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Flavivirus Modulation of Cellular Metabolism

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Abstract

Over the last decade, we have begun to appreciate how flaviviruses manipulate cellular metabolism to establish an optimal environment for their replication. These metabolic changes include the stimulation of glycolysis, in addition to lipid anabolic and catabolic pathways. These processes are thought to promote an energetically favorable state, in addition to modifying membrane lipid composition for viral replication and virion envelopment. Importantly, many of these processes can be pharmacologically inhibited as successful antiviral strategies, at least in cell culture. In this review, we discuss the mechanisms by which flaviviruses alter cellular metabolism, remaining questions, and opportunities for therapeutic development.

Introduction

Viral infections can modify many aspects of cellular physiology, including cell cycle, homeostatic pathways such as apoptosis and autophagy, and metabolic pathways. Viral strategies have evolved to either stimulate or inhibit the metabolic state of the cell depending on the desired outcome (productive or latent viral infection, or an altered cellular response to infection, reviewed in (9)). The flaviviruses are a closely related genus of (+) RNA viruses in the *Flaviviridae* family and include important human pathogens such as dengue virus (DENV), West Nile virus (WNV), Japanese encephalitis virus, yellow fever virus, tick-borne encephalitis virus, and Zika virus, among others. Most flaviviruses are transmitted to humans via an arthropod vector (tick or mosquito) and produce an acute cytolytic infection. As such, flavivirus modulation of cellular metabolism does not result in prolonged cellular survival and may, in some cases, contribute to cytopathic effect.

Our current understanding of the flaviviral benefits of modulating cellular metabolism includes the following conjectures. Flavivirus replication requires increased nucleotide pools and enzymatic cofactors, such as ATP for RNA helicase activity. As with all (+) RNA viruses, flaviviruses alter host membranes, in this case the endoplasmic reticulum (ER), to establish protected sites of replication. These replication compartments are thought to promote appropriate replicase scaffolding and concentration of replication substrates, in addition to shielding the RNA from cytosolic innate immune sensors and RNA degradation

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machinery (4, 5). Finally, as sites of replication and virion assembly are linked, modified ER lipid composition will be incorporated into virion envelopes, which may impact virion infectivity (33). The following studies have investigated flavivirus modulation of cellular metabolism. One caveat is that these studies were performed in different cell lines, which may respond differently to flavivirus infection, and thus these metabolic changes may not be viewed holistically.

DENV and Central Carbon Metabolism

DENV infection modulates and requires glycolysis for optimal virus replication (Fig. 1) (2, 7). Because most cancer cell lines, at baseline, have an altered central carbon metabolism, Fontaine et al. performed a metabolomic analysis of mock and DENV infected primary human foreskin fibroblasts (HFFs). Infection of HFFs with DENV virus resulted in a significant change in all classes of metabolites (amino acids, carbohydrates, lipids, and nucleotides) as compared to uninfected cells over the course of infection (7). DENV infection altered both glutamine and glucose utilization. Early glycolytic intermediates, such as glucose-6-phosphate and fructose-6-phosphate, increased in concentration over time, whereas late glycolytic intermediates decreased. While the authors were not able to determine whether these modulations were related to increased flux or decreased production of these intermediates, work from others has shown that the enzymatic activity of GAPDH is increased during DENV infection in an NS1-dependent manner (2). This suggests that DENV actively drives flux through the glycolytic pathway.

Infection with DENV resulted in a modest increase in cellular glucose concentration, which likely resulted from an increase in the expression of glucose transporter 1 and hexokinase 2, the first enzyme of glycolysis (7). Experimentally limiting glucose severely decreased viral replication, whereas limiting glutamine levels had only a modest effect on viral replication. Fontaine *et al.* further demonstrated that inhibition of glycolysis inhibited viral replication, thus showing that DENV replication requires active glycolysis for optimal virus replication. The authors also demonstrated the requirement for glycolysis in a second cell line of immortalized (but metabolically normal) endothelial cells, suggesting that the requirement for glycolysis is not cell-type specific. Glycolytic stimulation by DENV may enhance numerous processes, including glutamine production for increased ATP and nucleotide pools, in addition to citrate, which is a precursor for fatty acid synthesis, discussed later.

Modulation of lipids at the replication compartment (RC)

Flavivirus replication compartments (RCs) are convoluted invaginations of the endoplasmic reticulum (ER) membrane (8, 33). Numerous strategies exist to generate the membrane curvature of RCs, including modulation of membrane lipid content (5, 6). Insertion of polar lipids into membranes generates curvature, while some lipids, such as cholesterol, enhance membrane fluidity that facilitates the curvature of membranes by proteins (12). WNV infection increases cholesterol synthesis and accumulation at RCs. The cholesterol biosynthetic enzyme 3-hydroxymethylglutaryl-CoA reductase (HMGCR) is recruited to RCs. Inhibition of HMGCR inhibits WNV replication (18). In addition to potential roles of

cholesterol in RC formation, this redistribution of cholesterol to RCs perturbs lipid rafts that are important for antiviral interferon signaling (18).

DENV stimulates fatty acid biosynthesis to alter RC lipid composition (Fig. 1). A targeted siRNA screen utilizing the DENV2 replicon identified acetyl-coA Carboxylase 1 (ACC1/ACACA) and fatty acid synthase (FASN) as important cofactors for DENV-replication, suggesting an important role for *de novo* fatty acid synthesis in DENV replication (10). Pharmacological inhibition of FASN decreased DENV replication and infectious virus production. Infection with DENV caused a relocalization of FASN to RCs and increased *de novo* fatty acids synthesis in cellular crude biochemical fractions containing the DENV replicase. Yeast-two hybrid analysis identified DENV NS3 as binding FASN, and NS3 expression was sufficient to re-localize FASN to perinuclear regions associated with RC formation. Finally, the addition of purified NS3 was sufficient to increase FASN activity in cell lysates. Although NS3 can directly bind FASN, a role for the ras-related GTPase RAB18 in relocalization of Rab18 to ER-associated NS3 was uncovered. Both DENV replication and FASN relocalization required the active GTP-bound form of Rab18 (30).

Lipid synthetic enzymes further modify fatty acids to generate the vast majority of distinct membrane lipids. A lipidomic analysis of DENV-infected mosquito cells was performed to characterize global changes in cellular lipid composition, in addition to alterations in crude RC fractionations. (23). At a whole cell level, ~15% of metabolites were altered in DENV-infected cells. However, crude fractions containing the RCs were highly distinct, with ~85% of the metabolites altered. These alterations in lipid composition could be prevented by inhibition of FASN. In particular, the RCs were enriched in phospholipids (phosphatidylethanolamine) and sphingolipids (sphingomyelin and ceramide). These lipids have chemical properties linked to membrane curvature and may also be incorporated into virion envelopes, given the tight spatial linkage between replication and assembly (33).

Sphingomyelin also accumulates at WNV RCs and sphingomyelin synthase inhibitors reduce WNV replication (19). Although ceramide was increased in biochemical fractionation of insect cells that contained the DENV RCs, no enhancement of ceramide was observed in DENV RCs from mammalian cells and replication was refractory to inhibitors of ceramide synthesis. In contrast, ceramide did accumulate at WNV RCs and replication was sensitive to ceramide synthesis inhibitors indicating that there are mechanistic differences in RC formation between the flaviviruses (1).

Lipid Catabolism

Lipid droplets are cellular organelles that store neutral lipids (e.g. triglycerides and cholesterol esters), which can be used as a source for energy production. Upon conditions of starvation (or lipid overload), autophagosomes engulf portions of the lipid droplets and deliver them to lysosomes where free fatty acids are liberated from the triglycerides by the lysosomal acid lipase (27). The released free fatty acids are shunted to the mitochondria for β -oxidation resulting in increased ATP, as well as nicotinamide adenine dinucleotide phosphate (NADPH) and acetyl-CoA (28). DENV infection has multiple effects on lipid

droplets (Fig. 1). Early in infection, it is observed that lipid droplets are reabsorbed into the ER. This is hypothesized to provide lipids for ER expansion and RC formation (22).

Later in infection DENV induces a selective autophagy called lipophagy that mobilizes lipids from the lipid droplet for β -oxidation and energy generation (11). Many groups have shown that DENV infection induces a proviral autophagy *in vitro* and *in vivo* (3, 11, 14, 16, 17, 21). This induction was shown to be selective (e.g. targeted to specific cargo) in DENV infection with ~30% of autophagosomes associated with lipid droplets at 24 hours post infection. This resulted in the mobilization of lipids from the lipid droplet. When the media serum was low, as to limit uptake of extra-cellular lipids, the lipid droplets were depleted in DENV infection. The lipids accumulated in autophagosomes that are acidified, suggesting maturation, and were transported to mitochondria, leading to dramatic increases in the rate of β -oxidation and energy production. Although multiple roles for autophagy have been proposed for DENV replication, lipophagy is the key role. Defects in DENV replication caused by inhibiting autophagy pharmacologically or by small interfering RNAs targeting different essential autophagy components can be fully complemented by exogenous free fatty acids. This complementation requires β -oxidation, implying that energy production is the key role for lipophagy in DENV replication.

Why DENV replication requires increased β -oxidation remains unclear. As mentioned above, a number of processes in viral replication are ATP-dependent, which is a major product of β -oxidation. Additionally β -oxidation generates NADPH, which is a cofactor for FASN and thus may stimulate fatty acid synthesis. It is perplexing that DENV stimulates anabolic (fatty acid synthesis) and catabolic (lipophagy) processes at the same time. Generally, this does not happen in uninfected cells. Signals that stimulate autophagy typically inhibit fatty acid synthesis and vice-versa (13). This suggests DENV rewires cellular metabolic signaling.

Targeting metabolic proteins for anti-flaviviral therapy

Targeting cellular metabolism for antiviral therapies is appealing for a number of reasons. (i) Given the central role of metabolism in human health and disease, many metabolic inhibitors exist and more are under development for therapeutic purposes. (ii) Given the reliance of viruses on metabolism, there is potential for broad-spectrum antiviral activity. (iii) Host targeted inhibitors are likely to have high barriers to resistance. The drawbacks are the potential for toxicity and the possibility of a dampened/altered immune response, since many immune effector arms are regulated by their own metabolism. Virtually all of the processes discussed in this review have been targeted for anti-flaviviral efficacy in cell culture (32). Inhibition of cholesterol biosynthesis has anti-flaviviral properties (15, 20, 24, 25, 29). Similarly inhibitor of fatty acid synthesis are anti-flaviviral (10, 23, 26, 29, 30), in addition to downstream lipid synthetic enzymes (15, 19, 31). The current challenge is to translate these studies into animal models and eventually, infected patients.

Conclusions

Our understanding of the metabolic changes in flavivirus infection has increased over the past decade to appreciate alterations in glycolysis, lipid synthesis, and lipid catabolism involved in creating an optimal replication environment. Many questions remain. In addition to NS1 stimulation of GAPDH activity, are there other mechanisms by which DENV stimulates glycolysis? What are the key metabolites and their specific function in DENV infection? How does WNV relocate HMGCR to its RCs? What is the structure/function of how DENV NS3 stimulates fatty acid synthesis and what are the key functions of the altered lipids at DENV RCs? Is this altered lipid composition reflecting in virion envelope composition and does this impact virion infectivity? How does DENV induce lipophagy, yet still promote compartmentalized fatty acid synthesis? Finally, can we translate these findings into therapeutics?

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Highlights

- Flaviviruses modulate cellular metabolism to optimize their replication
- DENV stimulates glycolysis
- DENV induces lipid catabolism via selective autophagy
- DENV NS3 enhances fatty acid synthesis at replication compartments
- Replication compartments have altered lipid composition

Figure 1. Flavivirus modulation of metabolism

Early after infection, lipid droplets are reabsorbed into the ER. Later in infection, autophagosomes (green) liberate free fatty acids from lipid droplet triglycerides, which then undergo mitochondrial β -oxidation to produce ATP. In parallel, DENV stimulates glycolysis to produce ATP through the TCA cycle. This also generates citrate, which is a precursor for fatty acids biosynthesis. DENV NS3 recruits FASN to the ER in a Rab18-dependent fashion and stimulates fatty acid synthesis at RCs to modify the lipid composition. WNV recruits HMGCR to RCs to stimulate cholesterol synthesis. This establishes an optimal environment for flavivirus replication.