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Could NAMPT inhibition become a potential treatment option in hepatocellular carcinoma?

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Abbreviations:

AMPK	AMP-activated kinase
HCC	hepatocellular carcinoma
mTOR	mammalian target of rapamycin
NAD	nicotinamide adenine dinucleotide
NAMPT	nicotinamide phosphoribosyltransferase
NAPRT	nicotinic acid phosphoribosyltransferase
PARP	poly-ADP-ribosyltransferase
SIRT1	Sirtuin 1

1. Need for novel treatment options in HCC

Hepatocellular carcinoma (HCC) is still one of the leading causes of death caused by cancer worldwide [1]. Despite different curative treatment modalities available, advanced-stage HCC has a poor prognosis and high tumor recurrence rate. Since early stages of HCC are asymptomatic, many patients represent with non-resectable advanced-stage tumors. In every respect, early diagnosis of HCC is the key for a survival benefit because only in early stages treatment options such as resection, liver transplantation or nonsurgical approaches are available and lead to improved outcomes [2].

The pan-tyrosine kinase inhibitor Sorafenib is the only standard drug therapy available for patients with advanced HCC, with modest effectiveness at extending the overall survival of patients for 2-3 months. The mechanism by which Sorafenib acts on advanced HCC is not well understood, and no biomarkers have been identified to predict response to Sorafenib treatment in patients with HCC [3]. Moreover, resistance to Sorafenib and severe side effects further limit its clinical efficacy [3]. One factor which could play a role in the resistance to Sorafenib is Sirtuin 1 (SIRT1). Sirtuins are a family of nicotinamide adenine dinucleotide (NAD)-dependent enzymes that modulate distinct metabolic, energetic and stress response pathways. In a recent study, higher SIRT1 protein levels in HCC tissue were associated with worse outcome, and SIRT1 overexpression supported resistance to Sorafenib [4]. SIRT1 activity depends on intracellular NAD levels. Nicotinamide phosphoribosyltransferase (NAMPT) is the key enzyme in mammalian NAD salvage from nicotinamide and therefore regulates the activity of NAD-dependent enzymes, such as sirtuins or poly-ADP-ribosyltransferases (PARPs) (reviewed in [5], figure 1).

2. Inhibition of NAD biosynthesis in HCC

Cancer cells have a higher demand for NAD because of their rapid proliferation which is associated with the need for increased energy metabolism, ATP generation, nucleotide biosynthesis, DNA repair and increased activity of NAD consuming enzymes [6]. NAMPT has been extensively studied as a target of anti-cancer therapy. NAMPT is overexpressed in several types of tumor tissue and its expression often correlates with cancer therapy resistance and poor outcome in cancer patients [6]. NAMPT inhibitors as single agents have not been applied successfully in clinical studies so far [6]. Possible successful treatment combinations with NAMPT inhibitors include DNA damaging agents, inhibitors targeting enzymes involved in cellular stress responses or DNA repair and rescue with nicotinic acid in nicotinic acid phosphoribosyltransferase (NAPRT)-negative tumors to increase tolerance of non-tumor tissue to NAMPT inhibition [6]. Another promising possibility is the simultaneous inhibition of NAMPT and other enzymes providing precursors for NAD biosynthesis, e.g. CD73 [7].

We found that NAMPT expression is lower in hepatocarcinoma cell lines compared to primary hepatocytes. NAMPT enzymatic activity, however, is higher in HCC with the result of comparable NAD levels in HCC cell lines and primary hepatocytes [8]. Nevertheless, blocking NAMPT enzymatic activity by the specific inhibitor FK866 induced depletion of NAD, ATP and delayed cell death in human hepatocarcinoma cell lines. FK866 treatment induced AMP-activated kinase (AMPK) activation while the activity of mammalian target of rapamycin (mTOR) complex 1 and downstream targets was blocked [9]. We and others showed that the catalytic subunit of AMPK is significantly downregulated while mTOR complex 1 activation is higher in HCC compared to primary hepatocytes or normal liver tissue [9, 10]. Thus, increasing AMPK activity by inducing energy stress through NAMPT inhibition or by Metformin, an activator of AMPK, could help to antagonize the Warburg effect and could become a novel option for the treatment of HCC [11].

The polyphenol resveratrol was found to inhibit carcinogenesis in different types of cancer with a pleiotropic mode of action and has been considered in HCC prevention and treatment [12]. We found

that resveratrol treatment of hepatocarcinoma cell lines led to cell cycle arrest and apoptosis with concomitant reduced NAMPT activity and NAD levels, which resulted in reduced SIRT1 activity [8].

3. Extracellular NAMPT in HCC

In addition to its key role in intracellular NAD biosynthesis, NAMPT (also known as visfatin or PBEF in this context) is also found in human circulation. Extracellular NAMPT was found to act both as enzyme, converting nicotinamide to nicotinamide mononucleotide (NMN) and as cytokine (reviewed in [5]). Increased NAMPT serum concentrations have been associated with a variety of cancers and extracellular NAMPT was found to influence the microenvironment of cancer cells in a way to promote tumor progression and metastasis [13]. In patients with HCC, serum NAMPT levels were significantly correlated with stage progression and tumor enlargement. Extracellular NAMPT preferentially stimulated the proliferation of human HCC cell lines compared with normal hepatocytes via activation of ERK and AKT pathways, and glycogen synthase kinase-3β [14]. Similar results for extracellular NAMPT action have been found in breast cancer [15].

4. Boosting NAD levels in HCC

One of the cellular events preceding HCC development is oncogene-induced DNA damage. Expression of Unconventional Prefoldin RBP5 Interactor (URI) in hepatocytes was shown to reduce the expression of enzymes of de novo NAD biosynthesis. The resulting NAD depletion induced DNA damage by inhibiting the NAD-dependent DNA repair enzyme PARP-1, subsequent genotoxic stress, apoptosis and compensatory proliferation leading to the development of liver tumors in mice [16]. HCC development was blocked by the restoration of hepatocellular NAD pools by supplementing nicotinamide ribose, a precursor of NAD biosynthesis [16]. HCC is also driven by hepatocyte death during NAFLD progression. Studies on NASH patients and animal models revealed that there is a negative correlation of NAMPT expression and NAFLD progression. We could demonstrate that

during early stage NAFLD, NAD salvage via NAMPT is upregulated in mice [17], whereas in later disease stages NAMPT and NAD levels were reported to be downregulated (reviewed in [5]). Low NAD levels may then reduce the activity of PARP-1 thereby impairing genomic integrity as was described by Tummala et al. [16].

5. Conclusion

HCC is an extremely heterogeneous cancer, varying widely in etiology (genotoxic stress, metabolic disorders) and progression in individual patients [2]. Both, NAD depletion (for example via NAMPT inhibition) and NAD replenishment could be beneficial in the prevention and treatment of HCC, especially in case of Sorafenib resistance. Boosting NAD levels could be a preventive treatment, especially since recent studies on long-term supplementation of NAD precursors or intermediates did not show obvious toxicity or deleterious effects and increased NAD levels in mouse liver [18, 19]. Early therapeutic intervention prior to genomic instability may protect risk patients from tumor initiation and genotoxic-induced tumorigenesis.

Future studies focusing on inhibition of NAD biosynthesis in hepatocarcinoma animal models could help to elucidate questions regarding the timing of NAD inhibition (early vs. advanced stage tumors), finding biomarkers to predict response to NAMPT inhibitor treatment and test drug combinations that enhance the efficiency of NAD depletion. Complete inhibition of NAMPT is crucial to prevent tumor recurrence, as incomplete NAMPT inhibition, which impedes NAD+ metabolism but does not kill a tumor cell, can alter its phenotype to be more aggressive and metastatic [20].

Unraveling the role of NAMPT activity in HCC development and the function of NAMPT´s non-enzymatic activity in inflammation, angiogenesis and metastasis will pave the way for future novel NAMPT-based oncotherapy options for HCC.

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Figure legend

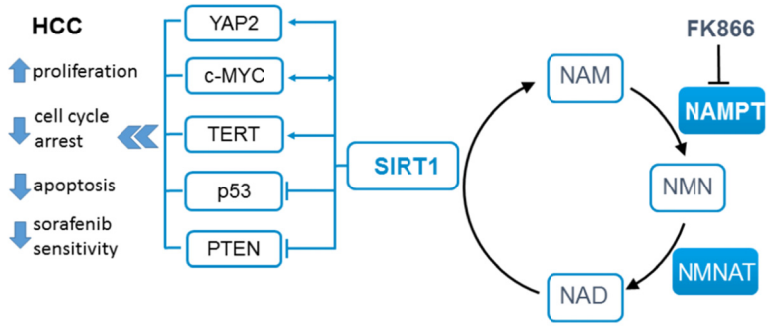

Figure 1: Potential mechanism of NAMPT-NAD-SIRT1 action in HCC

Nicotinamide phosphoribosyltransferase (NAMPT) catalyses the salvage of nicotinamide (NAM), a reaction product of Sirtuin (SIRT) 1, to nicotinamide mononucleotide (NMN). FK866 is a specific pharmacologic inhibitor of NAMPT enzymatic activity, which leads to depletion of intracellular nicotinamide adenine dinucleotide (NAD) levels. Conversion of NMN to NAD is carried out by nicotinamide mononucleotide adenylyltransferase (NMNAT) enzymes. NAD is a substrate for SIRT1. By deacetylating specific lysine residues, SIRT1 regulates the transcriptional activity or protein

stability of several crucial factors in hepatocarcinoma (HCC) tumorigenesis. YAP: Yes-associated protein; c-MYC: V-Myc Avian Myelocytomatosis Viral Oncogene Homolog; TERT: telomerase catalytic subunit; p53: tumor suppressor protein 53; PTEN: phosphatase and tensin homolog; Data from Wu et al. Tumor Biol. (2015) 36:4063–4074.