in aberrant cell growth, disruption of normal apoptotic pathways, dedifferentiation of hepatic cells, and the acquisition of other traits that are essential in carcinogenesis. For example, miR-21 is highly overexpressed in HCC and liver cancer cell lines and promotes hepatocyte growth by targeting PTEN [25]. MiR-221 is upregulated in 71% of HCC and controls cell cycle regulators p27 and p57 [26]. MiR-122 is a tumor-suppressor downregulated in 50–70% of HCC and correlates with loss of hepatocyte phenotype and increased invasiveness [27].