



Subcellular compartmentalization of NAD⁺ and its role in cancer: A sereNADE of metabolic melodies

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ABSTRACT

Nicotinamide adenine dinucleotide (NAD⁺) is an essential biomolecule involved in many critical processes. Its role as both a driver of energy production and a signaling molecule underscores its importance in health and disease. NAD⁺ signaling impacts multiple processes that are dysregulated in cancer, including DNA repair, cell proliferation, differentiation, redox regulation, and oxidative stress. Distribution of NAD⁺ is highly compartmentalized, with each subcellular NAD⁺ pool differentially regulated and preferentially involved in distinct NAD⁺-dependent signaling or metabolic events. Emerging evidence suggests that targeting NAD⁺ metabolism is likely to repress many specific mechanisms underlying tumor development and progression, including proliferation, survival, metabolic adaptations, invasive capabilities, heterotypic interactions with the tumor microenvironment, and stress response including notably DNA maintenance and repair. Here we provide a comprehensive overview of how compartmentalized NAD⁺ metabolism in mitochondria, nucleus, cytosol, and extracellular space impacts cancer formation and progression, along with a discussion of the therapeutic potential of NAD⁺-targeting drugs in cancer.

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1. Introduction

A critical hallmark in the multistep development and progression of cancer is the reprogramming of energy metabolism (Hanahan &

Weinberg, 2011). NAD⁺ is a fundamental signaling cofactor that regulates cancer metabolism through its coenzymatic function in redox reactions underlying essential bioenergetic pathways including glycolysis, the tricarboxylic acid (TCA) cycle, and oxidative

Abbreviations: ADP, Adenosine diphosphate; ATP, Adenosine triphosphate; cADPR, Cyclic ADP-ribose; CD38, Cluster of differentiation 38; CSC, Cancer stem cell; EMT, Epithelial-to-mesenchymal transition; ER, Endoplasmic reticulum; FDA, Food and Drug Administration; LDH, Lactate dehydrogenase; MSC, Mesenchymal stem cells; NA, Nicotinic acid; NAD⁺, Nicotinamide adenine dinucleotide (oxidized); NADH, Nicotinamide adenine dinucleotide (reduced); NADP⁺, Nicotinamide adenine dinucleotide phosphate (oxidized); NADPH, Nicotinamide adenine dinucleotide phosphate (reduced); NAM, Nicotinamide; NAMPT, Nicotinamide phosphoribosyltransferase; NAPRT, Nicotinic acid phosphoribosyltransferase; NAR, Nicotinic acid riboside; NMN, Nicotinamide mononucleotide; NMNAT, Nicotinamide mononucleotide adenyltransferase; NR, Nicotinamide riboside; NSCLC, Non-small cell lung cancer; HRR, Homologous recombination repair; PARP, Poly (ADP-ribose) polymerase; PPP, Pentose phosphate pathway; ROS, Reactive oxygen species; SIRT, Sirtuin; TCA, Tricarboxylic acid; Treg, Regulatory T cells; VAD, Vacor adenine dinucleotide.

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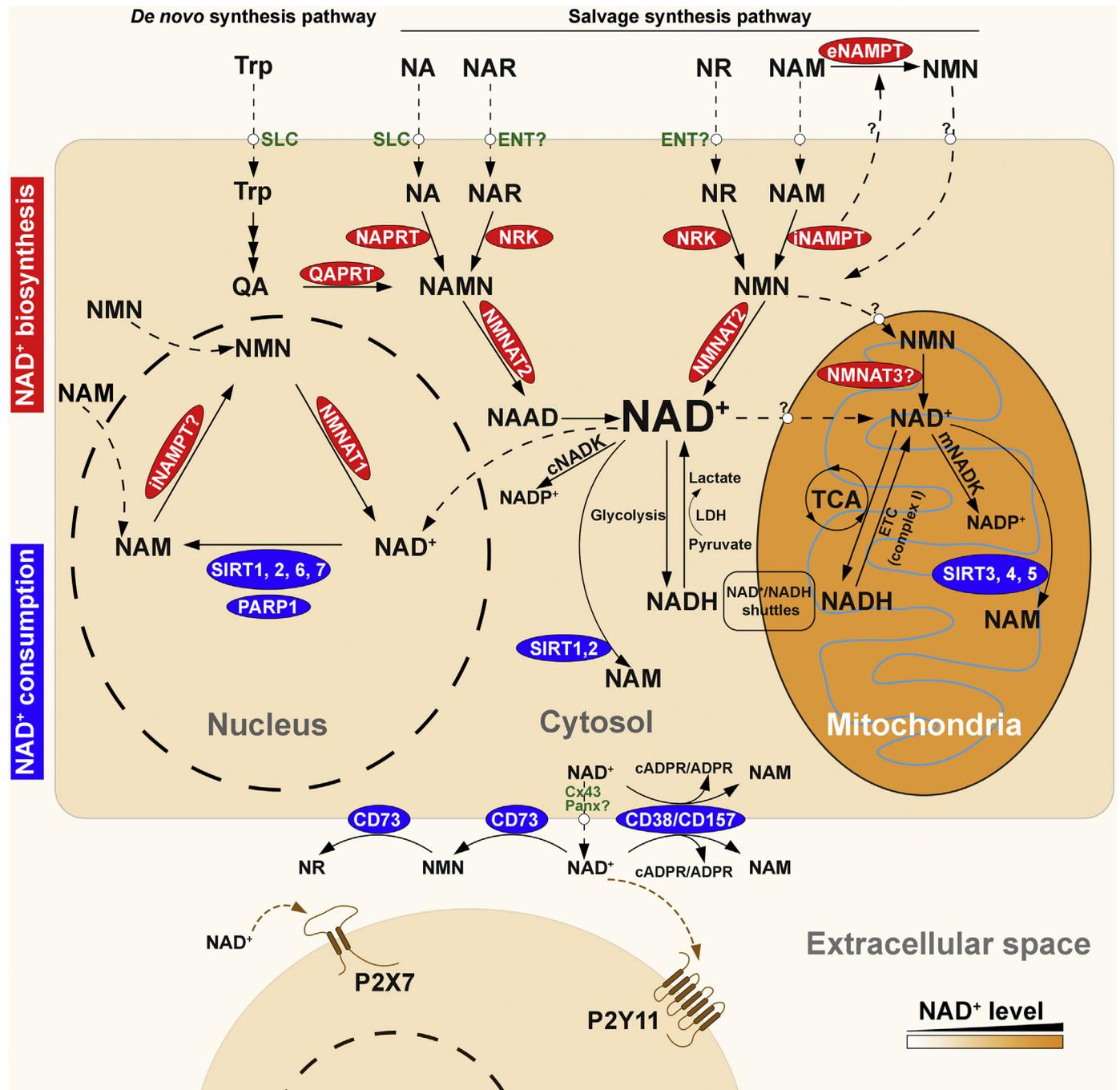


Fig. 1. Overview of the compartmentalized NAD⁺ metabolome in humans. NAD⁺ is synthesized via two major pathways: the *de novo* biosynthesis pathway from tryptophan (Trp), and the salvage pathway from various extracellular and intracellular precursors including nicotinic acid (NA), NA riboside (NAR), nicotinamide (NAM), and NAM riboside (NR). The precursors can cross cell membrane via different transporters, including solute carrier (SLC) transporters for Trp and NA, and equilibrative nucleoside transporters (ENTs) for NAR and NR. In the *de novo* synthesis pathway, Trp is degraded to quinolinic acid (QA), which can be converted to NA mononucleotide (NAMN) by quinolinic acid phosphoribosyltransferase (QAPRT). In the salvage synthesis pathway, NA and NR can be converted to their corresponding mononucleotides NAMN and nicotinamide mononucleotide (NMN) by nicotinic acid phosphoribosyltransferase (NAPRT) and nicotinamide phosphoribosyltransferase (NAMPT), respectively. NAMN and NMN can also be generated from phosphorylation of the nucleosides, NAR and NR, by NR kinases (NRKs). NMN may be generated from NAM by extracellular NAMPT (eNAMPT), although the secretion and function eNAMPT has not been elucidated, and whether or not NMN can enter cells is a subject of some debate in the field. The formation of dinucleotide NAAD or NAD⁺ from NAMN or NMN is catalyzed by nicotinamide mononucleotide adenylyltransferases (NMNATs). Human NMNAT comprises three isoforms that are localized in the nucleus (NMNAT1), cytosol (NMNAT2), and mitochondria (NMNAT3), although the presence and function of NMNAT3 is still under question. In cytosol, NAD⁺ can be reduced to NADH in glycolysis, and electrons from NADH can enter the mitochondrial electron transport chain (ETC) via glycerol-3-phosphate shuttle or malate-aspartate shuttle. NADH is oxidized to NAD⁺ when pyruvate is converted to lactate by lactate dehydrogenase (LDH). In mitochondria, NAD⁺ is reduced to NADH by tricarboxylic acid (TCA) cycle, and can be regenerated from NADH by the ETC complex I. Whether NAD⁺ or its precursors can enter mitochondria is still unclear. Cytosolic and mitochondrial NAD⁺ can be converted to NADP⁺ by cytosol NAD⁺ kinase (cNADK) and mitochondrial NADK (mNADK), respectively. NAD⁺ is consumed by poly (ADP-ribose) polymerases (PARPs) and sirtuins (SIRT) at different subcellular compartments. Intracellular NAD⁺ is released from cells through connexin 43 (Cx43) and possibly pannexin (Panx) hemi channels. Extracellular NAD⁺ can be degraded by membrane-bound glycohydrolases CD38/CD157 to cyclic ADP-ribose (cADPR) and ADPR, or by CD73 to NMN and further to NR. Extracellular NAD⁺ can modulate inflammatory response by activation of P2X7 and P2Y11 purinergic receptors.

phosphorylation. Continuous supplementation of NAD⁺ is essential for the functionality of glyceraldehyde-3-phosphate dehydrogenase in glycolysis (Busso et al., 2008). Serine biosynthesis from glucose requires NAD⁺-dependent 3-phosphoglycerate dehydrogenase that can be genomically amplified in cancer (Murphy et al., 2018; Possemato et al., 2011). NAD⁺ regulates multiple signaling pathways, including poly (ADP-ribose) polymerase (PARP)-mediated ADP-ribosylation and sirtuin (SIRT)-mediated deacetylation, both of which contribute to metabolic regulation, DNA damage repair, cell cycle progression, and epithelial-to-mesenchymal transition (EMT) (Imai, Armstrong, Kaeberlein, & Guarente, 2000; Schreiber, Dantzer, Ame, & De Murcia, 2006). NAD⁺ can also be converted by glycohydrolase CD38 (cluster of differentiation 38) to cyclic ADP-ribose (cADPR), a Ca²⁺-mobilizing second messenger that regulates multiple aspects of cancer biology, including cell survival, apoptosis, and inflammation (Camacho-Pereira et al., 2016; Chini, Chini, Netto, de Oliveira, & van Schooten, 2018). It is important to note that when NAD⁺ functions as a coenzyme, in redox reactions for example, NAD⁺ is converted to NADH and can be regenerated. In contrast, when NAD⁺ functions as a signaling molecule in NAD⁺-dependent signaling processes, NAD⁺ is continuously and irreversibly catabolized by cleavage of the glycosidic bond between nicotinamide (NAM) and the ADP-ribose moiety. Therefore, replenishment of NAD⁺ pool is particularly challenging for the rapidly dividing cancer cells. The emergence of NAD⁺ as a key molecule in tumorigenesis has prompted the development of therapeutic interventions targeting the NAD⁺ biosynthetic and consuming enzymes (Chiarugi, Dölle, Felici, & Ziegler, 2012; Chini, 2009; Ju et al., 2016; Sharif et al., 2019).

In mammalian cells, NAD⁺ has a relatively short half-life, about 1 h (Rechsteiner, Hillyard, & Olivera, 1976), and a limited diffusion radius due to its charge and cellular membrane barriers (Van Roermund, Elgersma, Singh, Wanders, & Tabak, 1995). It is conceivable that NAD⁺ concentration is not uniform within the cell and regulation of NAD⁺ concentration is “local”. This notion is supported by the finding that many enzymes involved in the biosynthesis or consumption of NAD⁺ are highly compartmentalized at subcellular level (Fig. 1). For example, nicotinamide mononucleotide adenyltransferase (NMNAT), the last enzyme in the NAD⁺ synthetic pathway, has three isoforms in mammals that are distinctly localized: NMNAT1, enriched in the nucleus; NMNAT2, in the cytosol and membrane structures; and NMNAT3, reported to be in the mitochondria (Berger, Lau, Dahlmann, & Ziegler, 2005; Brazill, Li, Zhu, & Zhai, 2017). Since most of the bioenergetic and NAD⁺-dependent signaling pathways occur within distinct subcellular compartments, locally accessible NAD⁺ is required for these metabolic processes (Alano et al., 2007; Nikiforov, Kulikova, & Ziegler, 2015). Several approaches have been attempted to visualize subcellular distribution of NAD⁺ pools. For example, targeted expression of PARP-1 can be used as a molecular NAD⁺ detector since the presence of NAD⁺ can be visualized by the formation of immunodetectable poly-ADP-ribose (Dölle, Niere, Lohndal, & Ziegler, 2010; VanLinden, Niere, Nikiforov, Ziegler, & Dölle, 2017). Moreover, several genetically encoded NAD⁺ biosensors have been developed, which contain a bipartite NAD⁺-binding domain derived from bacterial NAD⁺-dependent DNA ligase fused to a circularly permuted Venus fluorescent protein (cpVenus) (Cambronne et al., 2016). These NAD⁺ biosensors can be expressed either in nucleus or in cytoplasm and exhibit reduction in their fluorescent signals upon binding to NAD⁺. With these powerful molecular tools, NAD⁺ has been detected in the nucleus and almost all cytoplasmic organelles, including mitochondria, peroxisomes, endoplasmic reticulum (ER), and Golgi complex (Cambronne et al., 2016; Dölle et al., 2010; VanLinden et al., 2017).

In this review, we will focus on the compartmentalized regulation of NAD⁺ biosynthesis and metabolism in cancer development and progression, as well as on currently available small molecules targeting NAD⁺ and its associated biomolecules for cancer treatment. For a comprehensive understanding of the NAD⁺ metabolome in tumorigenesis, we refer readers to several excellent reviews recently published that

cover the topic extensively (Demarest et al., 2019; Sharif et al., 2019; Yaku, Okabe, Hikosaka, & Nakagawa, 2018).

2. Compartmentalized NAD⁺ pools in cancer

2.1. Mitochondrial NAD⁺ pool

Mitochondria house the largest NAD⁺ pool with concentrations of ~250 μM, which account for 50–70% of total cellular NAD⁺ in cardiac myocytes and neurons (Alano et al., 2007; Cambronne et al., 2016). Maintaining mitochondrial NAD⁺ levels is crucial to cell survival, especially when nuclear and cytosolic pools of NAD⁺ become depleted under stress conditions (Yang et al., 2007). Although the cytosolic NAD⁺ and mitochondrial NAD⁺ pools are connected by glycolysis and oxidative phosphorylation (Ying, 2008), the exact mechanism of mitochondrial NAD⁺ regeneration remains unclear. There are two possible sources of mitochondrial NAD⁺, imported from cytosol and locally synthesized in mitochondrial matrix. Numerous studies have gathered evidence supporting each source, although detailed mechanisms are still lacking. The most prominent outstanding question regarding the imported NAD⁺ is how NAD⁺ and its precursors, including NAM, nicotinamide riboside (NR), nicotinic acid (NA), nicotinic acid riboside (NAR), and nicotinamide mononucleotide (NMN), cross the mitochondrial membrane. One study shows that cytosolic NMN can be taken up by mitochondria and converted to mitochondrial NAD⁺ by NMNAT3 (Nikiforov, Dölle, Niere, & Ziegler, 2011), and partly supporting this notion is the fact that, so far, no mitochondrial NAD⁺ transporter has been identified in mammalian cells (Barile, Passarella, Danese, & Quagliariello, 1996; VanLinden et al., 2015). Additionally, supplementation of NR increases mitochondrial NAD⁺ level both in cultured cells and the mouse liver (Cantó et al., 2012). However, another group shows that providing precursors like NAM, NR, NA or NMN does not affect mitochondrial NAD⁺ content (Felici, Lapucci, Ramazzotti, & Chiarugi, 2013). Despite the lack of a confirmed transporter, supplying exogenous NAD⁺ has been shown to significantly increase mitochondrial NAD⁺ content (Felici et al., 2013; Pittelli et al., 2011), and a recent study using isotopic labeling of NR and NAR indicates that cytosolic NAD⁺ can be directly transported into mitochondria (Davila et al., 2018). Similar questions remain outstanding regarding the source of NAD⁺ from mitochondrial local synthesis. For example, the presence of mitochondrially localized enzymes essential for NAD⁺ synthesis remain unconfirmed. Nicotinamide phosphoribosyltransferase (NAMPT) catalyzes the rate-limiting step in the NAD⁺ salvage pathway. One study reported the localization of NAMPT in mitochondria (Yang et al., 2007), while another group could not confirm these results (Pittelli et al., 2010). Given a lack of consensus supporting the presence of mitochondrial NAD⁺ transporters or NAMPT in mammals, it is proposed that mitochondrial NAD⁺ is produced through NMN shuttling from the cytoplasm followed by NMNAT3 catalysis (Sharif et al., 2019; VanLinden et al., 2015). NMNAT3 is reported to have a mitochondrial localized splice variant FKSG76 and the ectopically expressed NMNAT3 localizes to mitochondria (Felici et al., 2013). However, although the mRNA transcripts can be detected, the presence of the endogenous protein is yet to be confirmed (Felici et al., 2013). Although one study suggests that NMNAT3 overexpression increases mitochondrial NAD⁺ (Son, Kwon, Son, & Cho, 2016), other studies have reported that NMNAT3 is dispensable for the maintenance of mitochondrial NAD⁺ level (Hikosaka et al., 2014; Yamamoto et al., 2016). It is likely that mitochondrial NAD⁺ comes from both local synthesis as well as transport from the cytosol. Each source serves as the backup for the other, and both sources together contribute to the tightly maintained NAD⁺ concentrations within mitochondria. Since local synthesis also requires the transport of NAD⁺ precursors, the concentration of mitochondrial NAD⁺ would be closely connected to the availability of cytosolic NAD⁺ and/or its precursors.

Whether transported or locally synthesized, mitochondrial NAD^+ serves an essential role in tumorigenesis. Mitochondrial NAD^+ is not only essential for bioenergetic processes underlying cell growth, proliferation and survival such as TCA cycle, oxidative phosphorylation, and fatty acid oxidation, but also serves as an obligatory co-substrate for mitochondrial-localized SIRT3 (SIRT3, 4 and 5) that have been implicated as master regulators of metabolic homeostasis (George & Ahmad, 2016; Stein & Imai, 2012). For example, SIRT3 has been shown to physically interact Complex I subunit 9 (NDUFA9, NADH:Ubiquinone Oxidoreductase Subunit A9) and selectively deacetylate and augment Complex I activity to maintain basal ATP production by oxidative phosphorylation (Ahn et al., 2008). SIRT4 or SIRT5 knockout animals exhibit significantly dysregulated glycolysis, gluconeogenesis, and fatty acid metabolism (Du et al., 2018; Wood et al., 2018). Interestingly, all mitochondrial SIRT3s have potential dual functions in cancer in that they can serve as either tumor suppressors or tumor promoters (Demarest et al., 2019).

SIRT3 is predominantly localized in the mitochondria, but is also reported to be a nuclear NAD^+ -dependent histone deacetylase that is translocated into mitochondria upon etoposide and UV-induced DNA damage putatively leading to derepression of nuclear genes involved in mitochondrial function (Scher, Vaquero, & Reinberg, 2007). SIRT3 has been shown to possess oncogenic function, for instance it inhibits apoptosis and promotes cell growth and division by enhancing pyruvate dehydrogenase activity in glycolysis (Ozden et al., 2014) (Fig. 2), facilitating mtDNA repair (Cheng et al., 2013), preserving mitochondrial membrane integrity (Kim et al., 2010; Yang et al., 2016), and increasing cell resilience to environmental stress (Tao et al., 2010; Torrens-Mas, Pons, Sastre-Serra, Oliver, & Roca, 2017). SIRT3 deacetylates and

stabilizes hydroxymethyltransferase 2 (SHMT2), a mitochondrial enzyme that catalyzes the reversible reaction of serine and tetrahydrofolate to generate glycine and 5,10-methylene tetrahydrofolate, promoting colorectal carcinogenesis (Wei et al., 2018). Silencing SIRT3 has been shown to increase ROS production and sensitize breast cancer cells to cytotoxic compounds (Torrens-Mas et al., 2017).

On the other hand, SIRT3 can also function to suppress tumor growth by negatively regulating the Warburg effect, destabilizing hypoxia-inducible factor-1 α (HIF1 α), and alleviating ROS burden (Bell, Emerling, Ricoult, & Guarente, 2011; Finley et al., 2011). A recent study shows that SIRT3 promotes FOXO3A by attenuating Wnt/ β -catenin pathway, thereby suppressing EMT in prostate cancer cells (Li, Quan, & Xia, 2018). Additionally, SIRT3 can downregulate 2-hydroxyglutarate (2HG) production through deacetylation of mitochondrial isocitrate dehydrogenase 2 (IDH2) to reverse the tumor-promoting mitochondrial metabolic profile and reduce colony formation and tumorigenesis of breast cancer cells (Dvorak, Zelenka, Smolkova, Vitek, & JeZek, 2017; Zou et al., 2017). Upregulation of mitochondrial NAD^+ by overexpressing mitochondrial enzyme nicotinamide nucleotide transhydrogenase (NNT) is accompanied by increased SIRT3 activity and compromised clonogenicity of glioblastoma tumor-initiating cells (Son et al., 2017), while downregulation of mitochondrial NAD^+ through dysregulation of mitochondrial Ca^{2+} signaling inhibits SIRT3, leading to deactivation of superoxide dismutase 2 (SOD2) and increased reactive oxygen species (ROS) production, facilitating metastasis of hepatocellular carcinoma cells (Ren et al., 2017).

SIRT4 mainly functions as an ADP-ribosyltransferase. Similar to SIRT3, SIRT4 has both oncogenic and tumor-suppressive activities (Huang & Zhu, 2018). It has been shown that SIRT4 promotes

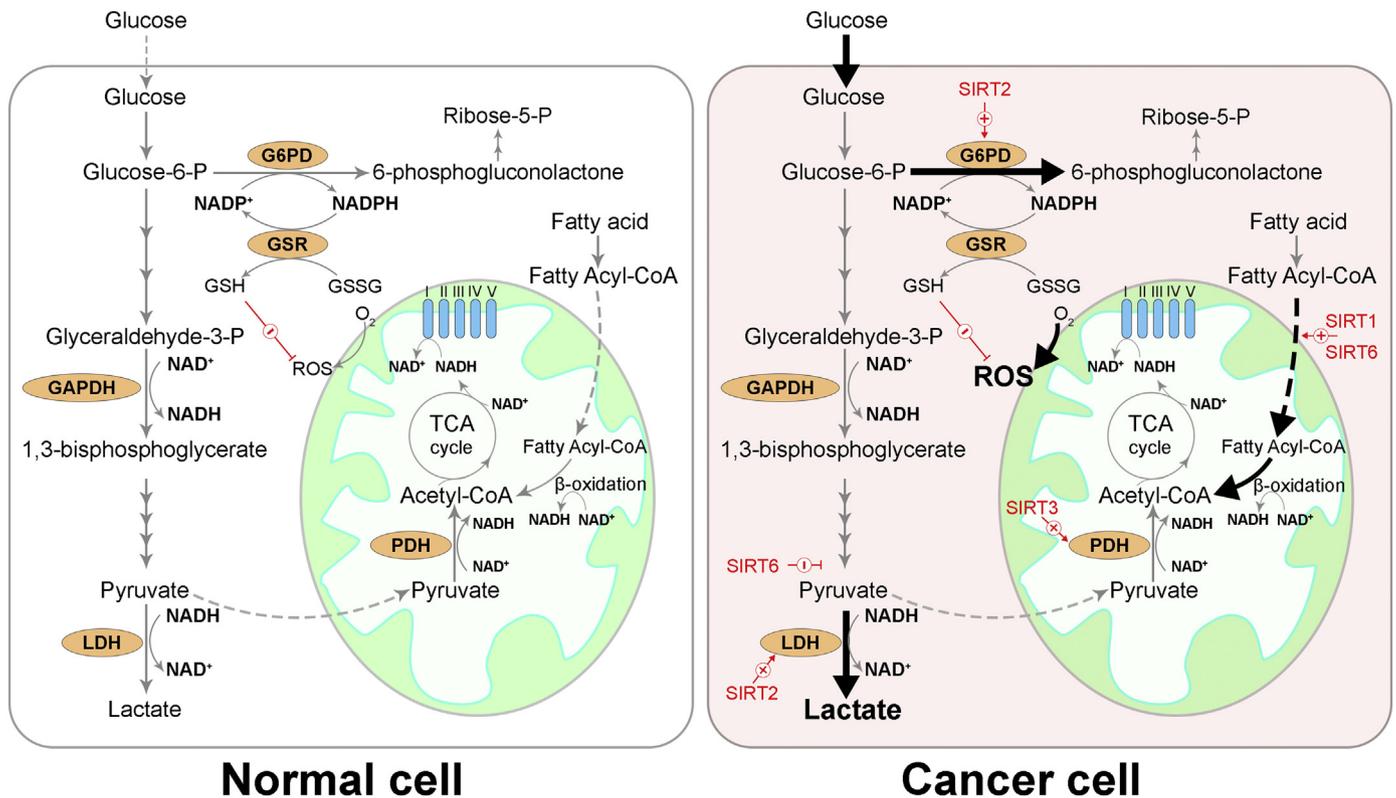


Fig. 2. Overview of NAD^+ in cancer metabolic reprogramming. Under normal conditions, glucose is metabolized by glycolysis and generates pyruvate, which is preferentially transported into mitochondria. Pyruvate dehydrogenase (PDH) converts pyruvate to acetyl-CoA, which is further metabolized by tricarboxylic acid (TCA) cycle, concomitantly converting NAD^+ to NADH. NADH and FADH_2 deliver high energy electrons to the electron transport chain (ETC) complexes (I-V) and power the production of ATP. Cancer cells adapt their metabolism to fulfill their increased demand for energy, biosynthetic intermediates, and to counter aerobic respiration-induced oxidative stress by: (1) increasing glucose uptake, (2) preferentially converting pyruvate to lactate by lactate dehydrogenase (LDH) even under aerobic conditions (Warburg effect) to provide metabolic building blocks, (3) diverting glycolysis to pentose phosphate pathway (PPP), where NADPH is produced to neutralize reactive oxygen species (ROS) and ribose-5-phosphate (ribose-5-P) is produced to support nucleic acid synthesis, (4) promoting fatty acid oxidation to provide acetyl-CoA. GAPDH: glyceraldehyde-3-phosphate dehydrogenase, G6PD: glucose-6-phosphate dehydrogenase, GSR: glutathione-disulfide reductase, GSSG: glutathione disulfide, GSH: glutathione.

tumorigenic transformation when induced under oncogenic stresses such as DNA damage and ER stress (Jeong, Hwang, & Seong, 2016), and maintains metabolic homeostasis (Wood et al., 2018). In contrast, evidence supporting the tumor-suppressive activity of SIRT4 comes from studies that show downregulation of SIRT4 inhibits apoptosis and desensitizes colorectal cancer cells to chemotherapy (Zhu et al., 2018), and that SIRT4 suppresses tumor growth by inhibiting glutamine metabolism (Csibi et al., 2013; Jeong et al., 2013; Wang et al., 2018).

SIRT5 plays an integral role in metabolic reprogramming during tumorigenesis (Bringman-Rodenbarger, Guo, Lyssiotis, & Lombard, 2018). SIRT5 is an inefficient deacetylase; instead it regulates its target protein activity by NAD⁺-dependent removal of malonyl, glutaryl, or succinyl groups from lysine residues (Du et al., 2011; Tan et al., 2014). For example, SIRT5 confers chemotherapy resistance to colorectal carcinoma through demalonylation of succinate dehydrogenase complex subunit A (SDHA), leading to accumulation of the oncometabolite succinate (Du et al., 2018). Additionally, SIRT5 deglutarylates and activates glutamate dehydrogenase 1 (GLUD1), permitting the entry of glutamine into the TCA cycle to support colorectal tumorigenesis (Wang et al., 2018). Moreover, SIRT5 desuccinylates and activates mitochondrial serine hydroxymethyltransferase 2 (SHMT2) that catalyzes serine catabolism, driving cancer cell proliferation (Yang et al., 2018). Notably, SIRT5-mediated desuccinylation also inhibits peroxisomal acyl-CoA oxidase 1 (ACOX1) activity, reduces oxidative stress, and protects against the development of hepatocellular carcinoma (Chen et al., 2018).

Recent studies highlight the role of mitochondrial NAD⁺ in stem cell function. It has been shown that NAD⁺ repletion by NR supplementation improves mitochondrial function and enhances stem cell function in aged mice (Zhang et al., 2016). Overexpressing NNT and NMNAT3 to restore mitochondrial NAD⁺ levels increases the lifespan of human mesenchymal stem cells (MSCs) by inhibiting cell senescence and also increases the reprogramming efficiency of aged somatic cells (Son et al., 2016). MSCs comprise tumor stroma, a key part of the tumor microenvironment, and are known to promote tumor progression and metastasis (Ridge, Sullivan, & Glynn, 2017). MSCs also possess immunomodulatory properties, including suppression of T-cell proliferation, potentially contributing to immunosuppression (Krueger, Thorek, Meeker, Isaacs, & Brennen, 2019; Shi et al., 2018). Thus, targeting mitochondrial NAD⁺ synthesis could potentially eliminate the tumor-promoting stromal cells that contribute to tumor progression and metastasis.

2.2. Nuclear NAD⁺ pool

The nuclear NAD⁺ pool is estimated to be smaller (~70–100 μM) than the mitochondrial NAD⁺ pool (Cambronne et al., 2016; Fjeld, Birdsong, & Goodman, 2003; Nakagawa, Lomb, Haigis, & Guarente, 2009). Nuclear NAD⁺ is thought to be mainly synthesized by nuclear NMNAT1, the most ubiquitously expressed NMNAT isoform (Lau, Niere, & Ziegler, 2009). Two-photon excitation microscopy shows specific elevation of NAD⁺ in the nucleus with ectopic expression of NMNAT1 (Zhang et al., 2012). Mutations in NMNAT1 have been found to cause Leber congenital amaurosis (LCA)9, a childhood blindness (Falk et al., 2012; Koenekoop et al., 2012). It was proposed that reduced NAD⁺ production is associated with the photoreceptor pathology in LCA9 (Koenekoop et al., 2012).

The nuclear role of NAD⁺ is centered around PARP-mediated ADP-ribosylation and SIRT-mediated deacetylation. PARP1 is the most abundantly expressed member of the PARP protein family and has been extensively studied in cancer, including DNA damage detection and repair, telomerase protection, chromatin modification, transcriptional regulation, p53-mediated apoptosis, and programmed necrosis (Brown, O'Carrigan, Jackson, & Yap, 2017; Chaudhuri & Nussenzweig, 2017; Gomez et al., 2006; Kim, Zhang, & Kraus, 2005; Sosna et al., 2014). Upon DNA damage, PARP1 activation consumes up to 80% nuclear NAD⁺ for massive ADP-ribosylation, especially poly ADP-ribosylation (PARylation) (Yu et al., 2002). A recent study suggests

PARP1 has dual functions in colorectal tumorigenesis, where it suppresses tumor initiation following DNA alkylation but paradoxically enhances inflammation-driven tumor progression (Dörsam et al., 2018). Mechanistic investigation reveals that PARP1 poly-ADP-ribosylates transcription factor E2F1 and restrains E2F1-induced apoptosis in cancer cells (Kumari et al., 2015). Activated PARP1 was also reported to possess a transcriptional-regulatory function (Schiewer et al., 2012; Schiewer & Knudsen, 2014) that promotes E2F1-mediated induction of DNA repair factors, thereby promoting progression to aggressive and treatment-resistant prostate cancer (Schiewer et al., 2018). For an in-depth discussion of the localization and function of other PARP enzymes, we refer readers to this review (Bai, 2015).

In addition to PARP, nuclear NAD⁺ also regulates many oncogenes and tumor suppressor genes through SIRT. Through various targets, nuclear SIRT (SIRT 1, 6 and 7) carry out multifaceted functions in regulating chromatin structure, genomic stability, cellular metabolism, and immune response that go awry in cancer cells (Chalkiadaki & Guarente, 2015; Zhu et al., 2018). Specifically, SIRT1 binds and deacetylates p53 upon DNA damage, resulting in repression of p53 transcriptional activity and subsequent disinhibition of cell cycle arrest (Vaziri et al., 2001). SIRT1 also deacetylates and inactivates E2F1, promoting abnormal cell proliferation and malignant transformation (Wang et al., 2006). Moreover, SIRT1 activity is required for O-GlcNAcylation-mediated cancer invasion and metastasis (Ferrer et al., 2017). Paradoxically, SIRT1 mutant mice exhibit compromised DNA repair, genome instability, and enhanced tumorigenesis (Wang et al., 2008), whereas SIRT1-overexpressing mice show delayed appearance of K-Ras^{G12V}-driven lung adenocarcinomas and extended survival (Costa-Machado et al., 2018), indicating a tumor-suppressive role of SIRT1. Notably, SIRT1 can shuttle between nuclear and cytoplasm as an adaptation to environmental stress. For example, in human bronchial epithelial cells where SIRT1 is enriched in cytoplasm under normal conditions, cigarette smoke exposure induces nuclear translocation of SIRT1, contributing to cell survival under oxidative stress but also likely promoting malignant transformation (Yanagisawa et al., 2018). Conversely, in primary fibroblast cells, where SIRT1 is predominantly nuclear localized, malignant transformation induces aberrant translocation of SIRT1 to cytoplasm, likely through regulation by PI3K (phosphoinositide 3-kinase)/IGF (insulin-like growth factor)-1R signaling (Byles et al., 2010). Inhibition of cytoplasmic SIRT1 promotes caspase-2-dependent apoptosis in breast cancer cells by promoting dissociation of its negative regulator, 14-3-3ζ (Andersen et al., 2011).

SIRT6 functions as a master regulator of metabolic programming and maintains genome integrity during tumorigenesis (Desantis, Lamanuzzi, & Vacca, 2018). SIRT6 has high tumorigenic activity, and high expression of SIRT6 in colon cancer samples has been shown to correlate with poor prognosis (Geng et al., 2018). SIRT6 regulates glucose homeostasis and suppresses the Warburg effect through inhibition of pyruvate kinase M2 (PKM2), the final rate-limiting step of glycolysis (Bhardwaj & Das, 2016; Sebastián et al., 2012) (Fig. 2). In an acidic tumor microenvironment, SIRT1 and SIRT6-mediated histone deacetylation downregulate acetyl-CoA carboxylase 2, (ACC2), a mitochondria-anchored enzyme that normally inhibits degradation of newly synthesized fatty acids, thereby facilitating a shift of the source of acetyl-CoA from glycolysis-derived pyruvate to fatty acid oxidation (Corbet et al., 2016) (Fig. 2). Additionally, SIRT6 can be mobilized to DNA damage sites by lamin A (Ghosh, Liu, Wang, Hao, & Zhou, 2015) or c-Jun N-terminal kinase (Van Meter et al., 2016), and promotes DNA repair by activating and recruiting PARP1 (Mao et al., 2011; Van Meter et al., 2016) and by mediating the rate-limiting step of DNA base excision repair through the 5'-deoxyribose phosphate (dRP) lyase activity of DNA polymerase β (Mostoslavsky et al., 2006). Ubiquitin-protein ligase E3A (UBE3A)-mediated SIRT6 degradation epigenetically activates annexin A2 and promotes cancer cell proliferation and invasion (Kohli, Bhardwaj, Kumari, & Das, 2018). Moreover, SIRT6-β-catenin signaling is involved in the EMT of ovarian cancer cells (Bae

et al., 2018). Interestingly, NAMPT has been found to be a direct substrate of SIRT6 - SIRT6-mediated deacetylation activates NAMPT and boosts intracellular NAD⁺ level in cancer cells, conferring resistance to oxidative stress (Sociali et al., 2018).

The pathophysiological role of SIRT7 in cancer is relatively poorly understood, due to its low enzymatic activity and few identified substrates (Blank & Grummt, 2017). SIRT7 is known to regulate ribosome biogenesis and RNA metabolism. For example, SIRT7 activates RNA polymerase II transcription by deacetylating cyclin dependent kinase 9 (CDK9), a subunit of the elongation factor P-TEFb, exhibiting oncogenic property (Blank et al., 2017). SIRT7 serves as a prognostic factor in colorectal cancer as it activates Raf-MEK-ERK pathway and induces EMT, promoting cell proliferation and invasion (Yu et al., 2014). However, SIRT7 also shows tumor-suppressive activity, as it inhibits breast cancer metastasis by promoting SMAD4 degradation and antagonizing TGF- β signaling (Tang et al., 2017).

The extensive network of PARP and SIRT proteins and their regulatory targets indicate the profound impact of nuclear NAD⁺. As PARP and SIRT are NAD⁺-consuming enzymes, their activity requires the replenishment of nuclear NAD⁺, mainly through nuclear NAD⁺ synthesis. It is therefore conceivable to influence PARP- or SIRT-dependent activity by manipulating nuclear NAD⁺ levels. Indeed, PARP1 activity is tightly regulated by the availability and accessibility of nuclear NAD⁺. It has recently been shown that under normal conditions, PARP1 has limited NAD⁺ access that accounts for the basal unstimulated PARP1 activity; while upon DNA damage, PARP1 interaction with DNA strand breaks leads to full NAD⁺ access to its catalytic site and promotes allosteric activation of PARP1 (Langelier, Zandarashvili, Aguiar, Black, & Pascal, 2018). Additionally, NMNAT1 can be recruited to target gene promoters by PARP1, and generates NAD⁺ to support PARP1 catalytic activity (Zhang et al., 2012). A recent study shows that NAD⁺ can directly regulate PARP1 activity by regulating the binding of PARP1 to the protein DBC1 (Deleted in Breast Cancer 1), a potential tumor suppressor with an NAD⁺ binding pocket. Depletion of NAD⁺ promotes the binding of PARP1 to DBC1, which inhibits PARP1 activity and leads to DNA damage accumulation (Li et al., 2017). This process may serve as a negative feedback mechanism that prevents excessive depletion of NAD⁺ by PARP1 upon DNA damage (Li et al., 2017). Likewise, SIRT1 activity can be directly regulated by nuclear NAD⁺. Increasing cellular NAD⁺ level by overexpressing NAMPT directly promotes the recruitment of SIRT1 catalytic domain to target gene and enhances transcriptional regulation (Revollo, Grimm, & Imai, S.-i., 2004).

2.3. Cytosolic NAD⁺ pool

The cytosolic NAD⁺ pool serves as a central hub that connects and regulates other NAD⁺ pools (Fig. 1). Cytosolic NAD⁺ is mainly synthesized by NMNAT2, which has been reported to be localized in the Golgi apparatus (Berger et al., 2005; Mayer et al., 2010). Cytosolic and nuclear NAD⁺ pools are closely connected as the levels of nuclear NAD⁺ and cytosolic NAD⁺ are comparable, and the small molecule NAMPT inhibitor FK866 produces a similar reduction of nuclear NAD⁺ and cytosolic NAD⁺ (Cambronne et al., 2016). It is likely that the NAD⁺ precursors, NAM and NMN, are shared between nucleus and cytosol. The final step, converting NMN to NAD⁺, is catalyzed locally by either the nuclear or cytosolic NMNAT. Recent studies found that depleting NMNAT2 significantly reduces cytosolic NAD⁺ but minimally affects nuclear NAD⁺, whereas nuclear NAD⁺ cannot completely replenish cytosolic NAD⁺ when NMNAT2 is depleted (Cambronne et al., 2016). These observations suggest that nuclear and cytosolic NAD⁺ pools are locally and specifically regulated by NMNAT1 and NMNAT2, respectively, in a compartment-specific manner.

Cytosolic NAD⁺/NADH together with other metabolic intermediates, including NADP⁺/NADPH (nicotinamide adenine dinucleotide phosphate) and FAD⁺/FADH₂ (flavin adenine dinucleotide), serve as key metabolic currency that is controlled by various oncogenes and tumor

suppressors during metabolic reprogramming of tumors and host immune cells (Pearce, Poffenberger, Chang, & Jones, 2013). Cytosolic NAD⁺ is pivotal for glycolysis in glucose metabolism, where glyceraldehyde-3-phosphate dehydrogenase converts glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate and NAD⁺ is reduced to NADH (Fig. 2). Since NADH cannot cross mitochondrial membrane, electrons from NADH can enter the mitochondrial electron transport chain via glycerol-3-phosphate shuttle where NAD⁺ is regenerated from reducing dihydroxyacetone phosphate to glycerol-3-phosphate, or via malate-aspartate shuttle, where NAD⁺ is regenerated from reducing oxaloacetate to malate (MacDonald, 1981; MacDonald, 1982). Cytosolic NAD⁺ is essential for cancer cell growth due to the elevated level of glycolytic flux for increased metabolic demands. However, cancer cells shift their glucose metabolism to aerobic glycolysis (the aforementioned Warburg effect), where they enhance glucose uptake but reduce oxidative phosphorylation, and energy is generated from cytosolic lactate fermentation (Warburg, Wind, & Negelein, 1927). This metabolic alteration provides several benefits. First, NAD⁺ can be regenerated from NADH during the conversion of pyruvate to lactate by lactate dehydrogenase (LDH) (Fig. 1), thereby maintaining active aerobic glycolysis (Vander Heiden, Cantley, & Thompson, 2009). Second, the Warburg effect minimizes ROS production from oxidative phosphorylation in mitochondria, promoting cell survival (Wang et al., 2019). Third, increased glucose uptake activates pentose phosphate pathway (PPP) and generates glycolytic intermediates, such as nucleic acids, amino acids, and lipids, as metabolic building blocks to meet the anabolic needs of cancer cell proliferation (Liberti & Locasale, 2016) (Fig. 2).

Increased demand of cytosolic NAD⁺ in glucose metabolism during cancer development indicate cancer cells need to prioritize the replenishment of cytosolic NAD⁺ pools (Vander Heiden et al., 2009), which can be achieved by balancing the activities of different NMNATs. A recent study shows that NMNAT1 and NMNAT2 compete for NMN, their common substrate, to promote the compartmentalized regulation of nuclear and cytosolic NAD⁺ pools. Specifically, in undifferentiated adipocytes, NMN is mostly converted by NMNAT1 to maintain nuclear NAD⁺ levels, required to activate PARP1 and inhibit transcriptional events involved in cell differentiation. During adipogenesis, NMNAT2 supports glucose metabolism by producing cytosolic NAD⁺ and the reduction of nuclear NAD⁺ disinhibits transcriptional program, permitting adipogenic differentiation (Ryu et al., 2018). Interestingly, the *NMNAT1* gene in cancerous tissue seems to be mostly mutated or deleted (source: www.cbioportal.org) (Cerami et al., 2012). Cancer cells with decreased *NMNAT1* expression exhibit low concentrations of nuclear NAD⁺ and reduced PARylation of multifunctional nuclear protein CCCTC-binding factor, leading to epigenetic silencing of tumor suppressor genes (Henderson, Miranda, & Emerson, 2017). *NMNAT1* has been reported to function as a DNA damage response gene and its inhibition delays DNA repair, thus potentially sensitizing cancer cells to DNA damage-inducing stressors; however it also increases rRNA transcription which contributes to cellular transformation, suggesting a context-dependent dual role in cancer (Song et al., 2013). Low levels of *NMNAT1* are considered a poor prognostic marker for renal cancer (Human Protein Atlas, www.proteinatlas.org) (Uhlen et al., 2017; Uhlén et al., 2015). By contrast, the *NMNAT2* gene appears to be amplified in most cancer types (source: www.cbioportal.org) (Cerami et al., 2012). Specifically, *NMNAT2* upregulation in colorectal cancer correlates with the invasive depth and TNM stage (Cui et al., 2016; Qi et al., 2018). The upregulated *NMNAT2* enzymatic activity in non-small cell lung cancer (NSCLC) can be triggered by SIRT3-mediated deacetylation (Li et al., 2013) or p53 signaling (Pan et al., 2014). Depletion of *NMNAT2* via shRNA in neuroblastoma cells inhibits cell growth, and reducing glucose availability decreases *NMNAT2* expression and activates PARP1 (Ryu et al., 2018). These observations support that putative mechanisms involving metabolic regulation of compartmentalized NAD⁺ synthesis could serve as adaptations during tumorigenesis to support glucose metabolism and fuel rapid cell growth and division.

SIRT2, a major cytosolic NAD⁺ consuming enzyme, was originally considered a tumor suppressor (Park et al., 2012). SIRT2 deacetylates and inhibits the peroxidase activity of peroxiredoxin-1 (Prdx-1), thereby sensitizing breast cancer cells to oxidative stress (Fiskus et al., 2016). Additionally, SIRT2 deacetylates HIF1 α in the cytosol, promoting hydroxylation and degradation of HIF1 α and suppressing hypoxia-dependent tumor growth (Seo et al., 2015). Mutations in *SIRT2* lead to genomic instability and promote tumorigenesis (Head et al., 2017; Kim et al., 2011). However, growing evidence demonstrates that SIRT2 promotes cancer growth by stabilizing the Myc family of oncoproteins (Liu et al., 2013) and potentiating Notch signaling (Zhao et al., 2014). Thiomyristoyl, a SIRT2-selective inhibitor, promotes c-Myc oncoprotein ubiquitination and degradation, exhibiting broad anticancer activity with minimal effect on non-cancerous cell lines and tumor-free mice (Jing et al., 2016).

Recent studies highlight the role of SIRT2 as a cytoplasmic nutrient sensor that directs metabolic activity to match the energy need for cancer growth, typically through regulating glycolysis and fatty acid synthesis. In glycolysis, many SIRT2 downstream deacetylation targets have been identified, including aldolase, enolase, LDH, and pyruvate kinase (Park et al., 2016). For example, SIRT2 deacetylates and activates LDH-A to accelerate glycolysis and lactate production, promoting pancreatic cancer growth (Zhao et al., 2013) (Fig. 2). Additionally, SIRT2 regulates PPP by activating glucose-6-phosphate dehydrogenase (G6PD) to boost NADPH production, thereby supporting leukemia cell proliferation (Xu, Wang, Li, & Wang, 2016) (Fig. 2). Moreover, SIRT2 mediates tetramerization of pyruvate kinase and regulates breast cancer progression (Park, Ozden, et al., 2016). In rapidly proliferating cells that undergo fatty acid synthesis, citrate produces from TCA cycle can be exported from mitochondria to cytosol and converted by ATP citrate lyase (ACLY) to cytosolic acetyl coenzyme A (acetyl-CoA). In lung cancer, upregulated acetylation of ACLY increases its protein stability, while SIRT2 deacetylates ACLY and promotes its ubiquitination, thereby inhibiting *de novo* lipid synthesis and cell proliferation (Lin et al., 2013). Since SIRT2 activity can be pharmacologically regulated by modulation of intracellular NAD⁺/NADH ratio (Seo et al., 2015), it becomes a promising target to control nutrient sensing and metabolism in cancer cells typically when its level is dysregulated in tumorigenesis.

Cytosolic *de novo* NAD⁺ synthesis from tryptophan metabolism (via the kynurenine pathway, KP) (Fig. 1) appears to be important for cells comprising the tumor microenvironment, including innate immunity-associated cells, such as macrophages, which can promote anti-tumor responses and enhance therapeutic outcomes (Moynihan & Irvine, 2017). The tryptophan-metabolizing enzyme indoleamine 2,3 dioxygenase 1 (IDO1) that catalyzes the initial step of KP is frequently overexpressed and activated in cancer and immunoregulatory cells (Prendergast, Malachowski, DuHadaway, & Muller, 2017). Macrophage effector responses have been shown to rely on the KP-produced NAD⁺ levels as their metabolic switch, and decreases in NAD⁺ are likely to at least partially underlie innate immune dysfunction (Minhas et al., 2019). Interestingly, KP activation induced by immune challenge paradoxically reduces NAD⁺ synthesis by inhibiting the conversion of quinolinic acid (QA) to NAD⁺ (Minhas et al., 2019). KP metabolites promote colorectal cancer (CRC) growth by activating PI3K-Akt signaling (Bishnupuri et al., 2019). Collectively these findings underscore the importance of cytosolic *de novo* NAD⁺ synthesis in cancer development and the clinical implications of IDO1 modulators in anti-cancer therapies.

NADPH is in high demand for cancer cell growth as it functions as a key cofactor in multiple metabolic pathways, and it neutralizes ROS burden during rapid cancer cell growth (Patra & Hay, 2014). Under oxidative or hypoxic stress, promoting NADPH production through TIGAR (TP53-induced glycolysis and apoptosis regulator)-mediated diversion of glycolysis into PPP increases cell survival (Bensaad et al., 2006; Ko et al., 2016; Wanka, Steinbach, & Rieger, 2012) (Fig. 2). Recent studies have highlighted the cancer-promoting role of NAD⁺ kinase (NADK) that converts cytosolic and mitochondrial NAD⁺ to NADP⁺, which can be further reduced to NADPH by G6PD in PPP (Tedeschi et al., 2016).

NADK has been found to be localized in both the cytosol (cNADK) and mitochondria (mNADK) (Ohashi, Kawai, & Murata, 2012). Activating mutations in cNADK has been reported to drive malignant transformation of pancreatic cells (Tsang et al., 2016). An NADK inhibitor prodrug, thionicotinamide, has shown potent cytotoxic effects to cancer cells by decreasing the cytosolic NADPH pool, and can help to sensitize cancer cells to other chemotherapeutic agents (Tedeschi et al., 2015). NADK activity is also likely to be dampened by conditions that decrease NAD⁺ concentrations (either by increased consumption or by inhibiting its synthesis), and the reduced level of NADP⁺ can potentially lead to replication stress, including compromised nucleotide synthesis and accompanying tumoricidal DNA breaks (Chiarugi et al., 2012).

2.4. Extracellular NAD⁺ pool

Under physiological conditions, the extracellular NAD⁺ concentration in mammalian serum is reported to be maintained between 0.1 and 0.5 μ M (Davies et al., 1999; O'Reilly & Niven, 2003). Extracellular and intracellular NAD⁺ pools are intricately connected (Fig. 1). First, extracellular NAD⁺ can be augmented by liberation of intracellular NAD⁺ upon cell damage or lysis (Adriouch et al., 2007; Haag et al., 2007). Intracellular NAD⁺ has been observed to be released from neuronal terminals by exocytosis, and functions as a neurotransmitter (Hwang et al., 2011; Mutafova-Yambolieva et al., 2007). A recent study demonstrates that prostate cancer cells release intracellular NAD⁺ into the culture media, indicating that NAD⁺ may serve as an autocrine or paracrine signaling molecule for nearby cancerous or noncancerous cells (Mottahedeh et al., 2018). Second, extracellular NAD⁺ helps to restore intracellular NAD⁺, as supplementation of extracellular NAD⁺ significantly elevates intracellular NAD⁺ level and counteracts cell death induced by the NAMPT inhibitor FK866 (Billington et al., 2008). It has been suggested that the direct exchange of intracellular and extracellular NAD⁺ pools can be achieved by connexin 43 hemi channels (Bruzzone, Guida, Zocchi, Franco, & De Flora, 2001), although the presence of connexin 43 hemi channels on the plasma membrane under physiological conditions has not been confirmed. On the other hand, pannexin hemi channels are possible candidates that would allow the exchange of NAD⁺ because of their channel conductance and permeability under physiological conditions (Wang & Dahl, 2018). Alternatively, extracellular NAD⁺ can be degraded to NMN, which is further metabolized by NR kinases (NRKs) to NR that can be imported as NAD⁺ precursors, likely through equilibrative nucleoside transporters (ENTs) (Nikiforov et al., 2011; Ratajczak et al., 2016).

Consumption of extracellular NAD⁺ mainly occurs through CD38, a ubiquitously expressed transmembrane NAD⁺ glycohydrolase (Chini et al., 2018). CD38 has a highly conserved homolog CD157 that may likely arise from a gene duplication event (Quarona et al., 2013). Induction of CD38 expression lowers NAD⁺ levels in prostate cancer cells and delays cancer development by arresting cell cycle progression and decreasing cell metabolism (Chmielewski et al., 2018). Increased expression of CD38 during aging may account for the age-related NAD⁺ decline and mitochondrial dysfunction (Camacho-Pereira et al., 2016). CD38 downregulates NAD⁺ in multiple ways. First, CD38 converts NAD⁺ to cADPR, an intracellular Ca²⁺-mobilizing messenger. Interestingly, CD38 has two opposing orientations, with its carboxyl-terminal catalytic domain facing extracellularly (type III) or intracellularly (type II). Therefore, CD38 is able to mobilize either extracellular or intracellular NAD⁺ pools, depending on its membrane topological transitions (Zhao, Lam, & Lee, 2012). Notably, a recent study shows that in primary and metastatic prostate cancer cells, CD38 expression specifically depletes extracellular NAD⁺ without altering the level of intracellular NAD⁺ (Mottahedeh et al., 2018). Second, CD38 also degrades extracellular NMN and generates NAM, which can cross plasma membrane and be converted to NAD⁺ through NAMPT and NMNAT (Camacho-Pereira et al., 2016).

Another important membrane-bound enzyme that degrades extracellular NAD⁺ is CD73. CD73 successively degrades NAD⁺ to NMN,

and further to NR (Garavaglia et al., 2012; Grozio et al., 2013) (Fig. 1). The conversion of extracellular NMN to NR is important to support intracellular NAD⁺ biosynthesis, and the downregulation or pharmacological inhibition of CD73 enhances FK866-induced cytotoxicity to cancer cells (Grozio et al., 2013; Sociali et al., 2016). Notably, CD73 also functions as an adhesion molecule that mediates cancer cell migration and invasion (Sadej & Skladanowski, 2012).

Extracellular NAD⁺ plays key roles in immune modulation. Inflammation can trigger the release of intracellular NAD⁺ into extracellular space, which in turn finetunes the immune response (Adriouch et al., 2007). Specifically, extracellular NAD⁺ acts as a pro-inflammatory cytokine that stimulates granulocytes through activation of P2Y11, augmenting chemotaxis (Moreschi et al., 2006). Additionally, evidences suggest the key regulatory role of extracellular NAD⁺ in anti-tumor T cell response. First, extracellular NAD⁺ activates the P2X7 purinergic receptor through ADP-ribosyltransferase 2 (ART2)-mediated ADP-ribosylation, leading to apoptosis of naïve T cells while facilitating the expansion of primed T cells (Adriouch et al., 2007; Haag et al., 2007). Second, Foxp3⁺ regulatory T cells (Treg) are specifically susceptible to NAD⁺-induced cell death (NICD), and systemic administration of NAD⁺ selectively depletes Tregs, promoting anti-tumor responses in mouse tumor xenograft models (Hubert et al., 2010). Third, T cells with reduced surface expression of the CD38 exhibited higher level of NAD⁺ and superior anti-tumor response, including enhanced oxidative phosphorylation, higher glutaminolysis, and altered mitochondrial dynamics (Chatterjee et al., 2018). Therefore, targeting CD38/NAD⁺ axis can potentially boost adoptive T cell therapy (ACT) to control tumor growth.

3. Targeting NAD⁺ metabolism in cancer therapy

Given the essential role of NAD⁺ in cell growth and other cancer-relevant processes, many enzymes in the NAD⁺ synthetic and metabolic pathways have been evaluated as targets for anti-cancer therapy. In this

section, we discuss the potential therapeutic outcomes of targeting NAD⁺ metabolism with the consideration of the distinct subcellular pools of NAD⁺ (Fig. 3).

3.1. Targeting NAD⁺ biosynthetic enzymes

3.1.1. NAMPT

Nicotinamide phosphoribosyltransferase (NAMPT) catalyzes the conversion of NAM to NMN, and is a rate limiting enzyme in NAD biosynthesis. NAMPT presents both intracellularly (iNAMPT) and extracellularly (eNAMPT). Intracellular NAMPT is reported to be localized in the nucleus and cytoplasm, while its presence in the mitochondria is controversial (Pittelli et al., 2010; Yang et al., 2007). It has been well-characterized that iNAMPT regulates intracellular NAD⁺ synthesis, thereby modulating cellular energetics and NAD⁺-dependent signaling (Garten et al., 2015). In contrast, the functional role of eNAMPT is poorly understood. Extracellular NAMPT not only has enzymatic activity that produces NMN, but also functions as a cytokine and known as pre-B cell colony-enhancing factor (PBEF) or visfatin that modulate immune response and lipid homeostasis (Grolla, Travelli, Genazzani, & Sethi, 2016; Jieyu et al., 2012). A wide range of stress signals, including ER and stress oxygen-glucose deprivation (OGD), promote eNAMPT excretion (Zhao et al., 2014). NAMPT has been recognized as a prognostic marker in cancer, where its expression level correlates with tumor behavior, stage, therapeutic efficacy, and patient survival (Gujar et al., 2016; Lucena-Cacace, Otero-Albiol, Jiménez-García, Muñoz-Galvan, & Carnero, 2018; Ohanna et al., 2018; Sharif et al., 2016). NAMPT signaling is also important for the tumor-promoting, pro-angiogenic function of tumor-associated neutrophils (TAN) (Pylaeva et al., 2019). Inhibition of NAMPT inhibits SIRT1 signaling and prevents transcription of pro-angiogenic genes in TAN (Pylaeva et al., 2019), potentially pointing to a targeting node in the tumor microenvironment. Additionally, a recent study indicates that NAMPT expression can be upregulated by the high

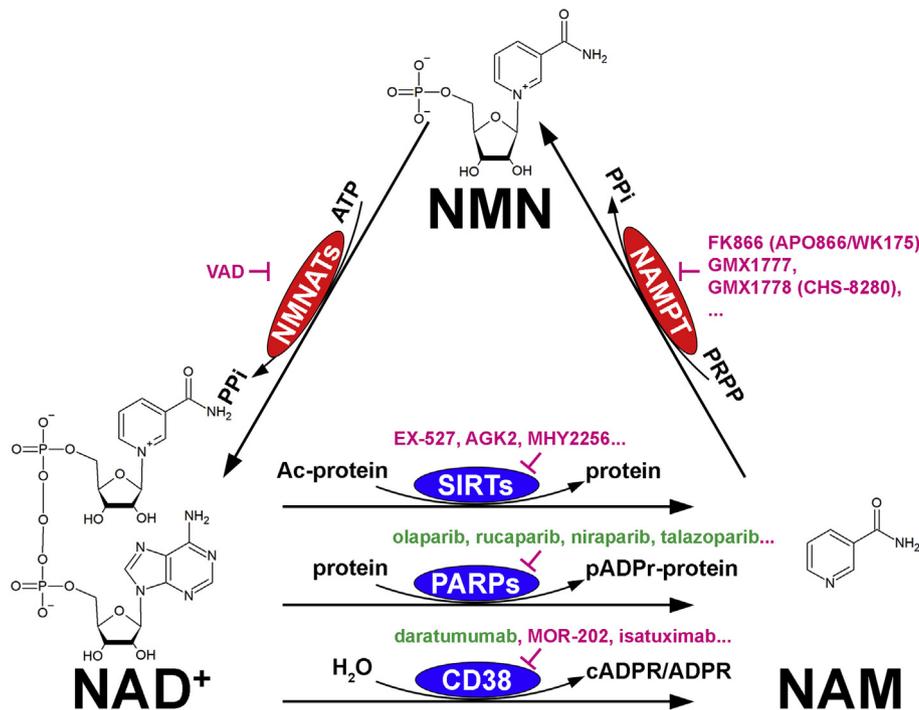


Fig. 3. Overview of pharmacological inhibitors targeting NAD⁺ metabolism. Nicotinamide phosphoribosyltransferase (NAMPT) catalyzes the transfer of the ribose 5-phosphate moiety of phosphoribosyl pyrophosphate (PRPP) to nicotinamide (NAM), producing nicotinamide mononucleotide (NMN) and pyrophosphate (PPi). Inhibitors targeting NAMPT include FK866, GMX1777, and GMX1778. Nicotinamide mononucleotide adenylyltransferase (NMNAT) catalyzes the transfer of the adenylate moiety of adenosine triphosphate (ATP) onto the NMN, producing NAD⁺ and PPI. An NAD⁺ analog, Vacor adenine dinucleotide (VAD), has shown potent inhibitory activity against NMNAT. NAD⁺ can be consumed by sirtuins (SIRT6) that catalyze the transfer of protein-bound acetyl (Ac) group onto the ADP-ribose moiety, producing O-acetyl-ADP-ribose (OAcADPR). NAD⁺ can also be consumed by poly (ADP-ribose) polymerases (PARPs) that catalyze the transfer of poly (ADP-ribose) moiety (pADPr) onto target protein. Moreover, NAD⁺ can be degraded by CD38 into cyclic ADP-ribose (cADPR) and ADPR. Several inhibitors targeting PARPs (olaparib, rucaparib, niraparib, talazoparib) and CD38 (daratumumab) are FDA-approved for treatment of specific cancers (drugs in green).

mobility group A (HMGA) proteins, and that the enhanced HMGA/NAMPT/NAD⁺ axis suppresses p53-mediated inhibition of p38 MAPK, activating NF- κ B signaling and promoting the pro-tumorigenic inflammatory senescence-associated secretory phenotype (SASP) (Nacarelli et al., 2019). Thus, targeting NAMPT may produce not only cell-autonomous tumor-inhibitory responses by reducing NAD⁺ availability in cancer cells but also inhibit the tumor-promoting paracrine signaling associated with the SASP. For the specific pathological roles of iNAMPT and eNAMPT in cancer, we refer readers to recent reviews with detailed discussions (Carbone et al., 2017; Dalamaga, Christodoulatos, & Mantzoros, 2018).

As NAMPT catalyzes the rate-limiting step in the synthesis of NAD⁺, inhibitors of NAMPT have been examined as anti-cancer agents. Three potent NAMPT inhibitors, FK866 (APO866/WK175), GMX1777, and GMX1778 (CHS-8280), have completed Phase I trials. By depletion of primarily cytosolic and nuclear NAD⁺, FK866 significantly attenuates glycolysis at the glyceraldehyde-3-phosphate dehydrogenase step (Tan et al., 2013), increases ROS production, induces apoptosis (Gehrke et al., 2014; Thakur et al., 2013), and inhibits the tumorigenicity of cancer stem cells (Gujar et al., 2016). FK866 delays tumor growth and increases radiation sensitivity in a mouse model of breast cancer through decreased NAD⁺ levels, pH and bioenergetics status (Muruganandham et al., 2005). FK866 combined with the protease inhibitor bortezomib synergistically depletes intracellular NAD⁺ to undetectable level in multiple myeloma cells and overcomes bortezomib resistance in anti-myeloma therapy (Cagnetta et al., 2013). GMX1777 is a prodrug of GMX1778, and has shown synergistic therapeutic benefits with other anti-cancer therapies in animal models, such as Pemetrexed in NSCLC (Chan et al., 2014) and radiation therapy in head and neck carcinoma (Kato et al., 2010).

Given the key role of NAMPT in NAD⁺ synthesis, inhibiting NAMPT is a double-edged sword, damaging both cancer and non-cancer cells that require NAD⁺. The impact on non-cancer cells results in unwanted on-target toxicity and indeed, all these NAMPT inhibitors have currently been discontinued for clinical evaluation due to systemic side effects, including thrombocytopenia, lymphopenia, cardiotoxicity, retinal toxicity, and gastrointestinal symptoms (Sampath, Zabka, Misner, O'Brien, & Dragovich, 2015). Despite these side effects, the potential of NAMPT as an anti-cancer target has yet to be fully realized, and several approaches have been considered to improve therapeutic outcome. First, several new NAMPT inhibitors have been developed, such as KPT-9274, LSN3154567, and OT-82, with broad spectrum anticancer activity as well as decreased toxicity to normal tissues (Aboud et al., 2016; Korotchikina et al., 2017; Zhao et al., 2017). Second, NAMPT inhibitors can be highly selective against certain cancer types. For example, FK866 exhibits selective toxicity towards gastric cancer cells marked by nicotinic acid phosphoribosyltransferase (NAPRT) deficiency and the EMT (Lee et al., 2018; Shames et al., 2013). Cancers carrying isocitrate dehydrogenase 1 (*IDH1*) mutations exhibit reduced level of NAPRT and exhibit enhanced sensitivity to NAD⁺ depletion by NAMPT inhibitors (Tateishi et al., 2015). Moreover, NSCLC with nucleotide excision repair (NER) deficiency shows exquisite sensitivity to NAMPT inhibition (Touat et al., 2018). Therefore, identification of predictive biomarkers may greatly help to improve therapeutic efficacy of these second-generation NAMPT inhibitors.

Combinatorial targeting may have particular benefit when targeting NAMPT, especially because cancer cells can develop resistance to NAMPT inhibitors through enhancing NAD⁺ *de novo* synthesis through upregulated quinolinic acid phosphoribosyltransferase (QAPRT), NAMPT mutations (Guo et al., 2017), or enhanced amino acid catabolism (such as tryptophan in *de novo* pathway and glutamine in salvage pathway) (Thongon et al., 2018). Moreover, therapeutic resistance has been attributed to the self-renewal and dedifferentiation abilities of tumor-initiating “cancer stem cells” (CSCs) residing within the bulk tumor (Gupta, Chaffer, & Weinberg, 2009). NAMPT overexpression in glioma cell lines has been shown to enrich the CD133-high/CD44-high

population that marks glioma stem cells, and its expression correlates with high levels of stem cell markers such as Nanog in patient-derived glioblastoma specimen (Lucena-Cacace, Otero-Albiol, Jiménez-García, Peinado-Serrano, & Carnero, 2017), pointing to its role in maintaining the treatment-refractory component of tumors. The persistence of the CSC population under therapeutic challenge has been attributed to metabolic and oxidative stress resistance due to their metabolic plasticity; however, such CSCs have also been reported to be acutely sensitive to mitochondrial metabolism-targeting drugs (Peiris-Pages, Martínez-Outschoorn, Pestell, Sotgia, & Lisanti, 2016). Thus, mitochondrial-specific targeting of tumor-associated NAMPT activity could circumvent the observed systemic toxicity of the first-generation inhibitors and provide enhanced therapeutic index. Notably, the dedifferentiation ability and damage recovery of cancer cells that have undergone therapy-relevant stresses (oxidative stress, glucose deprivation, hypoxia) depends critically on the labile, mitochondrially generated signaling molecule, hydrogen sulfide (H₂S) and its ability to upregulate NAMPT (Ostrakhovitch, Akakura, Sanokawa-Akakura, Goodwin, & Tabibzadeh, 2015; Sanokawa-Akakura, Ostrakhovitch, Akakura, Goodwin, & Tabibzadeh, 2014). The H₂S-induced dedifferentiation of the stress-challenged cancer cells can be inhibited by NAMPT suppression or by inactivation of the H₂S-producing enzymes, cystathionine beta synthase and cystathionase, which are also upregulated by NAMPT (Ostrakhovitch et al., 2015). The existence of such feedback regulation between NAMPT and other tumor-promoting signaling axes needs to be further investigated as they provide uniquely tumor-specific opportunities for combinatorial therapeutic targeting of NAMPT. Due to observed synergistic effects with FK866 and histone deacetylase complex (HDAC) inhibitors, recently dual NAMPT/HDAC inhibitors have been developed which possess nanomolar range IC₅₀s (half maximal inhibitory concentration) for both molecules, and exhibit apoptosis and autophagy in cancer cell lines as well as tumoricidal activity in xenograft tumor models *in vivo* (Chen et al., 2018; Dong et al., 2017). This type of combinatorial targeting exploits cancer-specific requirements for epigenome remodeling to transcribe tumor-driving genes and the essential role of NAMPT and NAD⁺ metabolism in such processes. Combining FK866 with the clinically relevant PARP inhibitor, Olaparib, results in synthetic lethality through suppression of the PARP substrate β -NAD⁺ in triple negative breast cancer cell lines as well as *in vivo* models, with negligible systemic effects of the combinatorial treatment (Bajrami et al., 2012). Thus, the true utility of NAMPT as a cancer target may lie in judicious synergistic combinations that will enable more effective co-targeting of known cancer-driving pathways through leveraging NAD⁺-dependent biology underlying these pathways. The added advantage of such combinatorial approaches is that their tumor specificity may serve to abrogate issues of systemic toxicity associated with single agent NAMPT targeting, through reduced dose requirements or better therapeutic index in tumors vs. normal tissue.

3.1.2. NAPRT

NAPRT converts nicotinic acid (NA) to nicotinic acid mononucleotide (NAMN) and serves as an alternative source for NAD⁺ production. Similar to NAMPT, NAPRT is located in both nuclear and cytoplasm, but has not been detected in mitochondria (Nikiforov et al., 2011; Piacente et al., 2017). NAPRT gene amplification is present in various cancer types, including ovarian, pancreatic, and prostate cancer, and commonly confers resistance to NAMPT inhibitor treatment (Piacente et al., 2017). Notably, the efficacy of NAPRT inhibitors largely depends on the NAD⁺ metabolic signature of the cancer. NAPRT inhibitor 2-hydroxynicotinic acid (2-HNA) sensitizes ovarian cancer cells to FK866, producing a significant decrease of cell viability (Piacente et al., 2017). By contrast, triple-negative breast cancer cells are not sensitive to 2-HNA, as they have low NAPRT-mediated NAD⁺ biosynthesis activity and instead primarily rely on LDH in aerobic glycolysis for the resistance to FK866 (Thongon et al., 2018).

3.1.3. NMNAT

All three human NMNAT isoforms (NMNAT1–3) have enzymatic activities and convert the last step of NAD⁺ synthesis from NMN (Berger et al., 2005). The aberrant regulation and critical role of NMNATs in tumor metabolism are indicative of their potential in cancer therapy. However, only a few compounds targeting NMNAT have been identified and characterized. Gallotannin, a polyphenolic plant metabolite, inhibits enzymatic activity of all three NMNAT isoforms and is highly potent against NMNAT1 (Berger et al., 2005). Several NAD⁺ synthetic analogs also shown selective inhibition against the different NMNAT isoforms (Petrelli, Felczak, & Cappellacci, 2011). A recent study identified Vacor adenine dinucleotide (VAD), an NAD⁺ analog converted from Vacor by NAMPT and NMNAT2, as a potent inhibitor of NMNAT enzymatic activity (Buonvicino et al., 2018). VAD rapidly depletes NAD⁺ pools, blocks glycolysis through inhibition of NAD⁺-dependent dehydrogenases, and significantly reduces the growth of NMNAT2-proficient neuroblastoma and melanoma xenografts (Buonvicino et al., 2018). A phenotypic screen using the Meso Scale Discovery (MSD)-based platform has identified several positive and negative regulators of NMNAT2 (Ali, Bradley, & Lu, 2017). Of note, NMNAT not only functions as an enzyme, but also acts as a chaperone (Ruan, Zhu, Li, Brazill, & Zhai, 2015; Zhai et al., 2008), which could contribute to meeting the increased demand in maintaining proteostasis in rapidly adapting cancer cells (Calderwood & Murshid, 2017). Chaperone inhibitors have shown great promise both preclinically and clinically in cancer therapy (Chatterjee & Burns, 2017). Therefore, depleting NMNAT2 may produce enhanced benefits by both inhibiting NAD⁺-dependent energy metabolism and signaling and disrupting protein homeostasis, thus simultaneously targeting two tumor-promoting mechanisms.

3.2. Targeting NAD⁺ consuming enzymes

3.2.1. SIRT

The involvement of different SIRT isoforms highlight the complex relationship between epigenetic regulation, transcription, DNA repair, cell cycle regulation, and energy metabolism in cancer cells. Increasing evidence supports the therapeutic potential of SIRT modulators in the treatment of cancer. Several recent reviews have provided comprehensive discussions on SIRT-targeting compounds, including their chemical structures, selectivity in different SIRT isoforms, and biological effects (Carafa et al., 2016; Hu, Jing, & Lin, 2014; O'Callaghan & Vassilopoulos, 2017). More recently, several novel SIRT inhibitors have been identified that exhibit potent anticancer activities. For example, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PG_{J2}) and its derivatives, J11-C1 and J19, markedly inhibit SIRT1 activity and induce cell death in ovarian cancer cells (Tae et al., 2018). A quinoxaline-based compound 4bb selectively reduces colon cancer cell viability without affecting normal fibroblasts (Ghosh et al., 2017). Moreover, MHY2256, a potent SIRT1 inhibitor, significantly inhibits growth of endometrial and breast cancer cells (De et al., 2018; Park et al., 2016). Recently, a new SIRT7 inhibitor (ID: 97491) has been identified that shows potent inhibitory effect on cancer growth by upregulating apoptosis (Kim et al., 2019). All these compounds work partially via promoting acetylation and activation of p53, thereby driving cell cycle arrest and inducing apoptotic or autophagic cell death (De et al., 2018; Ghosh et al., 2017; Kim et al., 2019; Tae et al., 2018).

3.2.2. PARP

PARPs play pivotal roles in NAD⁺-dependent DNA damage repair and transcriptional regulation, both of which are highly essential for cancer development. Therefore, PARP inhibitors have been widely studied in the context of their anticancer action (Dulaney, Marcrom, Stanley, & Yang, 2017; Sisay & Edessa, 2017). Four compounds, olaparib (Audeh et al., 2010; Fong et al., 2009; Ledermann et al., 2012; Robson et al., 2017; Tutt et al., 2010), rucaparib (Coleman et al., 2017; Swisher et al., 2017), niraparib (Mirza et al., 2016; Sandhu et al., 2013), and

talazoparib (de Bono et al., 2017; Litton et al., 2018) have been approved by the Food and Drug Administration (FDA) for treatment of gynecologic cancers (Fig. 3). Several other PARP inhibitors are under clinical trials for evaluation of efficacy and toxicity as monotherapies or in combination with radiation and other chemotherapeutics. PARP inhibitors are particularly effective in inducing synthetic lethality in BRCA1- and/or BRCA2-mutated cancers that are defective in homologous recombination repair (HRR) (Lord & Ashworth, 2017). The consequences of PARP inhibition are two-fold. First, PARP inhibition promotes progression of single-strand breaks to double-strand breaks, subsequently leading to cell death (Dziadkowiec, Gasiorowska, Nowak-Markwitz, & Jankowska, 2016). Second, PARP inhibition leads to deregulation of error-prone non-homologous end joining (NHEJ) which serves to introduce genomic instability (Patel, Sarkaria, & Kaufmann, 2011). Further mechanistic studies have shown that PARP inhibition reduces the availability of HRR factors by suppressing E2F1-mediated HR gene expression (Schiewer et al., 2018). Notably, PARP inhibitors can induce reversion mutations in HRR genes that restore HRR function and promote drug resistance (Edwards et al., 2008; Lin et al., 2019).

3.2.3. CD38

CD38 is a major NAD⁺ consumer, and its NAD⁺-dependent activity is required for cell growth. Recent studies indicated the role of CD38 in tumorigenesis. For example, CD38 promotes cervical cancer cell growth through reducing ROS levels and inhibiting apoptosis (Liao et al., 2017), and loss of CD38 in human lung adenocarcinoma cells inhibited cell growth, invasion, and xenograft growth in nude mice (Bu et al., 2017). CD38 also augments immunosuppression in cancer by inhibiting CD8⁺ T function (Chen et al., 2018; Feng et al., 2017). CD38 has been identified as a cell-surface marker for multiple myeloma (Chini et al., 2018), and CD38-targeting antibodies are specifically effective in relapsed/refractory multiple myeloma (RRMM) patients (Frerichs et al., 2018; van de Donk, Richardson, & Malavasi, 2018). Daratumumab is the first FDA-approved CD38-targeting drug with single-agent efficacy for RRMM (Dimopoulos et al., 2016; Lokhorst et al., 2015; Lonial et al., 2016; Mateos et al., 2018; Palumbo et al., 2016; Usmani et al., 2016). Other anti-CD38 antibodies, including isatuximab and MOR202, are currently in clinical trials, and preliminary data exhibit excellent infusion tolerability (Martin et al., 2017; Raab et al., 2016). CD38-targeting antibodies inhibit cancer development through multiple effector mechanisms. First, these antibodies possess classic Fc-dependent immune effector mechanisms for cancer cell lysis and removal, typically antibody-dependent cellular cytotoxicity (ADCC) (Frerichs et al., 2018; van de Donk et al., 2018). The effectiveness of the isatuximab-mediated ADCC correlates with cell-surface CD38 levels (Kriegsmann et al., 2018). Notably, since CD38 is highly expressed on multiple myeloma cells, with relatively low expression on normal myeloid or lymphoid cells (Chini et al., 2018; Krejcik et al., 2016), multiple myeloma cells are specifically susceptible to CD38 depletion. Second, targeting CD38 promotes T-cell expansion, enhances effector T cell function, and mitigates CD38⁺ Treg-mediated immunosuppression (Feng et al., 2017; Krejcik et al., 2016). Third, inhibition of CD38 expression on T cells upregulates NAD⁺ and activates T cells via promoting glutaminolysis, enhancing oxidative phosphorylation, and altering mitochondrial dynamics, thereby boosting the efficacy of adoptive T cell therapy (Chatterjee et al., 2018). Interestingly, a recent study demonstrates that cancer cells acquire resistance to PD-1/PD-L1 immune checkpoint inhibitors via upregulation of CD38 that blocks CD8⁺ T cell function through adenosine receptor signaling (Chen, Diao, et al., 2018), indicating potential benefits to employing CD38 inhibitors and PD-1/PD-L1 inhibitors in combination as anti-cancer therapy.

4. Concluding remarks

Decades of research has uncovered the key regulatory role of NAD⁺ in integrating multiple metabolic pathways underlying cancer

pathophysiology. In particular, NAD⁺ functions at the nexus of a crucial signaling hub that interlinks DNA repair, gene transcription, and bioenergetics, processes that govern critical steps in tumorigenesis, including cell cycle regulation, apoptosis, autophagy, redox homeostasis, and immune response. Notably, recent advances on our understanding of the subcellular distribution of NAD⁺ shed light on how different NAD⁺ pools are dynamically maintained, how they preferentially influence NAD⁺-dependent bioenergetic and signaling processes, and how they engage in crosstalk with critical tumor-driving pathways. The interplay of these factors in cancer cells as well as the tumor microenvironment drive cancer development and metastasis. Identification of druggable targets in the NAD⁺ metabolome shows promise for cancer treatment, as tumors have altered metabolic requirements that are heavily reliant on NAD⁺ for survival and growth. Further in-depth mechanistic studies with the consideration of subcellular compartment-specific NAD⁺ metabolic profile of different cancer types will bear promise to achieve maximal drug efficacy and minimize unwanted side effects.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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