Alterations of the nuclear transport system in hepatocellular carcinoma – New basis for therapeutic strategies

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Summary

Hepatocellular carcinoma (HCC) is among the most prevalent human malignancies worldwide with rising incidence in industrialised countries, few therapeutic options and poor prognosis. To expand and improve therapeutic strategies, identification of drug targets involved in several liver cancer-related pathways is crucial. Virtually all signal transduction cascades cross the nuclear envelope and therefore require components of the nuclear transport system (NTS), including nuclear transport receptors (e.g. importins and exportins) and the nuclear pore complex. Accordingly, members of the NTS represent promising targets for therapeutic intervention. Selective inhibitors of nuclear export have already entered clinical trials for various malignancies. Herein, we review the current knowledge regarding alterations of the NTS and their potential for targeted therapy in HCC.

Introduction

The predominant type of primary liver cancer is hepatocellular carcinoma (HCC), accounting for 85–90% of cases. Liver cancer is the fifth most frequent malignancy worldwide and represents a major global health problem as the second leading cause of cancer-related death. Furthermore, with an annual increase of 2% in the US, the incidence rate of liver cancer is increasing more rapidly than for any other solid tumour.1,2 The vast majority of HCC arises in the context of liver cirrhosis, caused by chronic liver damage initiating and sustaining chronic inflammation and fibrosis. Chronic liver damage is induced by the main risk factors for HCC, including hepatitis B virus (HBV) and/or hepatitis C Virus (HCV) infections, aflatoxin exposure, alcohol abuse leading to alcoholic steatohepatitis (ASH), and metabolic disorders (obesity, diabetes mellitus) resulting in non-alcoholic steatohepatitis (NASH),2 which is expected to contribute to a further substantial increase in HCC cases in the next decades. While the aetiology of the vast majority of HCCs (over 90%) is well defined, the therapeutic options remain limited, with less than 20% of patients with HCC amenable to curative treatment (partial hepatectomy and transplantation). For advanced HCC, the multikinase inhibitor Sorafenib represents the first approved systemic treatment.3 However, it has only provided mild improvements in patient outcomes, similar to other non-curative therapeutic options, such as transarterial chemoembolization (TACE) and selective internal radiotherapy (SIRT).4 In addition, as most HCC patients suffer from liver cirrhosis, aggressive treatment approaches are often not suitable because of patients’ severely impaired liver function and regenerative capacity. Thus, the prognosis for symptomatic HCC patients is extremely poor, with a five-year-survival of less than 5%. A better understanding of the disease-relevant molecular mechanisms, including chemotherapy resistance is indispensable for identifying novel drug targets and expanding the therapeutic repertoire. In this context, nuclear transport factors are about to emerge as promising candidates for targeted therapy with selective inhibitors already being tested in phase I clinical trials in solid tumours. Herein, members of the nuclear transport machinery (Fig. 1) will be reviewed in terms of their expression profile, (dys-)regulation, (patho-)physiological, prognostic and therapeutic significance in HCC. Altered signalling pathways (e.g. WNT/β-catenin, IL6-JAK/STAT, MAPK, PI3K-AKT/mTOR, TGF-β and p53) play a key role in HCC development and progression.5 These pathways are all intrinsically linked to the nuclear transport system (NTS), as they depend on translocation events across the nuclear envelope, e.g. by import of activated transcription factors or export of target gene products (Fig. 2). Providing

Keywords: Hepatocellular carcinoma; Nuclear transport; Nuclear pore complex.

Received 13 April 2017; received in revised form 20 June 2017; accepted 21 June 2017

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selective nucleo-cytoplasmic exchange of macromolecules, the NTS consists of the nuclear pore complex (NPC), as the trafficking gateway embedded in the nuclear envelope and nuclear transport receptors (NTRs) that shuttle cargos through the NPC. The NPC is composed of highly conserved, distinct structural modules: the inner pore ring, the cytoplasmic and nuclear rings as well as the cytoplasmic filaments and the nuclear basket (Fig. 1A, left panel).6 These subcomplexes are in turn built by subsets of the overall ~30 Nucleoporins (NUPs) with the corresponding individual structures being largely resolved 7–9 (Fig. 1A, right panel). About one third of all NUPs contain phenylalanine-glycine (FG)-rich repeat domains, extending into the central channel, that serve to build a meshwork implicated in transport selectivity.6,10 Nuclear transport receptors (NTRs), predominantly of the highly conserved karyopherin-β superfamily (e.g. importins and exportins), mediate translocation of cargos through the NPC by interacting with these FG-repeat domains.11 While proteins with a molecular weight below ~40 kDa can passively diffuse through the NPC, the transport of larger molecules depends on active transport by NTRs. Notably, several hundred to ~1000 transport events per NPC per second have been estimated.12 Members of the karyopherin-β family of NTRs are encoded by 20 genes comprising ten proteins serving in nuclear import, seven proteins that function in nuclear export, two proteins involved in bidirectional transport (although this very strict subdivision does not account for all of them) (Fig. 1B) and one protein (Ran-BP5) that remains to be characterised.13,14 Proteins of the karyopherin-α/importin-α family are considered as adaptors, encompassing seven members in humans (Fig. 1B), which connect importin-β to its cargos.

The best characterised classical protein import pathway involves the recognition of a nuclear localization signal (NLS) within a cargo protein sequence by an importin-α isoform and subsequent association with importin-β to form a trimeric cargo-NLS/importin-α/importin-β complex15,16 (Fig. 1C). Alternatively, importin-β can also bind directly to NLS cargo proteins independently of an importin-α adaptor protein (Fig. 1C). In either scenario the importin/cargo complex passes the NPC by interacting with FG-containing NUPs, before dissociating upon binding of Ras-related nuclear protein (Ran)GTP and releasing its transport substrate into the nucleoplasm.15 Importin-α isoforms are then re-shuttled to the cytoplasm by their specific exporter, exportin-2 (XPO2, cellular apoptosis susceptibility [CAS]) in association with Ran-GTP17,18 (Fig. 1C). Notably, some HCC relevant proteins (e.g. β-catenin and SMADs) do not require NTRs for their nuclear import but can interact directly with a subset of NUPs (=NTR-independent protein import (Fig. 2), discussed later).19

Cargo proteins harbouring a nuclear export signal (NES) bind to exportins and Ran-GTP to form an export complex in the nucleoplasm. The disassembly of the exported NTR/NES cargo complex is induced by the Ran-GTPase-activating protein (Ran-GAP) and additional proteins at the cytoplasmic face of the NPC15,20 (Fig. 1C). Probably the best characterised exportin is exportin-1 (XPO1, chromosome region maintenance 1 [CRM1]), which is thought to expel a large set of proteins from the nucleus.21 Beyond proteins, exportin-1 is also involved in the export of subsets of mRNAs (e.g. c-FOS) containing AU-rich elements,22 snRNAs, and tRNAs,23 while tRNAs are transported by exportin-t23 and premiRNAs by exportin-5,24 all of which depend on the Ran-GTP/Ran-GDP gradient. In contrast, nuclear export of bulk mRNA is provided by the non-karyopherin transport proteins NXF1/TAP–NXF1 (p15) in a Ran-GTP-independent manner.23,25

Members of the NTS, particularly NUPs, participate in a variety of essential cellular processes, such as cell differentiation, gene regulation, chromatin organization/modification, DNA repair, mitosis, and reprogramming.26–32 Accordingly, nuclear transport factors have been linked to a wide range of human disorders with astonishingly specific phenotypes, including autoimmune diseases (e.g. NUP210 and NUP62 as auto-antigens in primary biliary cirrhosis33), cardiovascular disorders (NUP155 mutation leading to atrial fibrillation34), triple-A [Achalasia-Addison-Alacrimia] syndrome by mutation in the nucleoporin Aladin,35 and a specific type of nephrotic syndrome associated with mutations in NUP93, NUP205, and exportin-5.36 Notably, these functions and phenotypes might come about through transport-dependent and –independent functions of NUPs and NTRs. The link between NUPs and cancer has been primarily studied in the context of NUP-containing fusion proteins (e.g. NUP98/HOXA9 or DEK-NUP214), resulting from chromosomal translocation typically occurring in haematological malignancies.36,37 However, neither NUP-containing fusion proteins nor recurrent point mutations in NUPs or karyopherin genes have been defined in HCC so far.

Dysregulation, function, and prognostic significance of nuclear transport factors in HCC

The karyopherins exportin-1, exportin-2, importin-β1 (KPNAB2), and importin-α5 (KPNAA1) were all overexpressed in HCC compared to non-tumorous liver tissue.38 Interestingly, in a transcriptomic HCC sub-classification study by Boyault et al.39 the karyopherins importin-β1 (KPNB1), exportin-1, importin-7, as well as the nucleoporins NUP155 and NUP107 were identified as highly expressed in a specific HCC subgroup (G3). This subgroup was characterised by TP53 mutations, lack of correlation to hepatitis B virus (HBV) infection, and overexpression of cell cycle regulating genes,39 already indicating a link between the nuclear transport machinery,
Fig. 1. The nuclear transport system. (A left panel) The nuclear pore complex (NPC) is embedded in the nuclear envelope and includes the indicated subcomplexes: cytoplasmic filaments, cytoplasmic ring, nuclear ring, inner pore ring, and nuclear basket. (A right panel) The subcomplexes are composed of subsets of nucleoporins (NUPs) indicated as colour-coded boxes: Y-complexes in dark yellow and dark purple; the inner ring complex in light blue; the transmembrane NUPs in orange; the NUP62 complex in green; cytoplasmic complexes in light yellow; and nuclear basket complexes are in light purple. (B) List of nuclear transport receptors (NTRs) sorted based on their transport directionality (import = blue, export = red, bi-directional = bold). (C) NTR-dependent import pathway either with a heterotrimERIC complex containing Importin-β, Importin-α and a cargo protein with a nuclear localization signal (NLS) or a heterodimeric complex containing Importin-β and a cargo protein with a NLS. Upon binding to Ran-GTP within the nucleus each of the complexes dissociate. Export pathway with a trimeric complex containing Ran-GTP, exportin-1 (XPO1/CRM1) and a cargo protein with a nuclear export signal. Upon conversion of Ran-GTP to Ran-GDP the complex dissociates in the cytoplasm. Also shown re-shuttling of Importin-α by exportin-2 (XPO2/CAS) together with Ran-GDP and of Importin-β by Ran-GTP alone from the nucleus to the cytoplasm. IPO, Importin; KPNA, Karyopherin alpha; KPNB, Karyopherin beta; NES, nuclear export signal; TNPO, Transportin.

the p53 pathway, and cell cycle regulation (discussed later). Downregulation of specific nuclear transport proteins, such as exportin-4 (XPO4)\textsuperscript{40} and NUP98,\textsuperscript{41} is also documented in liver cancer, consistent with a potential or proven tumour suppressive role of these factors in HCC.\textsuperscript{41,42} Moreover, upregulation (e.g. exportin-1 and exportin-2) and downregulation (e.g. exportin-4) correlate with tumour de-differentiation,\textsuperscript{38,43,44} proliferation\textsuperscript{38,44} and poor prognosis\textsuperscript{40,44–46} in HCC, indicating a prognostic value of deregulated transport factors. Notably, dysregulation of transport factors can already be observed in preneoplastic lesions (dysplastic nodules) as demonstrated for exportin-2\textsuperscript{38} or NUP88,\textsuperscript{47} suggesting that these are early events in hepatocarcinogenesis and therefore potential options for early intervention.

Functional characterisation of overexpressed NTRs in liver cancer suggests an anti-apoptotic role of the exportin-2/importin-α1 transport cycle, which is partially linked to the anti-apoptotic protein X-linked inhibitor of apoptosis (XIAP).\textsuperscript{38} However, the pro-survival role of exportin-2 seems to be context-dependent, since it can also be involved in apoptosis induction under certain conditions (e.g. toxin-treatment).\textsuperscript{48–50} Exportin-2 was also
linked to tumour cell migration and invasion in HCC, based on its importance for maintaining integrin-β1 expression as identified by a proteomics approach. Another aspect of the pro-migratory function of exportin-2 is connected to its role in microtubule interaction, as shown in breast cancer cells. Although not yet confirmed in HCC, importin-α1 promotes cell proliferation and tumourigenicity through upregulation of c-MYC and downregulation of forhead box O3a (FOXO3a).

In contrast to many studies that have focused on identifying and characterising upregulated karyopherins in cancer, relatively little is known about the mechanisms leading to their overexpression. For exportin-2 and importin-α1 it was shown that both karyopherins are transcriptionally repressed by the tumour suppressor protein p53 in HCC, in a p21-dependent manner. Thus, a relief of repression upon p53 inactivation either functionally (e.g. via MDM4 overexpression or interaction with the HBx protein), or genetically (by mutations in the TP53 gene) leads to coordinated exportin-2 and importin-α1 overexpression, presumably not only in HCC. A similar scenario could be relevant for exportin-1 upregulation, since exportin-1 was also demonstrated to be a p53 repression target in lung fibroblasts and in cervical cancer cell lines. The former study also identified importin-7 as a p53 repression target, while c-MYC was found to be an inducer of importin-7, importin-5, importin-4, importin-11, and transportin-1 (importin-β2).

Gene amplification may also contribute to high levels of karyopherins such as exportin-2, since the corresponding gene XP02 resides in the 20q13.3 region which is frequently amplified in HCC. For importin-β1 and importin-α1, E2F-driven increased transcription upon RB inactivation was reported. Although this phenomenon was identified in cervical carcinoma cells it may also contribute to dysregulation of both transport factors in HCC, as RB1 is recurrently deleted in HCCs. Other transcription factors postulated to regulate karyopherin-beta genes are SP1, nuclear factor (erythroid-derived 2)-like 2 (Nrf2/NFE2L2), nescent helix-loop-helix 1 (Hen-1/NHLH1), ras responsive element binding protein 1 (RREB-1), and nuclear transcription factor-Y (NF-Y). Of which SP1 and Nrf2 have been implicated in hepatocarcinogenesis. Beyond transcriptional activation, dysregulation of karyopherins can also occur post-transcriptionally, which is exemplified by importin-α1 mRNA being a target of miRNA-26b. Although this post-transcriptional regulatory mechanism was observed in ovarian cancer, miRNA-26b is also downregulated in HCC and therefore could be relevant for importin-α1 overexpression in liver cancer as well.

HCC relevant signalling cascades utilize NTR-dependent and –independent transport pathways

In the following passage, we will discuss how HCC relevant signalling cascades using NTR-dependent and –independent transport pathways are associated with particular members of the nuclear transport system (Fig. 2 and Table 1). If not yet defined in HCC we also include data derived from non-hepatic cell lines. The emphasis will be on the nuclear import and export mechanisms of key transcription factors in a selection of signalling cascades. Beyond these cascades, Table 1 also provides information about additional signalling pathways that are not discussed herein.

**p53 tumour suppressor pathway (NTR-dependent)**

The tumour suppressor protein p53 is a major barrier against liver tumour development and progression. It is mutated in 18–35% of HCCs. Upon different kinds of stress (e.g. DNA damage, oncogene activation, etc.) the transcription factor p53 is stabilized by escaping (MDM2-mediated degradation and translocating to the nucleus. There p53 trans-activates subsets of its multiple target genes, leading to a variety of cellular outcomes (e.g. cell cycle arrest, senescence, apoptosis, metabolic changes, etc.). Therefore, nuclear import is obviously pivotal to p53’s function. In unstressed cells, ubiquitylation of lysines 319–321 of NLS I protects p53 from binding to importin-α3, thereby preventing its nuclear import and exposing p53 to proteosomal degradation. During a stress response, post-translational modifications of MDM2 and p53 result in reduced ubiquitylation, which allows NLS I and importin-α3 to interact, enabling nuclear import of p53. This adds a new aspect to the prevailing model, suggesting that stress-induced tetramerization of nuclear p53 results in masking p53’s NES and thereby causes an export block, by preventing the interaction with exportin-1 and retaining p53 in the nucleus. While active import via importin-α3 occurs at early stages, the export block appears to be relevant at later stages of the p53 mediated stress response. Also, the subcellular localisation of regulators of the p53-MDM2 loop requires consideration, as shown for the polycomb group (PcG) member RING1 and YY1-binding protein (RYBP), which is downregulated in HCC and correlated with patient outcome and responsiveness to chemotherapy. By identifying and mutating three functional monopartite NLSs in RYBP, Tan et al. could demonstrate that the cytoplasmically retained RYBpmut is more potent in inhibiting MDM2-mediated polyubiquitination and degradation of p53 than predominantly nuclear-localized wild-type RYBP. Consequently, RYBpmut reduced cell proliferation and induced apoptosis more efficiently than the corresponding wild-type protein, even though not entirely in a

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**Key point**

Dysregulation of nuclear transport factors is of functional and prognostic significance and has been linked with up-regulation of tumour promoting pathways and downregulation of tumour suppressive pathways.
p53-dependent manner. Consistent with its increased tumour-suppressive function the authors suggest utilising RYBPmut in a therapeutic approach.

Another level at which members of the nuclear transport machinery are linked to the p53 pathway is in p53 target gene selection. In a transport-independent function exportin-2 can associate with chromatin at specific p53 target gene promoter regions (e.g. TP53-AIP), which is required for the full induction of these genes. Furthermore, based on a targeted RNAi screen NUP98 was shown to regulate the induction of p21 (CDKN1A), a cell cycle regulator and key effector of the p53 response on a post-transcriptional level.

Mechanistically, NUP98 associates with the 3′UTR of p21 mRNA to protect it from exosomal degradation. In accordance with a potential tumour suppressive function, NUP98 expression was lower in ~25% of HCCs compared to non-tumorous liver tissue. However, since NUP98 and p21 mRNA were correlated to each other, a subset of HCCs also showed higher expression of both factors, suggesting a dual role of NUP98 in liver cancer possibly through p21. The complexity of p21 in cancer biology, ranging from tumour suppressive to oncogenic functions, involves many determinants including its subcellular localisation.

With few exceptions nuclear p21 is widely considered to exhibit tumour suppressive functions, for instance, by mediating cell cycle arrest or senescence, particularly upon p53 induction. In contrast, cytoplasmic p21 is correlated with poor prognosis in several malignancies and considered oncogenic because of its anti-apoptotic function via inhibition of procaspase 3, caspase 8, and caspase 10, among other mechanisms. Since the nuclear export of p21 is exportin-1-dependent and overexpression of exportin-1 observed in HCC favours the cytoplasmic accumulation and thereby pro-tumourigenic function of p21.

WNT/β-catenin (NTR-independent)

Aberrant activation of the WNT signalling cascade is one of the main drivers of hepatocarcinogenesis, most frequently due to a missense mutation in exon 3 of the CTNNB1 gene (10–33%). A critical step in this pathway is the nuclear accumulation of β-catenin and its association with lymphoid enhancer factor (LEF)/T cell factor (TCF), converting the latter into a transcriptional activator and driving expression of pro-tumourigenic genes, such as MYC or CCND1. The nuclear translocation of β-catenin represents one of the most studied import pathways, which is also independent of the Ran-GAP cycle. Not containing a typical NLS, β-catenin interacts directly with NPC components, specifically the FG-repeats of NUP62, NUP98, NUP153, and NUP358. As suggested by deletion studies, the C-terminus and the Armadillo repeats 10–12 represent the regions of β-catenin required for its nuclear import. However, it still remains a matter of debate if there is another import pathway for β-catenin that is NTR- and Ran-dependent and involves a yet to be defined cytoplasmic binding partner. Nuclear export of β-catenin (devoid of a classical NES) relies on largely the same regions that are required for its import and involves direct interaction with Ran-BP3. Although Ran-BP3 is a co-factor of exportin-1, the export of β-catenin is exportin-1-independent. These findings further support the prevailing model that transport of β-catenin in either direction does not rely on karyopherins.

NF-κB pathway (NTR-dependent)

HCC represents a prototype of inflammation-associated cancer. The nuclear factor kappa B (NF-κB) pathway, with its fundamental role in inflammation and immune response, has been strongly linked to liver tumourigenesis. However, NF-κB plays a dual role in HCC with context-dependent pro- and anti-tumourigenic effects. Accordingly, NF-κB proteins represent a key group of transcription factors that control the expression of genes involved in various important cellular processes, including apoptosis, cell survival, stress and immune response, differen-
NF-κB operates as a dimer composed of various combinations of the following five subunits: p65 (RELA), c-REL, and RELB, as well as p50 and p52 (lacking transcriptional activation domains). The predominant heterodimer in most cell types is p50/p65. Inhibitors of NF-κB (IκBs), such as IκBα and IκBβ, retain NF-κB in the cytoplasm by masking the NLS within the respective NF-κB subunits. A classical NLS is found in p50 and p65. Upon activation, an IκB kinase complex phosphorylates IκB, resulting in ubiquitination and proteosomal degradation of IκB. Consequently, the NLSs of p50 and p65 are unmasked, and the dimers are rapidly translocated into the nucleus, where they initiate transcription by binding regulatory DNA sequences of responsive genes. Importin-α3 and importin-α4 are the main importin-α isoforms involved in the nuclear import of NF-κB p50/p65 heterodimers upon TNF-α-stimulation. Interestingly, p52 protein directly interacts with importin-α5, -α4, -α5 and -α6, and c-REL binds to importin-α5, -α6 and -α7, while RELB interacts with importin-α5 and -α6. Moreover, the specificity of a given heterodimer is dictated by only one of the respective subunits, as the import of the p52/RELB heterodimers is mediated exclusively by the NLS of RELB, while p52 mediates the nuclear import of p52/p65 heterodimers. Beyond NTRs, NF-κB signalling has been linked to NUP88 based on the observation that low levels of NUP88 increase NF-κB nuclear export. In turn, NUP88 overexpression that is frequently found in HCC, and other tumour entities, contributes to NF-κB nuclear accumulation resulting from impaired nuclear export. A recent report suggested another role of NUP88 in malignant transformation, not directly related to NF-κB. The authors identified NUP88 in association with NUP98 and ribonucleic acid export 1 (RAE1) as an inhibitor of the pre-mitotic activity of the anaphase-promoting complex (APC/C). Consequently, overexpression and cytoplasmic accumulation of NUP88 sequestered NUP98–RAE1, preventing the interaction with APC/C and thereby impairing mitotic checkpoint control, inducing

**Table 1. The link between (liver-) cancer relevant pathways/proteins and members of the nuclear transport system are listed.**

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*Own unpublished data (Mackmull et al.).
abnormal chromosome segregation, aneuploidy, and chromosomal instability. 

**Ras/MAPK and PI3K/Akt signalling**

Several HCC relevant receptor tyrosine kinases (RTK), such as the insulin-like growth factor receptor 1 (IGF-R1), signal through the Ras/Raf/MEK/ERK pathway and/or the phosphoinositide 3-kinase (PI3K)/Akt pathway, which induce a variety of pro-tumourigenic downstream effects. The former typically drives proliferation and cell survival, whilst the latter increases protein synthesis and glucose metabolism. The nuclear translocation of extracellular signal-regulated kinase (ERK1/2) is critical for the Ras/Raf/MEK/ERK kinase cascade. During unstimulated conditions, ERK kinase 1/2 (MEK1/2) anchors ERK1/2 in the cytoplasm. Upon stimulation, MEK1/2 phosphorylates ERK1/2 leading to a conformational change of ERK1/2, subsequent separation from MEK1/2, and interaction with importin-7. Importin-7 recognizes a nuclear transport sequence containing a phosphorylated Ser-Pro-Ser (SPS) motif in the kinase insert domain of ERK1/2, resulting in nuclear import of ERK1/2 in an importin-α independent manner. However, NTR-independent nuclear import, at least for ERK2, has also been reported. The nuclear export of ERK2 was shown to be partially dependent on exportin-1.

Increasing protein synthesis by the PI3K/Akt cascade involves activation of the mammalian target of rapamycin complex 1 (mTORC1) through inhibition of TSC1 and TSC2 by phosphorylated AKT. Activated mTORC1 in turn phosphorylates the eukaryotic translation initiation factor 4E (eIF4E) binding proteins (4E-BPs), releasing them from the potent oncogene eIF4E, which induces mRNA translation. eIF4E rearranges components of the cytoplasmic side of the NPC, leading to increased export of eIF4E target mRNAs (e.g., CyclinD1, c-MYC, MDM2). In detail, eIF4E reduces NUP358/Ran-BP2, relocates NUP214, and increases Ran-BP1, as well as the RNA export factors GLE1 and DEAD-box helicase 19 (DDX19). Conversely, NUP358/Ran-BP2 overexpression inhibits eIF4E target mRNA export and impedes malignant transformation by eIF4E.

**Targeting the nuclear transport system as a therapeutic approach**

The expression profile, functional effects, and early dysregulation of a subset of NTRs in HCC make them attractive therapeutic targets. Firstly, we discuss exportin-1, which is overexpressed not only in HCC but also in other solid tumours (e.g., ovarian and cervical cancer) and haematological neoplasms, such as multiple myeloma, chronic myelogenous leukaemia, acute myeloid leukaemia (AML), chronic lymphocytic leukaemia, and non-Hodgkin’s lymphoma. In fact, haematological malignancies are currently the primary group of neoplasms where anti-exportin-1 compounds are being tested in a pre-clinical and clinical setting (discussed later). The current concept of targeting exportin-1, is based on its involvement in nuclear export of tumour suppressor and growth inhibiting proteins, such as p53, p21, p27, FOXO, and IκB. Thus, overexpression of exportin-1 in tumours leads to increased export of these factors and impairment of their tumour suppressing and growth inhibiting functions. This in turn can be prevented by disrupting the interaction between exportin-1 and its transport substrates in a therapeutic approach. Unfortunately, the earliest generation of exportin-1 inhibitors, such as leptomycin B (LMB) showed only minimal efficacy and severe toxicity, most likely due to off-target effects. While disappointing from a clinical perspective, leptomycin B became a highly valuable tool for nuclear transport research.

Recently, a promising new class of slowly reversible small molecule covalent inhibitors of exportin-1, so called selective inhibitors of nuclear export (SINE), were designed. SINE inhibit the interaction between exportin-1 and its transport substrates by covalently binding to the Cys528 residue located in the NES-binding pocket. Selinexor, the most prominent SINE compound, is orally bioavailable, shows proportional pharmacokinetics with no evidence of drug accumulation, and broad anti-tumour activity. Extensive pre-clinical testing revealed that Selinexor, while not significantly affecting normal haematopoietic cells, causes increased cell death in T cell acute lymphoblastic leukaemia and AML cells lines, primary cells, and murine xenograft models. On-target effects were detectable in AML xenograft models as indicated by reductions in exportin-1 protein and nuclear accumulation of p53 and nucleophosmin 1 (NPM1). Furthermore, in recent phase I studies it was shown that Selinexor can be administered safely in combination with fludarabine and cytarabine in paediatric relapsed or refractory acute leukaemia, or as a single therapeutic in patients with solid tumours in advanced stages. The efficacy of Selinexor in the latter study was monitored by analyses of tumour biopsies, evaluating cleaved caspase3, p53, Apoptag, Ki67, and FOXO3A. The response rates in these studies were promising enough to be further explored in phase II trials. Another recent study demonstrated that Selinexor shows synthetic lethality with oncogenic KRAS in non-small cell lung cancer (NSCLC). The authors pinpointed the accumulation of nuclear k-B and associated inhibition of the transcriptional activity of NF-kB as the primary mechanism of Selinexor sensitivity. Moreover, yes-associated protein (YAP1) activation via mutant follistatin like 5 (FSTL5) was demonstrated to render tumours resistant to Selinexor, which could be circumvented by co-
administration of a YAP1-TEA domain transcription factor (TEAD) inhibitor.\textsuperscript{122} Since YAP1 plays an important role in liver tumourigenesis,\textsuperscript{123} YAP1-mediated resistance to Selinexor needs to be considered in HCC trials. Initial \textit{in vitro} and \textit{in vivo} findings in HCC cell lines and xenograft models using Selinexor are encouraging.\textsuperscript{105} Selinexor treatment in HCC cell lines leads to cell cycle arrest, induction of apoptosis, increase of p53, p27, and p53 upregulated modulator of apoptosis (PUMA) expression while inhibiting the expression of c-MYC and c-MET. Finally, oral application of Selinexor reduced tumour growth in HCC xenograft models with only mild toxicity.\textsuperscript{105}

Based on the aforementioned studies, it appears that inhibiting exportin-1-dependent nuclear export has only a minor effect on non-tumourous tissue. This in turn suggests that the dependency of cancer cells on this nuclear export pathway is much stronger than in non-transformed cells, to some extent reminiscent of the phenomenon called “oncogene addiction”.\textsuperscript{124} Furthermore, the “addiction” of liver cancer cells may also be transferred to other members of the NTS, such as exportin-2, since its depletion strikingly reduces the viability of HCC cell lines,\textsuperscript{38} while not significantly affecting non-tumourous liver cells.\textsuperscript{46} Thus, despite the fundamental and general function of the nuclear transport machinery, cancer-specific effects can be achieved by targeting dysregulated NTS members.

Final remarks and outlook

There is accumulating evidence indicating that dysregulation of nuclear transport factors has both functional and prognostic significance in hepatocarcinogenesis. Furthermore, referring to properties of ideal drug targets,\textsuperscript{125} nuclear transport factors such as exportin-1 seem to fulfill several of the following criteria: “the target is disease modifying, modulation of the target is less important under physiological conditions, it can be assayed using high throughput screening, target/disease-specific biomarkers exist to monitor therapeutic efficacy, and prediction of potential side effects according to phenotype data is favourable”. As there is great potential in targeting the nuclear transport system in liver carcinogenesis, well-designed clinical trials with genomic and immunohistochemical tumour preselection are required. For instance, exportin-1 inhibitors are expected to be most effective in tumours showing high exportin-1 expression and low levels of nuclear (wild-type) p53, p21, p27, FOXO, and 1-κB, which could be analyzed by immunohistochemistry (IHC) in pretreatment biopsies. In addition, potential converse effects of SINE compounds could be anticipated, since some protumourigenic proteins (e.g., STATs) are exported by exportin-1 as well, and may therefore further accumulate in the nucleus. In this context, it is important to note that NTR-cargo specificities dictated by the interactome of relevant karyopherins have not yet been comprehensively analysed in liver cancer cells. These analyses will not only reveal cargo specificities, but also redundancies, with the latter being most relevant for potential resistance mechanisms employed by cancer cells to escape anti-NTR treatment strategies. Initial studies in this direction are under way and will complement future functional and mechanistic approaches to define the settings in which SINE and other upcoming anti-transport compounds are most effective in HCC.

Financial support

S.S. acknowledges funding by the German Research Foundation (DFG grant Si-1487/3-1 and SFB/TR209 B04), Hella-Buehler-Foundation, and the Heidelberg Research Center for Molecular Medicine (HRCMM).

Conflict of interest

The authors of this manuscript have no conflicts of interest to declare.

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

Authors’ contributions

All authors contributed to the conception and writing of the manuscript.

Acknowledgements

We thank Marie-Therese Mackmull and Kerstin Holzer for their support. We apologize to those authors whose work we were unable to cite due to space limitations.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at \url{http://dx.doi.org/10.1016/j.jhep.2017.06.021}.

References

Review


