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Intricate Roles of Mammalian Sirtuins in Defense against Viral Pathogens

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For a number of years, sirtuin enzymes have been appreciated as effective "sensors" of the cellular environment to rapidly transmit information to diverse cellular pathways. Much effort was placed into exploring their roles in human cancers and aging. However, a growing body of literature brings these enzymes to the spotlight in the field of virology. Here, we discuss sirtuin functions in the context of viral infection, which provide regulatory points for therapeutic intervention against pathogens.

The discovery of host proteins that provide defense against viral pathogens is a research area of significant interest, being relevant both for understanding basic mechanisms of host defense and for developing alternative antiviral therapeutics. Given that most currently available antiviral drugs target virally encoded proteins, they tend to be specific for certain types of viral infections and to have their efficacy compromised by drug-resistant viral strains. Recent studies have pointed to mammalian sirtuins (SIRTs) as important defense factors against viruses. As the *Escherichia coli* sirtuin homolog also functions in defense against bacteriophages (1), studying sirtuin function holds promise to have a broad impact in the field of infectious disease.

The seven mammalian SIRTs (SIRT1 to SIRT7) are ubiquitously expressed in most cells and tissues and are evolutionarily conserved. Sirtuins are NAD^+-dependent enzymes, primarily known as lysine deacetylases, and derive their name from the founder of the family, the *Saccharomyces cerevisiae* transcriptional regulator Sir2 protein. Although their functions remain to be fully characterized, a growing body of literature indicates that SIRTs impact a wide range of cellular pathways. This breadth of function is partly derived from their diverse intracellular localizations, with SIRT1, SIRT6, and SIRT7 being nuclear; SIRT2 being cytoplasmic; and SIRT3, SIRT4, and SIRT5 being predominantly mitochondrial (Fig. 1). Equally important is the still-evolving understanding that, in addition to their deacetylase activity, these proteins can have alternative enzymatic activities, including ADP ribosylation (SIRT1, SIRT4, and SIRT6), desuccinylation and demalonylation (SIRT5), delipoylation (SIRT4), and demyristoylation and depalmitoylation (SIRT6) (reviewed in reference 2). Given these diverse activities and localizations, sirtuins are core regulators of transcription and metabolism. Therefore, sirtuins have the ability to control numerous cellular pathways required throughout the viral life cycle. Here, we highlight recent research on characterizing sirtuin functions during viral infection and place these findings into the broader context of developing improved antiviral therapeutics.

ROLES OF SIRTUINS IN GENE EXPRESSION DURING VIRAL INFECTION

Sirtuins are known to regulate gene expression primarily by controlling the modification status of histones and transcription factors. In the nucleus, the SIRT1-mediated removal of acetyl groups from lysine residues on histones (H3K9ac, H3K14ac, or H4K16ac) modulates heterochromatin formation, thereby impacting gene expression (reviewed in reference 2). SIRT6 deacetylates H3K9ac, targeting NF-κB-dependent gene expression, while H3K18ac deacetylation by SIRT7 promotes oncogenic transformation. Although primarily cytoplasmic, SIRT2 was shown to regulate DNA compaction through deacetylation of H4K16ac during the G_2/M transition. Sirtuins also target important transcription factors, such as p53, NF-κB, and FOXO1, directly affecting the expression of their target genes. Through these actions, sirtuins may be able to impact the outcome of viral infection by modulating both host and viral gene expression. In turn, viruses are known to manipulate host epigenetic and transcription machineries by hijacking SIRT-regulated pathways. This has been well illustrated during infection with human immunodeficiency virus (HIV). SIRT1 was shown to deacetylate the HIV protein Tat, enabling HIV transactivation (3). However, Tat can also inhibit SIRT1, activating NF-κB-responsive gene transcription and promoting $CD4^+$ T-cell hyperactivation that is favorable for viral replication (4, 5). Another elegant mechanism aimed at modulating gene expression is seen with influenza A virus, which has evolved to encode NS1, a protein mimic of histone H3 tail that can suppress transcription of antiviral genes (6). As NS1 can be acetylated and methylated at the lysine site equivalent to the mammalian histone H3K4 (6), it remains to be investigated if these modifications are modulated by sirtuins. Moreover, sirtuin inhibition was shown to increase influenza A virus titers, while activation reduced titers (1).

Through their modulation of histone modifications on viral nucleosomes, sirtuins can also be critical regulatory hubs during infections with DNA viruses. Chromatin assembly around viral DNA has been documented for several nuclear-replicating viruses, including hepatitis B virus (HBV), human cytomegalovirus (HCMV), varicella-zoster virus (VZV), human papillomavirus (HPV), and herpes simplex virus 1 (HSV-1). As a result, transcription from the viral genome can be regulated by histone modifications. For example, recruitment of SIRT1

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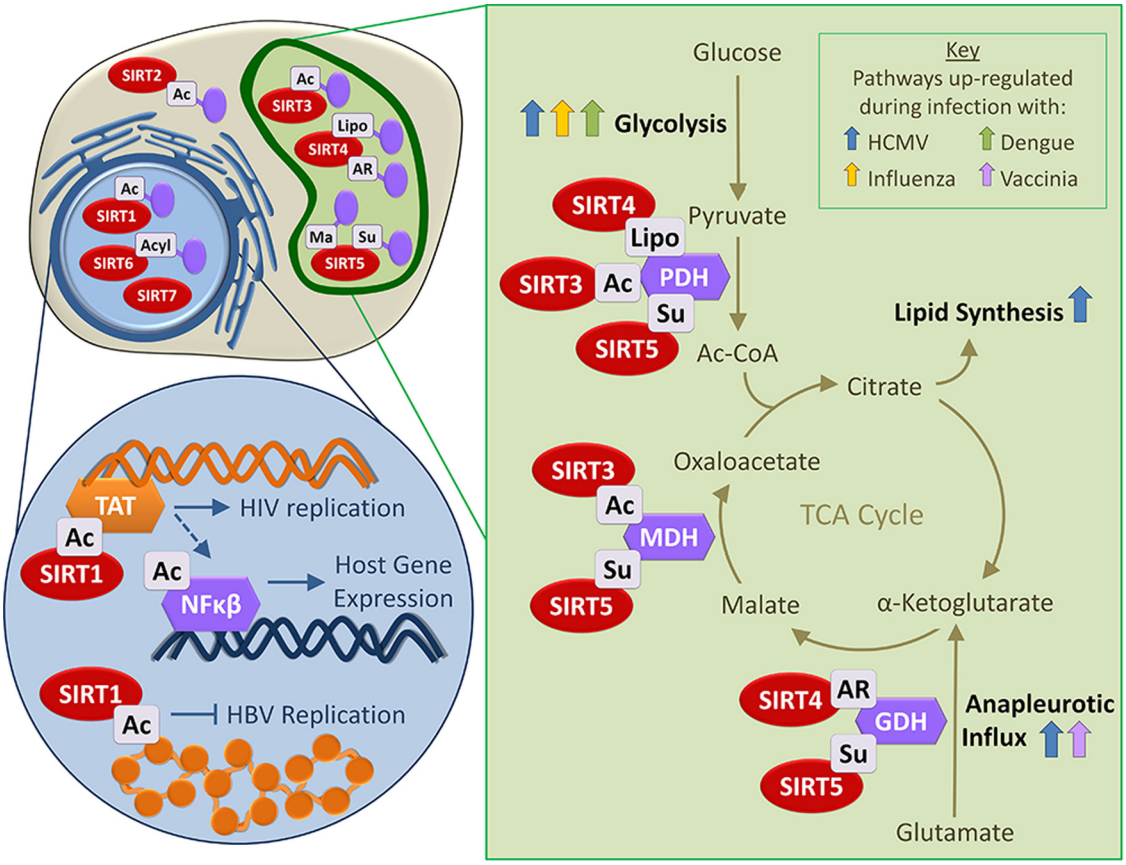

FIG 1 Enzymatic activity and subcellular localization of mammalian sirtuins. SIRT1 (deacetylase), SIRT6 (demyristoylase and depalmitoylase), and SIRT7 (deacetylase) are nuclear; SIRT2 (deacetylase and demyristoylase) is predominantly cytoplasmic; SIRT3 (deacetylase), SIRT4 (ADP-ribosyltransferase and lipoamidase), and SIRT5 (desuccinylase and demalonylase) are mitochondrial. In the nucleus, the role of SIRT1 is depicted during HIV and HBV infections. On the right, virus-induced changes to metabolic pathways are shown for several viruses, along with mitochondrial sirtuin substrates that have critical roles in the dysregulated pathways. Ac-CoA, acetyl coenzyme A; PDH, pyruvate dehydrogenase; MDH, malate dehydrogenase; GDH, glutamate dehydrogenase; Lipo, lipoyl; Ac, acetyl; Su, succinyl; AR, ADP-ribose.

onto HBV covalently closed circular DNA (cccDNA) was observed late in infection, coinciding with a decline in viral replication (7).

Histone modifications on viral nucleosomes are also finely tuned in a temporal manner during HCMV infection. HCMV nucleosomal structures were shown to have elevated transcriptionally active marks late in infection compared to prominent repressive patterns early in infection. Additionally, H4K16 acetylation, associated with cell cycle progression and transcriptional repression, and a known substrate of SIRT1 and SIRT2, decreased during HCMV infection (8). Reduction in the levels of these SIRTs was reported to trigger increased HCMV yields (1). Because sirtuins can deacetylate histones on both viral and host chromatin, their effect on viral replication needs further exploration from the intertwined perspectives of the virus and host. As an example of viral genome regulation, reactivation of Kaposi's sarcoma-associated herpesvirus (KSHV) was observed upon treatment with sirtuin inhibitors; this increased active (H3K4me3) and decreased repressive (H3K27me3) histone marks at the viral transcription activator promoter (9).

Along with chromatin regulation, DNA viruses can modulate the activity of various host transcription factors through manipulation of sirtuin levels and their interactions. For example, HSV-1 inhibits apoptosis to promote neuronal cell survival by modulating SIRT1 interaction and colocalization with p53 (10). Since multiple deacetylases can target important transcription factors, it remains to be determined whether sirtuins' roles are redundant or dependent on specific conditions, such as cell type. From the host perspective, sirtuins may represent a first line of defense against viral infection. SIRT1 was demonstrated to promote sumoylation and stabilization of PML in vesicular stomatitis virus (VSV)-infected cells, which explains the increase in VSV titers upon SIRT1 knockout (11).

The diversity and abundance of sirtuin substrates, which include both histone and nonhistone proteins, introduce complexity to the interpretation of their functions during infection. This illustrates the need for further insight into the spatial and temporal sirtuin-mediated events during infection, helping to define mechanisms through which hosts and viruses battle for control over sirtuin functions to either inhibit or promote viral replication.

SIRTUIN REGULATION OF HOST CELL METABOLISM DURING VIRAL INFECTION

Among the human sirtuins, the three mitochondrial SIRTs (SIRT3, SIRT4, and SIRT5) act as sensors of their environment

to regulate essential metabolic pathways, many of which are known to be altered during viral infection. SIRT3 is a major mitochondrial deacetylase with numerous substrates, having the ability to impact a wide range of critical metabolic processes, such as fatty acid oxidation and the tricarboxylic acid (TCA) cycle (12). SIRT4 was identified as an ADP-ribosyltransferase (13) and more recently as a lipoamidase that inhibits the pyruvate dehydrogenase complex (14). In addition to acting as a weak deacetylase, SIRT5 was characterized as a desuccinylase and demalonylase, regulating many metabolic pathways, including the urea cycle via its substrate CPS1 (15, 16). Although the investigations of mitochondrial sirtuin functions during viral infection have only recently emerged and are still limited, the interface between SIRT functions, dynamic changes in metabolism, and viral infection holds significant promise to understanding both the biology and pathogenicity of infections. Indeed, small interfering RNA (siRNA)-mediated knockdown of each mitochondrial sirtuin was reported to lead to increased viral titers for several DNA viruses (1). The observed variation in the magnitude of the viral titers is likely indicative of the different metabolic needs of the tested viruses and the specific sirtuin regulation of those pathways.

One of the metabolic signatures of viral infection is the increase in glucose uptake and glycolytic flux. Using mass spectrometry, an elegant example was shown during infection with HCMV, where the efflux of additional carbon is shuttled through the TCA cycle to increase lipid biosynthesis, required for the envelopment of infectious virions (17, 18). Influenza virus infection is also known to increase glycolytic flux at 12 h postinfection, the point of virus release and onset of virus-mediated apoptosis, which is necessary for viral replication (19). Highlighting the global importance of this metabolic pathway during infection, dengue virus infection was also shown to increase glycolytic flux and nucleotide synthesis, both critical for viral replication (20). Sirtuins are optimally positioned to regulate enzymes of the glycolytic and TCA cycle pathways, including the pyruvate dehydrogenase complex under the control of SIRT3, SIRT4, and SIRT5 (12, 14, 16). Malate dehydrogenase in the TCA cycle is a substrate of both SIRT3 and SIRT5 for deacetylation and desuccinylation, respectively, pointing to possible sirtuin cross talk and fine-tuned regulation of metabolic processes (12, 15). Another metabolic reprogramming signature of infection is the anapleurotic influx into the TCA cycle, requiring glutamate dehydrogenase, a substrate of both SIRT4 and SIRT5 (13, 15, 17, 18). This need for glutamine as a carbon source for the TCA cycle was reported for both HCMV and vaccinia virus infections (17, 18, 20).

Given these findings regarding sirtuin functions and virus-induced metabolic changes, better understanding of viral metabolic signatures promises to aid the development of improved antiviral therapies. These therapies will likely need to be tailored to each virus. For example, even closely related viruses, such as the herpesviruses HCMV and HSV-1, trigger different metabolic changes. This knowledge was essential for the understanding that nucleotide analogs, but not fatty acid synthesis inhibitors, are effective against HSV-1 but not HCMV and vice versa (18). Even more nuanced, this metabolic reprogramming can vary by cell type. In a metabolomics study of HIV-1 infection in $CD4^+$ T cells and model macrophages, increased glucose uptake and glycolytic flux were observed only in $CD4^+$ T cells, while macrophages had striking reductions in both uptake and flux (21). These studies demonstrate that within one virus family, or even one type of viral infection, divergent metabolic responses can arise, illustrating the need for tailored antiviral therapeutics. Sirtuins are promising targets for this because of their specific connection to numerous metabolic processes impacted during infection.

PERSPECTIVES

In summary, sirtuins offer a remarkably rich diversity of regulatory points. Their NAD^+-dependent activities allow them to transmit information about changes in the environment to major cellular pathways for rapid and effective responses. With the discovery that all sirtuins can impact the replication of DNA and RNA viruses, we expect to learn more about their universal roles in the immune response, the stress response, and other types of defense responses utilized by an organism to prevent disease caused by pathogens. Therefore, being able to target sirtuins provides a valuable antiviral therapeutic strategy. Maybe the simplest way to control this family of enzymes is through regulation of NAD^+ levels. It is possible that certain viruses have already acquired this capacity; for example, HSV-1 infection leads to reduced NAD^+ levels, likely inhibiting sirtuins (18). However, simply regulating NAD^+ levels is probably not preferred. From a host defense and clinical perspective, the simultaneous regulation of all sirtuins could lead to global stress responses and cytotoxicity. Additionally, from a virus perspective, the transcriptional functions of certain sirtuins appear necessary for the regulation of viral gene expression. So, the fine-tuning of sirtuin regulation at the level of one enzyme or one given enzyme function may be the more promising way forward to take advantage of sirtuin defense properties. However, to achieve this level of precision in sirtuin regulation, it is critical to first understand the basic mechanisms for their housekeeping and antiviral functions. The constantly evolving knowledge regarding the sirtuin catalytic activities indicates that there is still a way to go before fully understanding their repertoire of functions. The integration of molecular and cellular biology with modern proteomic and metabolomic approaches can provide mechanistic insights into sirtuin functions. There is great value in future studies aimed at defining sirtuin substrates in the context of infection and the unique epigenetic and metabolic reprogramming by different viruses. To bridge these findings with clinical applications, such studies would benefit from considering sirtuin cross talk and roles in other human diseases, including cancers. Furthermore, as sirtuins may impact the susceptibility of a host to viral or bacterial infections, it will be informative to further investigate their roles during superinfection or infection with opportunistic pathogens.

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