Targeting of NAD biosynthesis enzymes for the treatment of bacterial infections and neurodegenerative diseases

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ANTIMICROBIAL DRUG TARGETS

The NAD(P) biosynthesis pathways in human and microbial cells represent a generous source of enzyme targets for drug development. In almost all living organisms, NMNAT, NADS, and NADK (NadD, NadE, and NadF in bacteria) form a three-step pathway common to de novo and salvage routes for NAD(P) biosynthesis. These targets individually represent promising broad-spectrum candidates against a large variety of bacteria. Their validation is based on (1) their essentiality and conservation among pathogenic microorganisms, (2) differential structural features or metabolic contribution to NAD(P) homeostasis between microbial and human cell types. Many such enzymes’ 3D structures are growing in the PDB, allowing rational design of selective antibacterials even when orthologous enzymes drive NAD synthesis in both human host and infecting microbe, e.g. by targeting subtle structural differences in substrate binding pockets.

NAD biosynthesis in major bacterial pathogens

NAD biosynthesis in human

NAD biosynthesis in bacteria

Narrow-spectrum antibiotics against niche pathogens can be developed based on targeting essential NAD formation by action on enzymes that are lacking (or diverse) in the human host. Such targets are identified through bioinformatics among the hundreds of sequenced bacterial genomes now available. For example, in silico pathway reconstruction through comparative genomics shows that some microbial (and also eukaryotic) pathogens lack the de novo pathway and strictly use pre-formed pyridine rings; such pathogens offer additional valuable targets implicated in NAD salvaging that we are currently pursuing. Some other pathogens lack NadD and rely solely on amided salvage pathways. Among the most virulent of this niche are Francisella tularensis and Acinetobacter baumannii, where NadD is replaced by the bifunctional NadM/NudD enzyme. NadM is widely considered as a target for narrow-spectrum antibiotics.

NAD biosynthesis in Francisella tularensis

NAD biosynthesis in some bacterial and eukaryotic pathogens:

- Streptococcus and Staphylococcus
- Anacardiaceae flavonoids
- Members of the Eubacteriaceae
- Isocitrate dehydrogenase
- Thiol-specific enzymes

In this context, NAMPT and NMNAT emerge as major drug targets. Together they form a two-step “amidated” pathway driving Nam recycling toward NAD resynthesis that plays as a major contributor to cellular NAD homeostasis in mammals. NAMPT is a pleiotropic protein under circadian rhythm control, with functions either intracellular as “peacemaker” enzyme regulating the metabolic flux of NAD synthesis, or extracellular as circulating secreted cytokine. NMNAT is usually abundant within the cell and not rate-limiting, but still pivotal because it is the sole enzyme leading to NAD synthesis within the whole pathway. Both NAMPT and NMNAT are vital in mammals when NAD is consumed and needs to be adequately replenished to guarantee cell survival, thus providing a valuable standpoint for therapies based on their drug targeting.

NAD SYNTHESES AND AXON DEGENERATION

In mammalian axons, the above two-step NAD synthesis pathway plays a recognized pivotal role. The axoplasm contains NAMPT and mainly one out of three NADM isoforms, NMNAT2, showing an essential and protective role for axon integrity and survival. Indeed, its gene ablation in mouse resulted in embryonic death due to lack of peripheral innervation.

In vivo, NMNAT2 is constantly delivered to the axon by fast axonal anterograde transport, but has a short half-life; therefore, when axons are injured and new protein can no longer be delivered locally, NMNAT2 levels fall below a critical threshold, triggering axon degeneration. This process, known as ‘Wallerian degeneration’ after Augustus Waller’s first description in 1850, is characterized by mitochondrial swelling and axonal cytoskeleton degradation. The role of NAD biosynthesis in this process was first revealed about 25 years ago by studies on the spontaneous mutant mouse Wd1. The Wd1 mutation causes a tenfold delay in Wallerian degeneration by generating stable overexpression of a protein chimera in which full-length NMNAT1 is fused to an N-terminal sequence that relocates the nuclear protein to the axon. As NMNAT1 is stable, it may compensate for loss of NMNAT2 after an injury, thus maintaining axonal integrity. Being NAMPT activity indispensable for axon protection, its formed product NAD was early postulated as the key mediator of axon integrity. Indeed, NAD, its precursors and over-expression of its biosynthetic enzymes were all shown to confer neuroprotection. Recently, several lines of evidence questioned about axon survival being regulated by the supply of NAD or by another action of NAMPT. First, its substrate NMN was found to accumulate in degenerating axons after NMNAT2 loss. Second, drug inhibition of axonal NAMPT was found to be neuroprotective despite lowering both NNN and NAD. Third, ectopic expression of NNN deaminase, a bacterial NMN-scavenging enzyme, prolonged survival of injured axons. These data indicate that NAD (the NMNAT product) is less important than NNN (the NMNAT substrate) to initiate axon degeneration, and disclose new strategies for the treatment of axonopathies based on limiting NNN accumulation by drug targeting of NAMPT. Currently, NAMPT is also being targeted in cancer. In contrast to many cancer chemotherapeutics which damage axons, drugs targeting NAMPT are axon-protective.

NAD-TARGETING IN NON-INFECTIONOUS HUMAN DISORDERS

In humans, NAD metabolism becomes altered in several non-infectious physiopathological conditions such as aging, obesity, diabetes, cancer, autoimmunity, and neurodegenerative disorders, whose incidence and prevalence are rising in our aging societies. Depending on whether proliferative or degenerative conditions are considered, two opposite strategies are required, respectively based on differential inhibition of cell growth or its stimulation (cell regeneration). Overall, the abovementioned conditions share higher physiological NAD degradation due to enhanced NAD-consuming reactions. This focused most current investigations on the NAD biosynthesis cell machinery with the aim to keep healthy NAD levels. Thus, drug targeting of key NAD biosynthetic enzymes in mammals, by appropriately modulating NAD homeostasis, is of recognized therapeutic value. This strategy must address the issues of drug differential toxicity and side-effects at the human organism level.