Editorial

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Maternal obesity: A severe risk factor in hepatocarcinogenesis?

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See Article, page 603–615

While one third of the world's population is overweight or obese, the impact of obesity as an epigenetic trait affecting not only people's health but also the health of unborn children is highly questionable. Obesity confers a higher risk of developing metabolic diseases, such as non-alcoholic fatty liver disease (NAFLD), and hepatocellular carcinoma (HCC).[1] Besides that, the offspring of obese mothers are known to be more susceptible to NAFLD, but no relationship has been established between obesity in mothers and the risk of their progeny developing HCC.[2] There is abundant evidence for intergenerational inheritance of epigenetic states occurring in plants and animals.[3] This inheritance presupposes an external stimulus — that can be metabolic — which stably modifies parental cell fate and can be transmitted from the parents to the fetus. When cellular memory is imprinted in the DNA of germ-line cells, cell reprogramming is stable over the generations, and becomes transgenerational. When affecting somatic cells of the mother, the inheritance of cell memory can be inter-generational, affecting only the immediate progeny. Maternal or paternal short RNAs have been implicated in inter- or trans-generational inheritance of epigenetic states.[4] In this issue of *Journal of Hepatology*, Sun *et al.* proposed an inter-generational inheritance of HCC susceptibility in mice fed a high-fat-diet (HFD), revealing a new avenue for cancer research, at the crossroads of metabolism and epigenetics.

Sun *et al.* addressed the role of a maternal multi-generational HFD exposure on the development of HCC in offspring.[5] Male offspring were fed with a HFD and tumors were induced by diethylnitrosamine (DEN) – a widely used chemical carcinogen that induces chronic liver damage and liver carcinoma.[6] These mice were compared to mice fed with normal chow with and without DEN treatment. The analyses were performed over 3 generations. They identified a set of miRNAs which are differentially regulated. Specifically, they identified miR-27a-3p and its downstream targets *Acsl1* (Acyl-CoA synthetase long chain family member 1) and *Aldh2* (aldehyde dehydrogenase 2 family member) as important factors in fatty liver disease and subsequently HCC formation (Fig. 1). Interestingly maternal obesity due to HFD increased the susceptibility of the offspring to tumor formation and reduced survival in the second and third generation. RNA sequencing of liver tumors from these mice revealed alterations in genes responsible for the lipid and/or amino acid metabolism. The authors focused on the genes *Acsl1* and *Aldh2* which were gradually downregulated in the offspring; this downregulation has also been linked to poor survival in patients with liver cancer based on the TCGA database. Additional hepatic miRNA levels were examined by RNA sequencing to identify possible regulators. Several miRNAs were gradually altered in the offspring, including miR-27a-3p, which is suggested to decrease the survival rate of patients with HCC when expressed at elevated levels, according to the TCGA database. Bioinformatic analysis indicated that miR-27a-3p might regulate transcription of *Acsl1* and *Aldh2,* which was verified by *in vitro* and *in vivo* analysis.

The elevated level of miR-27a-3p in offspring assumes a transportation of miRNAs from the mother to the offspring by small extracellular vesicles (sEVs). The transport of miRNAs including miR-27a-3p by sEVs has previously been demonstrated. PMT (proneural-to-mesenchymal transition) is a common process in the progression of glioblastoma which results in increased radiotherapy resistance. PMT is triggered by tumor-associated macrophages releasing sEVs. These sEVs send miR-27a-3p, miR-22-3p and miR-221-3p to glioma stem cells promoting the mesenchymal phenotype via the RelB/p50 and STAT3 pathways.[7] The interplay of the NF-κB pathway was also shown in the context of acute lung injury. miR-27a-3p targets NFKB1 and thereby functions as a regulator of M2 macrophage polarization. In this study, mesenchymal stem cell-derived sEVs alleviated acute lung injury through elevated levels of miR-27a-3p in alveolar macrophages, resulting in the promotion of M2 macrophages.[8] sEVs are also reported to be involved in stem cell maintenance, self-renewal, and differentiation. sEV-derived miRNAs mimic the function of parental stem cells in regulating the maintenance and differentiation of stem cells, controlling the intercellular regulation of gene expression, and potentially even affecting cell fate. miR-27a-3p was highly expressed in human adipose tissue stromal/stem cells and is reported to be involved in the regulation of osteogenesis.[9]

A deregulation of metabolic pathways has been implicated in the onset and progression of HCCs. In a study by Zahid and

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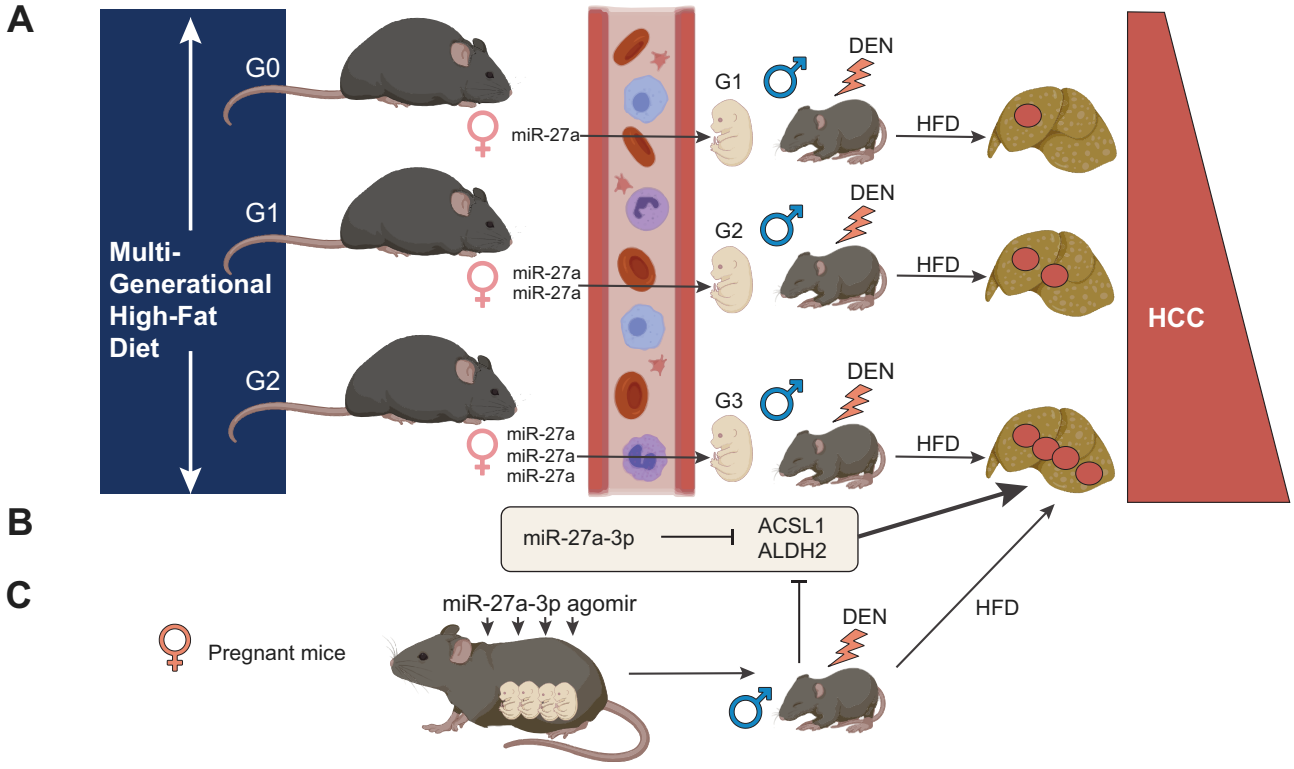

Fig. 1. Multi-generational HFD predisposes the offspring to chemical hepatocarcinogenesis. *In vivo* experiments showing that (A) HFD-fed female mice secrete miR-27a-3p and this increases with the generations (G0 to G2), leading to a higher HCC burden in G3 male mice compared to G1; (B) miR-27a-3p represses the expression of Acsl1 and Aldh2, responsible for this increased tumor burden; (C) Injection of miR-27a-3p agomir to pregnant female mice leads to the same phenotypic consequences in the offspring. HCC, hepatocellular carcinoma; HFD, high-fat diet.

colleagues, a gene signature coding for catabolic enzymes in patients with HCC identified ALDH2 and ADH1A (alcohol dehydrogenase 1A), both key regulators of alcohol metabolism, as key in the development of HCC. Using *in silico* analyses they claimed that *ADH1A* and *ALDH2* were transcriptionally suppressed by HDAC1 (histone deacetylase 1) downstream of mTORC1 signaling, which was associated with poor survival and an aggressive disease state.[10] It was also shown that ALDH2 mRNA and protein levels were significantly lower in tumor tissues than normal tissues and were also lower in tissues that exhibited increased migratory capacity. ALDH2 altered the redox status of cells by regulating acetaldehyde levels and stimulating the AMP-activated protein kinase signaling pathway.[11] Transgenic *Aldh2*-deficient mice were more susceptible to CCl_4 and alcohol-induced liver fibrosis and subsequent HCC formation. *Aldh2* deficiency results in increased amounts of harmful oxidized mitochondrial DNA transported via EVs, which can lead to the activation of oncogenic pathways in neighboring cells.[12]

Several studies have demonstrated that ACSL1 is involved in aberrant lipid metabolism in liver cancer. HULC, a long non-coding RNA, modulates lipid metabolism by activating ACSL1.[13] Another modulator of lipid metabolism is miR205, which targets ACSL1;[14] NF-κB-ACSL1 signaling has also been suggested to be involved in abnormal lipid metabolism in liver cancer cells.[15] Consequently, ACSL1 was reported as a potential prognostic gene in HCC.[16] These studies point to the fact that miR-27a-3p is not the only player upstream of *Acsl1* and *Aldh2*. It is more likely part of a wider signaling network which ultimately influences susceptibility to HCC formation.

Furthermore, Sun and colleagues analyzed the expression of miR-27a-3p/Acsl1/Aldh2 in patient samples. A small cohort of 27 fatty liver-associated HCCs and 27 non-fatty liver-associated HCCs and corresponding non-tumor tissues were analyzed by immunohistochemistry. In accordance with the reported HFD mouse model, similar results were reported in fatty liver-associated HCCs, suggesting that their mouse model is a powerful tool for the analyses of fatty liver disease and subsequent HCC development. The positive area for miR-27a-3p was increased in human HCC compared to non-tumor tissue. The miR-27a-3p positive area was increased in fatty liver-associated HCCs *vs.* non-fatty liver-associated HCCs, as well as in non-tumorous fatty liver tissue compared to non-fatty liver. Because of increased miR-27a-3p levels, the analyzed positive area of Acsl1 and Aldh2 was lower in liver tumors compared to non-tumor tissue. This effect was more dramatic in fatty liver-associated HCCs and also in the fatty liver itself. A more detailed analysis of a bigger patient cohort consisting of HCCs associated with different kinds of chronic liver disease would be of interest. It would also be interesting to know whether miR-27a-3p/Acsl1/Aldh2 levels differ depending on the etiology of fatty liver disease, or if this identified axis is a universal link in fatty liver and fatty liver-associated HCCs.

This study provides new information on how maternal stress could influence progeny, with consequences for HCC development. The mode of inheritance is not a classical intergenerational one. It is multi-generational, as the susceptibility to HCC gradually increases over generations, requiring maintenance of HFD status in mothers over the generations to reach a

sufficient level of miR-27a-3p to efficiently activate the miR-27a-3p/Acsl1/Aldh2 axis. However, several issues still remain. First, the mechanism of inheritance is unclear: In the mother, which cell type is responsible for miR-27a-3p synthesis and secretion? The transportation of miR-27a-3p from the mother to the fetus by sEVs also remains to be confirmed. Second, to what extent is the maternal transmission of the miR-27a-3p/Acsl1/Aldh2 axis conserved between mice and humans? If it is, there is further evidence for the need to prevent junk food consumption.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

SC and AL contributed equally to write the editorial. SC designed and realized the figure.

Supplementary data

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