EBioMedicine 46 (2019) 21–22

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

EBioMedicine
Published by THE LANCET

Commentary

# Uncovering the mechanism of action of aspirin in HCC chemoprevention

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*Keywords:*
Aspirin
Hepatocellular carcinoma
Chemoprevention

Hepatocellular carcinoma (HCC) is reported to be the second most common cause of cancer-related death in the world and arises almost always in the context of chronic inflammation and hepatic fibrosis or cirrhosis [1]. The still growing incidence and limited options for curative treatment of HCC in patients with chronic liver disease warrant the development of HCC chemoprevention. Recently, several epidemiological and experimental studies have indicated that the anti-inflammatory agent aspirin may be associated with a decrease of hepatocarcinogenesis. Sahasrabuddhe *et al* reported a significantly reduced risk of HCC development in patients with regular intake of aspirin [2], while a population-based prospective study by Simon *et al* indicated a dose- and duration-dependency of aspirin-associated decrease in HCC incidence [3]. Complementing these clinical observations, experimental studies revealed antiproliferative effects of aspirin alone, as well as synergistic anti-tumor effects in combination with sorafenib in hepatoma cell lines [4,5]. Moreover, treatment with aspirin reduced HCC tumor growth in HBV transgenic mice [6] and showed preventive effects on hepatic fibrogenesis and carcinogenesis in a mouse model of non-alcoholic fatty liver disease [7].

Several studies indicate these putative cancer-preventive effects of aspirin to depend on its anti-inflammatory properties, for example by targeting NFκB signaling [4,8]. However, the precise molecular mode of action remains largely unclear. Considering the reports on its simultaneous anti-fibrotic and anti-cancer effects in the liver, Wang *et al* hypothesized in their recent article, published in EBioMedicine [9] that aspirin targets P4HA2, a key enzyme in collagen synthesis, that has not only been implicated in liver fibrogenesis but also in HCC development [10]. In confirmation of recent studies [4], *in-vitro* treatment of hepatoma cells with aspirin decreased cell proliferation and colony formation. Similarly, aspirin treatment of mice that were subcutaneously xenografted with HepG2 cells led to reduced collagen deposition and hampered tumor growth, compared to non-treated mice. Interestingly, aspirin treatment decreased P4HA2 expression in HepG2 cells both *in-vivo* and *in-vitro*. Luciferase-reporter assays of the promoter region of P4HA2 further revealed dose-dependent reduction of P4HA2 transcription upon aspirin treatment. By elegant truncation of the applied plasmid Wang et al. identified two NFκB binding fragments within the P4HA2 promoter, potentially responsible for aspirin treatment effects. In line with these results, NFκB activation by TNFα induced and inhibition by PDTC suppressed P4HA2 expression. Moreover, aspirin treated mice showed decreased NFκB/p65 expression. Wang and colleagues further identified a second potential signaling pathway linking aspirin treatment effects with P4HA2. Thus, using computational analyses they predicted let7g as a P4HA2 targeting microRNA (miRNA) and confirmed its regulatory properties on P4HA2 expression by luciferase reporter assay. The decrease of a let7g binding lncRNA, LMCD1-AS1, in aspirin treated mice further connected these P4HA2 regulatory miRNA with aspirin's molecular mode of action. Indeed, aspirin treated mice showed increased let7g and LMCD1-AS1 expression compared to the control group.

Collectively, Wang et al. provide evidence for P4HA2 to be regulated by aspirin in hepatoma cells and to serve as a potential mediator of aspirin's reported tumor-preventive and antifibrotic effects. A potential limitation of this study is the main usage of cell lines rather than primary cells for the mechanistic studies. Considering previous reports of antiproliferative effects of aspirin in hepatoma cell lines, the administration of aspirin in primary HCC spheroids or PDX models, mimicking the original HCC microenvironment would provide additional evidence for aspirin's antiproliferative actions in HCC.

However, the finding of a molecular pathway linking aspirin with a key regulator of fibrogenesis, hereby affecting *in-vivo* collagen-deposition and tumor growth is novel and of potential clinical relevance. Thus, despite the enormous unmet medical need considering advanced fibrosis as the most important risk factor for HCC, yet no antifibrotic therapies are available. Moreover, recent studies linking aspirin's antitumor effects with its function as an antiplatelet drug may limit its clinical application in patients with chronic liver disease that are at high risk of bleeding [7]. The clinical impact of this article therefore comprises the discovery of a specifically targetable molecular signaling pathway, potentially enabling simultaneous anti-tumor and anti-fibrotic effects without platelet inhibition. Future studies should address the consequential hypothesis of anti-P4HA2 therapies preventing liver fibrogenesis and hepatocarcinogenesis.

DOI of original article: https://doi.org/10.1016/j.ebiom.2019.06.048.
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https://doi.org/10.1016/j.ebiom.2019.07.047

## Author contributions

All authors contributed equally in literature research, data analysis and interpretation. NR wrote the manuscript. Both authors approved the final manuscript to be submitted.

## Declaration of Competing Interest

The authors declare no conflict of interest.

## Acknowledgments

This work was supported in part by the US National Institutes of Health (NIH) grant U19-AI123862 and by the National Institute of Allergy & Infectious Diseases (NIAID) and National Cancer Institute (NCI) grants R03AI131066 and 1R21CA209940-01A1 (TFB), by ARC Foundation for Cancer Research (TheraHCC, TheraHCC2.0 IHUARC IHU201301187 and IHUARC2019 IHU201901299 to TFB) and by the European Union (ERC-AdG-2014-671231-HEPCIR and H2020-2015-667273-HEP-CAR to TFB). This work has been published under the framework of the LABEX ANR-10-LABX-0028_HEPSYS (TFB) and benefits from funding from the state managed by the French National Research Agency as part of the Investments for the Future Program.

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