

Translation reprogramming as a novel therapeutic target in MAFLD

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Abstract

Approved pharmacotherapies for metabolic-dysfunction-associated fatty liver disease (MAFLD) are lacking. Novel approaches and therapeutic targets that are likely to translate to clinical benefit are required. Therapeutic agents that target components of the translation machinery hold promise as novel drugs that can overcome the well-known disease heterogeneity, as dysregulation of mRNA translation is a frequent feature independent of the MAFLD drivers. In this Review, we discuss the role of mRNA translation in MAFLD, with a particular focus on the implications and challenges to ‘translate’ these results to the clinic, and provide an overview of similar recent efforts in other diseases.

Introduction

Metabolic-dysfunction-associated fatty liver disease (MAFLD) is the most common chronic liver disease, affecting up to quarter of the global population, with well-described adverse clinical outcomes (1-3). MAFLD is a leading and the fastest-growing global cause of end-stage liver disease, cancer and liver transplantation, with an estimated 20 million people likely to eventually die from liver disease (4). While HCC classically develops in the context of cirrhosis, it is increasingly described in non-cirrhotic MAFLD patients. MAFLD is also associated with adverse extrahepatic outcomes such as cardiovascular disease, diabetes, chronic kidney disease, extra-hepatic cancers, osteoarthritis, obstructive sleep apnoea, gallbladder disease and psoriasis(5). MAFLD is associated with significant impairment of patient-reported outcomes and impairs quality of life including increased fatigue and decreased mental well-being compared to the general population as well as other causes of chronic liver disease such as chronic viral Hepatitis B and C(6). Finally, MAFLD represents a significant economic burden to society.

As with other complex traits, the pathogenesis and clinical manifestations of MAFLD is the outcome of gene-environment interactions (7, 8), that is impacted by ethnicity, gender, biological and chronological age (9). Lifestyle modification remains the cornerstone management for patients with MAFLD, which can ameliorate the risk of hepatic and non-hepatic related complications, including attenuating the accompanying metabolic dysfunction comorbidities and cardiovascular risk.

To date, the results of the drug discovery pipeline in MAFLD have been below expectations, with multiple trials unsuccessful, although their mode of actions were strongly supported by preclinical and early clinical evaluations (10). This failure is likely explained at least partially by the underlying heterogeneity of MAFLD and their main disease drivers, making a one-size fits all approach unlikely. The details of the pipeline of the investigational

candidate drugs for MAFLD and potential reasons of the suboptimal performance of these drugs in clinical trials have been subject of multiple recent reviews (5, 11-13). Transformational changes and novel targets and concepts are needed to address MAFLD-related morbidity and mortality (12, 14).

Dysregulation of mRNA translation is a ubiquitous feature of MAFLD. Novel insights into how mRNA translation is implicated in MAFLD could substantially accelerate the rate of discovery and clinical development. In this review, we discuss why mRNA translation may play a critical role in the pathophysiology of MAFLD and the possibility to be exploited as a therapeutic target for MAFLD. We structured this review into the following sections: (i) the principals of mRNA translation, (ii) key determinants of mRNA translation in health and disease, (iii) alterations in mRNA translation in MAFLD– current evidence and (iv) pharmacological interventions of mRNA translation.

mRNA translation

Protein levels are known to be regulated at transcriptional, translational, and post-translational levels. Messenger RNA (mRNA) translation involves an orchestrated interaction of transfer ribonucleic acid (tRNA), ribosomes, auxiliary factors and mRNA. According to a growing body of evidence, there is a fundamental role for regulating the translation in controlling gene expression, as multiple studies have demonstrated that protein levels display low concordance with the cellular proteome at steady-state mRNA (15-17).

The process of translation occurs in three stages: initiation, elongation, and termination, which is then followed by ribosome recycling. Translation initiation refers to as the major rate-limiting step of protein synthesis and a frequent target of regulation (18). Translation initiation is a multistep process that involves coalescing the mRNA, the initiator tRNA, and the ribosome. It starts with the assembly of the 43S preinitiation complex, consisting of the 40S subunit, plus

methionyl initiator tRNA and various eukaryotic initiation factors (eIFs)(19). Although translation initiation is considered the most vital regulatory step, there is increasing evidence of the role of other steps as well, in particular elongation (20). Moreover, translation regulation may occur in a more complex and integrated fashion in vivo, thereby impacting several steps simultaneously (20).

The relevance for appropriate translational regulation for human health is evidenced by the fact that mistranslation has been linked to a wide range of human diseases, including cancer, immunodeficiency, age-related degenerative conditions, and metabolic diseases, including MAFLD and neurological disorders(21). With the advancement in genome-wide translome profiling studies, realising the role of alteration in translation and aberrant protein synthesis in human diseases has become even more evident (21, 22). For example, a recent study using mRNAs bound to ribosome-nascent chain complex sequencing of high-fat-diet-induced mouse fatty liver demonstrated a pivotal role for the mRNA translation in regulating hepatic steatosis(23).

Broadly, the translation-related human disorders can be categorized into four main categories: those involving ribosomopathies, deregulated tRNA synthesis or function, deregulation of the integrated stress response (ISR) pathway and deregulation of the mammalian target of rapamycin (mTOR) pathway(21). The main objective of this classification is to simplify the description of the underlying mechanisms of translation-related human diseases. However, considerable overlaps seem to exist between these four categories. For instance, deregulation of tRNA can trigger the activation of the ISR and inhibition of the mTOR pathway(24). On the other hand, targets of the latter pathways have pivotal roles in tRNA and ribosomal biogenesis(25).

Determinants of translation kinetics

As translation requires the synchronization of different players, from mRNA and tRNA to ribosomal proteins and proteostasis machinery, numerous variables influence the rate of translation and its downstream effects. These comprise codon context and usage, tRNA abundance, mRNA secondary structure and protein sequence (21).

Codon usage

The most commonly studied factor in controlling the rate of translation is the frequency of codons in the mRNA transcriptome, referred to as codon usage or bias. The genetic code is considered degenerate or redundant, as the same amino acid is being encoded by multiple codons(26). Synonymous codons are often used at unequal frequencies that can vary over an order of magnitude between common and rare codons and delineate an organism's codon usage(26).

Synonymous codons were long viewed as “silent” mutations as they do not induce any change in amino acids. However, growing evidence is revealing that the use of “silent” is a misnomer as synonymous mutations are associated with human diseases, by influencing translational processes and even fidelity, and subsequently can modulate protein folding and activity. Synonymous variant in the fibroblast growth factor 21 (FGF21) can for instance drive the development of hepatic inflammation in a patient with MAFLD(27).

tRNA abundance

tRNAs represent 10–15% of the total RNA in a cell with >500 human tRNA genes known and are vital molecules for translation, acting as nexus molecules between mRNAs and proteins and representing an additional layer of regulation of translation and protein synthesis. The composition of the tRNA pool is dynamic and can vary substantially in response to environmental cues or disease. Emerging evidence suggests that tRNAs have a more

fundamental role in a stress response process by functioning directly as signalling molecules in adaptive translation(28).

tRNA abundance is considered the main factor contributing to the decoding rates of specific codons and both determine codon optimality and thus translation efficiency and protein synthesis. Codon optimality is an estimation of differential codon recognition and usage(29). Generally, optimal codons represent common codons that are efficiently decoded by abundant tRNA. While nonoptimal codons consist of rare codons or a lower abundant tRNA and decoded more slowly compared to the optimal codons(30). Therefore, codon optimality accounts for the correlated differences in codon usage and the availability of the decoding tRNA.

Ribosomal proteins

Ribosomal proteins represent another layer that plays a pivotal role in protein translation and ribosome assembly. The human ribosomal P complex, which consists of the acidic ribosomal P proteins RPLP0, RPLP1, and RPLP2 (RPLP proteins), recruits translational factors, facilitating protein synthesis (31). In particular, RPLP0, formerly known as P0, is a specific multifunctional protein and has been recently identified as a novel cellular stress-responsive element. It acts as a regulatory element responding to environmental fluctuations and can propagate a signal from ribosomes perturbed by amino acid starvation to general control nonderepressible 2 (GCN2) leading to initiation of the integrated stress response (ISR), linking ISR to translational stress(32, 33). RPLP0 can be released from the ribosome upon nucleolar stress (34). High expression levels of RPLP0 have been detected in human tumours, including hepatocellular carcinoma(35). RPLP0 was demonstrated to be required for efficient protein translation of selective proteins that are mainly involved in metabolic processes (33). In human cells, disrupting the P protein complex does not lead to a change in ribosomal function, overall protein synthesis, or mRNA translation(33). A central role for RPLP0 in

mediating FGF21 resistance in human MAFLD via mistranslation of this protein was recently identified (27).

Translation kinetics regulates co-translational protein folding

The optimal translation kinetics require the balance of the trade-off of the non-uniform speed of translation and protein synthesis and the ribosome pausing that can be required for appropriate co-translational protein folding and translational fidelity, ensuring the protein quality (**Figure 1**). A growing number of examples demonstrate the important role of this balance in modulating the pathophysiology of multiple human diseases. For protein folding diseases, such as cystic fibrosis and certain neurodegenerative diseases, there are beneficial effects to slowing down translation, allowing more time for the endoplasmic reticulum (ER) to fine-tune the folding efficiency without altering its protein sequence (36). It seems promising to evaluate this therapeutic strategy also in MAFLD as hepatocellular injury (steatohepatitis) in MAFLD is characterized by ER stress and a dysfunctional unfolded protein response (37).

Translational reprogramming during stress adaptation

Stress adaptation may contribute to shaping the progression of chronic inflammatory condition such as MAFLD. Translation plays a crucial role in shaping the proteome during adaptation to various types of stress, with the mammalian target of rapamycin (mTOR) and the ISR are central cellular hubs in regulating this(38) (**Figure 2**).

Integrated stress response (ISR)

ISR is a homeostatic mechanism induced by ER stress and provides a regulatory loop to modulate translation in response to diverse cell stresses to restore cellular homeostasis. Mammals have four kinases-GCN2, RNA-dependent protein kinase (PKR), PKR-like ER kinase (PERK), and heme-regulated eIF2 α kinase (HRI)—that are specifically activated by discrete stress signals and universally phosphorylate translation initiation factor (eIF2 α)(39).

In particular, during nutrient stress, the GCN2 phosphorylates eIF2 α , initiating the ISR. This leads to a reduction in the rate of global 5' cap-dependent events and a shutdown of global protein synthesis but also the induction of preferential translation of the mRNAs of stress-related genes. When stress is prolonged or cannot be resolved, this leads to chronic ER stress, a unique program, chronic ISR program is characterized by persistently elevated mRNA translation of the vast majority of mRNAs, which were translationally up-regulated during acute ER stress(40).

Dysregulation of the ISR has been linked to various diseases including cancer, diabetes, MAFLD, alcoholic hepatitis and inflammation(41). However, it remains unclear whether ISR contributes to disease pathogenesis or represents an innate defence mechanism against metabolic stresses. Clarifying these aspects and deciphering the mechanisms of selective mRNA translation that occur under cell stress holds great promise for the identification of new targets in the treatment of MAFLD.

mTOR pathway

Activation of the mTOR pathway in response to nutrients or growth factors is a master regulator of RNA translation mostly at the level of initiation and subsequently protein synthesis through regulating the phosphorylation or activity of several translation factors. Activation of mTOR, as the catalytic subunit of the mechanistic target of rapamycin complex 1 (mTORC1), substantially promotes RNA translation to enhance protein synthesis. Following that, the suppression of mTORC1 activity, either by the withdrawal of nutrients or mitogens or pharmacologically, leads to the rapid dephosphorylation of eIF4E-binding proteins (4E-BP1–3) and subsequent global suppression of mRNA translation and inhibition of protein synthesis(42).

The hyperactivation of the mTOR pathway has been implicated in a number of diseases, including MAFLD. Various reports have demonstrated that hyperactivation of the mTOR signalling pathway is responsible for the activation of sterol regulatory element-binding protein 1 (SREBP-1c) to enhance hepatic de novo lipogenesis in response to feeding and insulin, which is a pivotal component of pathologies of metabolic dysfunction in MAFLD(43).

Translation based therapeutic strategies

The intricate nature of the translation process renders it susceptible to deregulation at multiple levels and targeting mRNA translation can be a very productive therapeutic strategy for MAFLD due to a multitude of reasons. First, because protein synthesis is one of the most energy-demanding cellular processes, translation rates are very tightly regulated, with high specificity in translation regulation signalling (44-46). Second, translation plays an orchestra role in fine-tuning the cell response during stress adaptation. In a stressful situation, the tight regulation of translation allows for limiting the translation capacity of the cell on the production of what is crucially needed for survival. On the other hand, with translation being the last step in gene expression, regulating translation allows for quicker adaptive responses to a stress situation than any upstream steps in the gene expression pathway. Subsequently, translation is linking nutrient availability, cellular metabolism adjustment with inflammation. Third, as the components of the translation machinery integrate almost all dysregulated signals, targeting the components of this machinery holds promise for overcoming a major hurdle associated with the disease heterogeneity. Indeed, MAFLD heterogeneity is thought to be one of the major obstacles in identifying a pharmacotherapy for the disease and for applying targeted therapies in the clinic(10). Fourth, conceptually, translation inhibitors may preferentially inhibit pathogenic or activated cells while preserving normal cells to a degree with acceptable safety.

Two broad strategies can be envisioned to improve the precision and therapeutic window of targeting protein translation in MAFLD. The first approach could focus on identifying translational regulators that are preferentially activated or overexpressed in MAFLD or MAFLD related advanced liver injury compared to normal livers. The second approach may involve identifying translationally regulated genes that are overexpressed or activated in MAFLD or MAFLD related advanced liver injury but not in normal livers. The translome remodelers that reprogram protein output to activate stress adaptations provide a therapeutic window to selectively target MAFLD. Below, we summarise translation based therapeutic strategies and some of the insights from other diseases, with referral to potential application in MAFLD, as appropriate.

Translation inhibitors

A growing number of translation inhibitors have been developed to inhibit mainly translational initiation or elongation (**Figure 3**). Broadly, proteins with short half-lives may be particularly affected by translation inhibitors. An array of drugs targeting protein translation have been approved for clinical use in many diseases such as cancer and cystic fibrosis (e.g. Rapamycin, Everolimus and Homoharringtonine). Many others are in clinical trials (e.g. Tomivosertib (selective translation regulation inhibitor that targets MNK1 and MNK2 (MNK1/2))(47), selinexor (selective inhibitor of nuclear export and translation)(48) and Prexasertib (Checkpoint kinase 1 (CHK1) inhibitor which inhibits the mTOR pathway, activates 4E-BP1 and inhibits protein synthesis)(48).

Pharmacological modulation of protein folding

Slowing translation improves appropriate protein folding, and is reported in multiple diseases such as neurodegenerative diseases and cystic fibrosis. Slowing ribosome velocity via Ribosomal Protein L12 (RPL12) silencing as a corrector to facilitate the folding and function

of the mutant CF transmembrane conductance regulator (CFTR) and has a synergistic effect with the clinically approved drugs lumacaftor and tezacaftor (pharmacologic modulators of CFTR protein)(36, 49). Drugs such as Salubrinal, Guanabenz, and Sephin1 (inhibitors of eIF2 α dephosphorylation) seem also to action by slowing down translation and protein synthesis, allowing increased time for proper protein folding, and therefore protecting the cells from the injurious effects of proteotoxicity(50, 51). Whether, this approach can be useful to treat MAFLD, characterised by proteotoxicity is yet to be explored.

Pharmacological modulation of ISR signalling

In addition, the pharmacological modulation of ISR signalling, either activation or inhibition, emerges as another promising therapeutic strategy for the treatment of ISR-mediated diseases. A recent study showed that constitutive repressor of eIF2 α phosphorylation (CReP) mediated ISR activation protects mice from high fat diet-induced adiposity, hepatic steatosis and insulin resistance via FGF21 induction(52). Pharmacological activation of ISR signalling can be attained either by eIF2 α phosphorylation through activating eIF2 α kinases or by phosphatase inhibitors mediated inhibition of eIF2 α dephosphorylation. Some examples of eIF2 α kinase activators include CCT020312 and BEPP monohydrochloride (a selective PERK activator)(53) and histidinol and arginine deiminase (GCN2 activators)(54, 55).

On the other hand, the inhibition of chronic ISR activity is another potential therapeutic approach. For instance, targeting the ISR via the inhibition of the growth arrest and DNA damage-inducible protein (GADD34), which catalyzes the dephosphorylation of eIF2 α demonstrated promise results as a therapeutic strategy in murine models of numerous neurodegenerative diseases that are characterized by chronic eIF2 α phosphorylation (56). Some of the drug compounds have been shown to inhibit eIF2 α kinase include GSK2606414 and GSK2656157 (selective PERK inhibitors that prevent its autophosphorylation)(57)

(**Figure 3**). Recently, a small-molecule ISR inhibitor, ISRIB, was identified to rescue protein translation and prevent the aggregation of inactive translation initiation complexes into stress granules in the presence of P-eIF2 α that can be exploited to treat neurodegenerative diseases (58-60). Targeting mTORC1 has also been demonstrated to be a highly promising strategy in the treatment of various diseases including cancer(61). Whether pharmacological modulation of ISR and mTOR signalling can be exploited to treat MAFLD is yet to be determined.

Mistranslation: A novel conceptual framework for considering FGF21 resistance

Particularly in the liver, it has been shown that ISR activation results in a dramatic induction of FGF21(62). FGF21 is a liver-derived hormone with pleiotropic beneficial effects on metabolism and regulates interactions between energy metabolism and stress responses(63). The intact FGF-21 is a small protein comprising 181 amino acids. β -Klotho forms a binary complex with FGF receptor 1c (FGFR1c) and functions as an obligate co-receptor for FGF21.

The administration of recombinant FGF-21 lowered plasma glucose and insulin levels, reduced hepatic and circulating triglyceride and cholesterol levels, and improved insulin sensitivity, energy expenditure, hepatic steatosis, and obesity in a range of insulin-resistant animal models(63). Paradoxically, despite beneficial effects in rodents, elevated FGF21 levels are reported in metabolic diseases, implying the existence of FGF21 resistance (64-67). In humans, high FGF21 is associated with the incidence of metabolic syndrome, MAFLD and cardiovascular events (68-70). A murine study showed that hepatic FGF21 production increased following intake of a high-fat diet for 1 day and that the increases in plasma FGF21 levels preceded hyperinsulinemia, hyperglycemia, and weight gain, excluding that the increases in circulating FGF21 result from obesity or diet-induced metabolic disturbances(71). Consistently, FGF21 is reduced in response to corrective interventions such as lifestyle modification and bariatric surgery(63). In two studies, a decrease in hepatic FGF21 production induced by PCPA, a tryptophan hydroxylase inhibitor or Whey protein isolate led to

suppression of insulin resistance and hyperglycemia in mice fed a high-fat diet(71, 72). Similarly, statin decreases hepatic FGF21 levels in a dose-dependent manner in humans and mice(69, 73). Reductions in FGF21 was reported in association with decreased liver fat(74).

Whether, FGF21 levels and function might be dysregulated in human because of either FGF21 resistance in peripheral tissues (downregulation of receptors) or abnormality in FGF21 secretion is unclear. We have very recently shown via a genetic approach that increased FGF21 production via mistranslation has a key initiating or causative role in FGF21 resistance, that is regulated by FGF21 polymorphism(27). Thus, the FGF21 resistance could be considered a defensive mechanism, rather than a pathological impairment, used by responsive tissues against abnormally increased FGF21. The excessively sustained up-regulated FGF21 may induce a vicious loop of FGF21 resistance. These findings suggest that restoring the abnormally increased FGF21 levels to a physiologic range might be a new therapeutic approach for MAFLD.

Can mild suppression of FGF21 be a novel therapeutic approach for MAFLD?

Given the strikingly beneficial pharmacology of FGF21 in animal models, several FGF21 analogues and mimetics have developed and progressed to early phases of clinical trials for treating metabolic disease and demonstrated the therapeutic potential as a novel metabolic therapy(75). Nonetheless, safety concerns associated with targeting the FGF21 pathway have also emerged and there have been serious adverse effects recorded including sizable bone loss, female infertility, short growth, and increase blood pressure and pulse rate (44, 76-79). However, significantly lower FGF21 concentrations in the bloodstream are sufficient to achieve positive therapeutic effects than the concentrations achieved in clinical trials, which were associated with the development of FGF21-related toxicities in mice (80). It is worth considering whether maintaining constant low levels of serum FGF21 via regulating mRNA

translation could be the more efficient and safe therapeutic approach. Further studies are required to explore this approach.

Challenges and open questions

Although very promising, it goes without saying that translation based drugs strategy is not without challenges. Along with global mechanisms of translational regulation, it has been increasingly recognised that certain subsets of mRNAs are differently influenced by translation signalling. The molecular mechanisms of specific translation that are dictating this selectivity are poorly characterized. Similarly, the basis of the cell type and context dependent-specific regulation of mRNA translation and kinetics are still largely unrecognized. Another pivotal path for future studies is to characterize the localization and upstream regulation of mRNA translation in hepatocytes and other liver cells from MAFLD patients that are likely to be different from that of normal cells.

Conclusions

Concordant with the growing burden of MAFLD as the most common chronic liver disease, the health, societal and economic burden of MAFLD is soaring. To date, there is no pharmacological therapy for MAFLD and a new strategy for innovative therapy is needed. While translation inhibitors have gradually entered clinical development for other diseases, this is still less developed for MAFLD. The elucidation of the molecular mechanisms underlying specific mRNA translation will help in a better understanding of MAFLD pathogenesis. It will be critical to integrate the recent translome technologies into studies of MAFLD development and progression to more comprehensively define the compendium of genes dysregulated under disease conditions as well as the upstream factors controlling these changes. This can pave the path for novel rationale therapeutic approaches to tackle the growing burden of this disease.

Figures legends

Figure 1: Translation kinetics regulates co-translational protein folding.

Figure 2: Translation based therapeutic strategies for MAFLD.

Figure 3: An overview of translation inhibitors.

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