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Commentary

Sirtuins and inflammation: Friends or foes?

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ABSTRACT

Lysine acetylation/deacetylation has been recognized as an important posttranslational modification regulating numerous cellular processes. Sirtuins represent novel players in these complex regulatory circuits. These NAD-dependent lysine-deacetylases have attracted much interest based on their role in the regulation of lifespan in lower organisms, and their capacity to interfere with cell growth, proliferation and survival in response to stress. Their absolute requirement for NAD suggests that these enzymes may represent an important molecular link between metabolism and several human disorders such as diabetes and cancer. More recently, the identification of several transcription factors known to play a role in the immune system as sirtuin substrates has suggested that this family of enzymes may also play an important role in the regulation of inflammation, a pathological situation with clear links to metabolism and aging in humans. We review herein the possible links between nuclear sirtuins and the regulation of an immune response, and discuss the possible strategies that may lead to the development of novel therapeutic approaches to treat inflammation by targeting sirtuin activity.

1. Introduction

The activity, stability, and intracellular location of virtually all proteins are regulated by post-translational modifications (PTMs). This very diverse set of modifications include phosphorylations, acetylations, sumoylations, ubiquitinations, ADP-ribosylations, nitrations and many others [1]. PTMs are highly dynamic processes and most of them result from the activity of antagonizing enzymes (e.g. kinases vs. phosphatases, acetylases vs. deacetylases, etc.). Numerous studies have highlighted the important role of PTMs in signaling pathways, allowing a crosstalk between the cell and its environment and endowing the cell with a certain flexibility towards change [1]. In this context, nicotinamide adenine dinucleotide (NAD) has gained a renewed interest as an important substrate for a series of enzymes catalyzing a set of post-translational modifications, such as deacetylation or ADP-ribosylation. In contrast to its role in energy metabolism, the involvement of NAD in these regulatory processes is based on its ability to act as an ADP-ribose donor, thus requiring NAD resynthesis to avoid depletion of the intracellular NAD pool [2]. The central role of NAD in both energy metabolism and protein modification has been shown to have important physiological consequences, in particular in the control of cell metabolism, cell death and longevity [3].

NAD has been linked to lifespan extension through the activity of a family of deacetylases whose activity is strictly dependent on its availability [4]. NAD-dependent deacetylases, or sirtuins, have indeed been shown to promote longevity in several model organisms [5,6] and in mammalian cell cultures [7], bringing NAD to the forefront of the pharmaceutical struggle against aging [8]. This family comprises seven members (SIRT1 to SIRT7) that localize to the nucleus (SIRT1,6 and 7), the cytoplasm (SIRT2) and the mitochondria (SIRT3,4 and5) even though some of them can shuttle between these compartments. To date, SIRT4 is the only member that has been shown to lack a deacetylase activity, catalyzing instead an ADP-ribosylation reaction [9]. The deacetylase activity of the other members of the family consists in the removal of the lysine-linked acetyl group of a target protein [10] (Fig. 1). During sirtuin-mediated deacetylation, an NAD molecule is hydrolyzed, releasing a metabolite resulting from the condensation of the acetyl group with the ADP-ribose moiety of NAD (OAADPR) [10]. The other moiety of NAD, nicotinamide, is also released and acts as an end-product inhibitor of the deacetylation reaction [10]. Although sirtuins share many substrates with classical histone deacetylases (HDACs), their unique NAD-dependency links the activity of these enzymes to the intracellular

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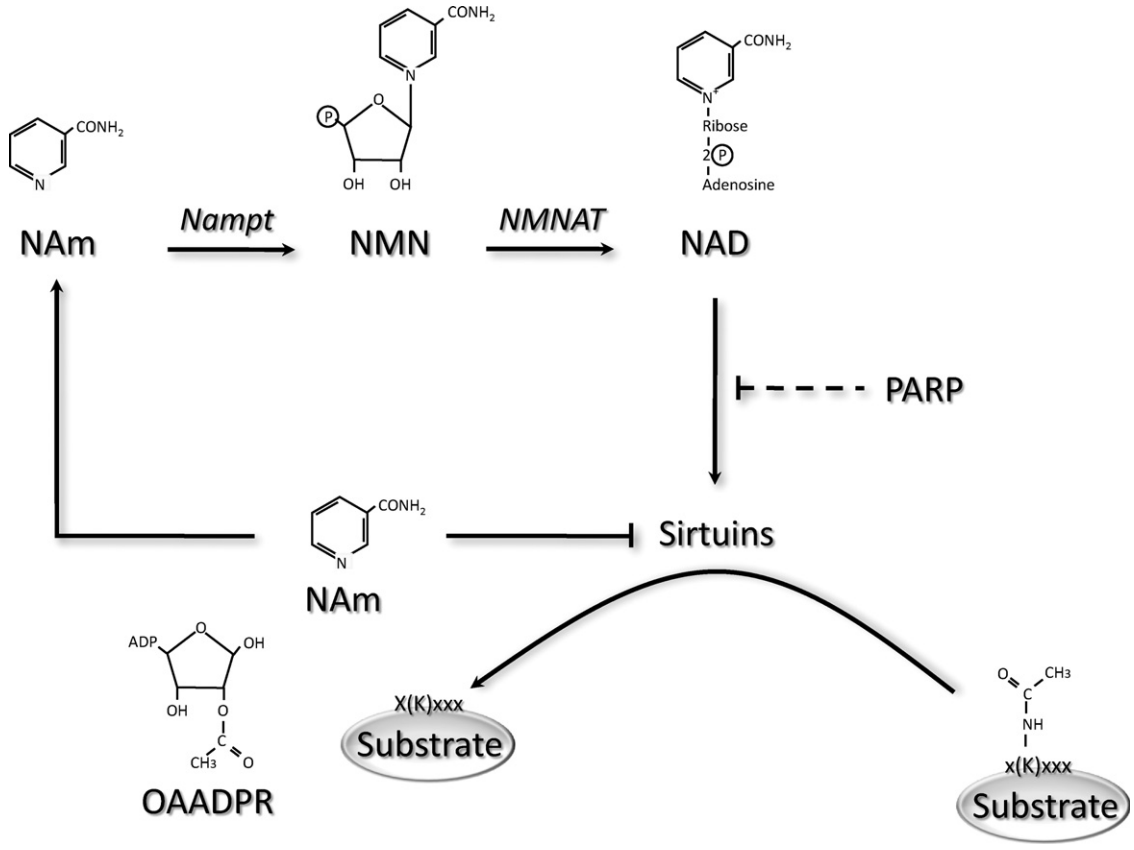

Fig. 1. Schematic representation of nicotinamide recycling to NAD and of the reaction catalyzed by sirtuins. Nicotinamide (NAm) is the major NAD precursor for mammalian cells both in vivo and in vitro. It can be converted to NAD through two successive enzymatic reactions: the first one is rate-limiting and is catalyzed by nicotinamide phosphoribosyl transferase (Nampt). Nicotinamide mononucleotide arises from this reaction and is converted to NAD by nicotinamide/nicotinic acid mononucleotide adenylyl transferase (NMNAT). In the presence of NAD, sirtuins can remove the acetyl group on specific lysines of a given substrate and transfer it on the ADP ribose moiety of an NAD molecule. The reaction also releases the deacetylated substrate and nicotinamide that can be recycled to NAD. If nicotinamide is not cleared, however, it can inhibit deacetylation. This reaction can also be indirectly impaired by the activation of members of the PARP family that deplete the NAD cellular pool and contribute to nicotinamide accumulation.

concentration of available NAD, leading several authors to consider sirtuins as "metabolic sensors" [11]. Accordingly, sirtuins have been shown to regulate the cellular response to nutrient availability, intracellular glucose metabolism and cell survival in response to several forms of stress [11,12]. The discovery that SIRT1 activity can regulate the circadian machinery [13] that potently impacts metabolism, further argues for this sirtuin's pivotal role in cellular metabolism, as discussed in several recently published reviews [11,12].

An additional and potentially important role for sirtuins in regulating an immune response has been recently uncovered. Several experimental observations had previously established a potential link between NAD metabolism and inflammation. In particular, expression of Nampt, the enzyme catalyzing the first and limiting reaction allowing NAD biosysnthesis from nicotinamide, has been found overexpressed in several cell lines and tissues during an inflammatory response [14]. Although high rates of NAD biosynthesis may represent a biological response to meet an increased metabolic rate, this observation is also compatible with an important role for NAD-dependent enzymes in controlling immune reactions. A series of recent observations concur with this hypothesis, and clearly demonstrate a direct role for sirtuins in controlling the expression of several inflammatory mediators. Although a conclusive role for sirtuins as pro or anti-inflammatory regulators is still a matter of debate, several recent reports have clearly established that acetylation and sirtuin dependent deacetylation of several transcription factors with well established immunoregulatory functions, play an important role in immune cells, warranting a brief review of the literature on this new emerging field.

2. SIRT1

2.1. SIRT1 negatively regulates NFkB activity

The NFkB transcription factor plays a pivotal role in immune function [15]. The observation that several lysines of NFkB p65 subunit can be acetylated [15] has highlighted the potential regulatory role of lysine acetylation on NFkB function. Among these, K310 acetylation confers superior transcriptional activity, while representing a substrate for SIRT1 [16]. SIRT1 acts therefore as a negative regulator of NFkB activity through the deacetylation of the p65 lysine 310. This NFkB-SIRT1 negative loop has been described in several experimental models, confirming its biological relevance. Kwon and coworkers have recently confirmed and expanded these results, by showing that this pathway is targeted and exploited by the HIV virus. The authors wished to understand the mechanism by which the HIV transcriptional activator Tat can positively regulate NFkB activity and induce IL-2 expression in infected lymphocytes [17]. In their work, they elegantly demonstrate that Tat inhibits SIRT1 enzymatic activity by binding to its acetyl-lysine-binding domain. Functional SIRT1 inhibition leads to NFkB hyperacetylation, which in turn causes an increase in IL-2 production and T cell activation, a condition that renders T cells permissive to the virus. This SIRT1-NFkB pathway has subsequently been shown to be active in several tissues and cell types, in

which a reduction in SIRT1 protein levels and/or activity is associated with an NFkB-dependent sustained inflammatory response. A decrease in SIRT1 activity has for example been reported in the lungs of smokers and of patients suffering from chronic obstructive pulmonary disease (COPD) [18], correlating with increased inflammatory gene expression and p65 K310 acetylation. An oxidative modification of the SIRT1 protein is thought to lead to an increased SIRT1 decay in cells exposed to cigarette smoke [19], possibly explaining the failure to restore adequate SIRT1 activity in this model by increasing the intracellular NAD pool. [20]. In organs such as the liver or in adipose tissue that play a major role in metabolic homeostasis, SIRT1 activity can be affected by diet. In most studies, high fat diet (HFD), in which approximately 40–60% calories are provided from fat, is accompanied by a decrease in SIRT1 activity [21,22], while caloric restriction (CR), that consists of a moderate caloric limitation (to approximately 60% of a standard regimen), tends to activate SIRT1 [23]. Thus SIRT1$^{+/-}$ mice develop hepatic steatosis under a HFD, with a steatosis-associated increase in pro-inflammatory cytokine production and macrophage infiltration [24]. The same inflammatory activation is evident in SIRT1-depleted adipocytes, together with an increase in insulin resistance [22]. The putative role for NFkB in this model is supported by the observation of an NFkB hyperacetylation status [22] and by the finding that NFkB knockdown reverses these metabolic changes [22]. Importantly, free fatty acids have been shown to lead to a similar reduction in SIRT1 activity, by downregulating AMP-activated protein kinase (AMPK) [21]. This study, conducted by Yang and coworkers, shows that, like SIRT1, AMPK is a metabolic sensor that can reduce NFkB activation and that the anti-inflammatory properties of AMPK are strictly dependent on its ability to induce SIRT1-dependent p65 deacetylation [21], further supporting the physiological relevance of an SIRT1-NFkB anti-inflammatory axis. Interestingly a recent report proposes that the inflammatory character of fatty acids depends on their nature: *n*-6 unsaturated fatty acids being pro-inflammatory while the *n*-3 subset has anti-inflammatory properties possibly linked to their ability to induce SIRT1 expression [25]. Besides from its dietary origin, hepatic steatosis can also be caused by excessive alcoholic absorption. Ethanol-derived metabolites have also been shown to decrease SIRT1 levels in macrophages [26]. NFkB hyperacetylation originates from this SIRT1 depletion and causes a pro-inflammatory cytokine release [26] that can reduce insulin sensitivity in co-cultured adipocytes [27]. It must be noted that inflammatory cytokines can be released by macrophages but also by non-immune cells such as hepatocytes and adipocytes. This raises the question of whether the inflammatory state observed in the liver and in the adipose tissue is of immune or hepatic/adipose origin. A similar question has been elegantly addressed by Li's team, who focused on SIRT1's role in maintaining liver homeostasis. In two successive papers, Li's team has knocked out SIRT1 expression in hepatocytes [28] and in macrophages [29], and has assessed how this would affect liver steatosis following a HFD. Purushotham's liver specific SIRT1-KO mice (LKO) have impaired lipid homeostasis and are more prone to developing liver steatosis when fed a HFD, as compared to wt littermates [28]. Steatosis is accompanied by liver inflammation, with an increase in TNFα and IL-1β synthesis and in total macrophage markers such as F4.80. However, it must be noted that Guarente's team has observed an opposite outcome in a similar model of liver-specific SIRT1 deletion, with SIRT1 absence proving to have a beneficial effect on several metabolic parameters following HFD, even if no effect on NFkB acetylation or liver inflammation was reported [30]. The increase in F4.80 expression in Purushotham's study suggests an important macrophage infiltration and this prompted the authors to disrupt SIRT1 in macrophages and assess the consequences of HFD feeding [29]. Macrophage-specific SIRT1 deletion (Mac-SIRT1 KO) has remarkable effects on several organs and on the entire organism. HFD reduces insulin sensitivity in Mac-SIRT1 KO mice while exacerbating inflammatory cytokine expression, both systemically and locally. SIRT1 deficiency clearly correlates with NFkB hyperacetylation and NFkB knockdown blunts the increase in cytokine production observed in macrophages from SIRT1 KO mice. It is therefore tempting to conclude that, as previously stated, lack of SIRT1 activity results in hyperactive NFkB status and contributes to the development of inflammation in these models.

In line with the previous conclusion, several studies have shown that systemic SIRT1 activation decreases inflammation by reducing NFkB activity [31,32]. SIRT1 in vivo overexpression has been achieved by Pfluger and colleagues, who generated a transgenic mouse carrying an extra copy of the SIRT1 gene under its own promoter [31]. Alternatively, SIRT1 overactivation can result from the removal of endogenous SIRT1 inhibitors, such as deleted in breast cancer-1 (DBC1) [32], a nuclear protein recently shown to bind and negatively regulate SIRT1 activity [33,34]. Focusing on the liver, both of these studies showed that SIRT1 activation confers protection against HFD-induced steatosis and the associated inflammation. The authors also conclude that SIRT1 anti-inflammatory effects can be explained by the inhibition of NFkB, since they observe an impaired p65 activation following TNFα stimulation [31,32].

Although numerous reports have illustrated the negative regulatory influence of SIRT1 on NF-kB, the underlying molecular mechanism has not been completely elucidated. Recently, however, two potential mechanisms by which SIRT1 can negatively affect NFkB activity have been uncovered. In a search for proteins able to modulate RelA transcriptional activity, Huang and coworkers have identified Brd4 as a bromodomain-containing protein able to specifically bind and co-activate K310-acetylated RelA [35]. Brd4 allows the recruitment of the CDK9 kinase at the site of transcription, an event associated with the phosphorylation of the RNA pol II [35] (Fig. 2). Notably, not all NF-kB-dependent genes are similarly regulated by Brd4, suggesting that the acetylation state of RelA K310 offers a new and subtle mechanism for modulating NFkB activity. In a separate study, the same group has recently demonstrated a role for the acetylation of the lysine 310 of RelA in modulating protein stability [36]. Acetylation of this SIRT1 substrate impairs the Set9-mediated methylation of proximal lysine residues (314 and 315), a posttranslational modification that targets chromatin-associated RelA for ubiquitin-mediated degradation. Acetylated RelA is therefore protected from degradation, a novel mechanism that explains the negative influence of SIRT1 on NFkB function [36].

2.2. SIRT1 inhibits AP-1 by deacetylating c-Jun and c-Fos

c-Jun and c-Fos are the main components of the dimeric transcription factor AP-1. In a recent study, Gao and coworkers showed that c-Jun transcriptional activity can be downregulated by SIRT1 in vitro [37]. A second study rapidly followed, showing that c-Jun is actively deacetylated by SIRT1 in vivo [38], while a third team reported that both the subunits c-Jun and c-Fos are targeted by SIRT1 [39]. These events play a major role in regulating the function of several immune cells. Indeed, in macrophages, SIRT1 activation was reported to decrease the transcription of COX-2, a typical AP-1 dependent pro-inflammatory gene [39]. In T lymphocytes, AP-1 deacetylation by SIRT1 underlies T cell anergy and permits peripheral tolerance, preventing uncontrolled T cell proliferation and cytokine production [38]. As a consequence, SIRT1$^{-/-}$ mice display an increased T cell responsiveness in vitro and are predisposed to develop autoimmune disorders, a conclusion further confirmed by an independent study [40].

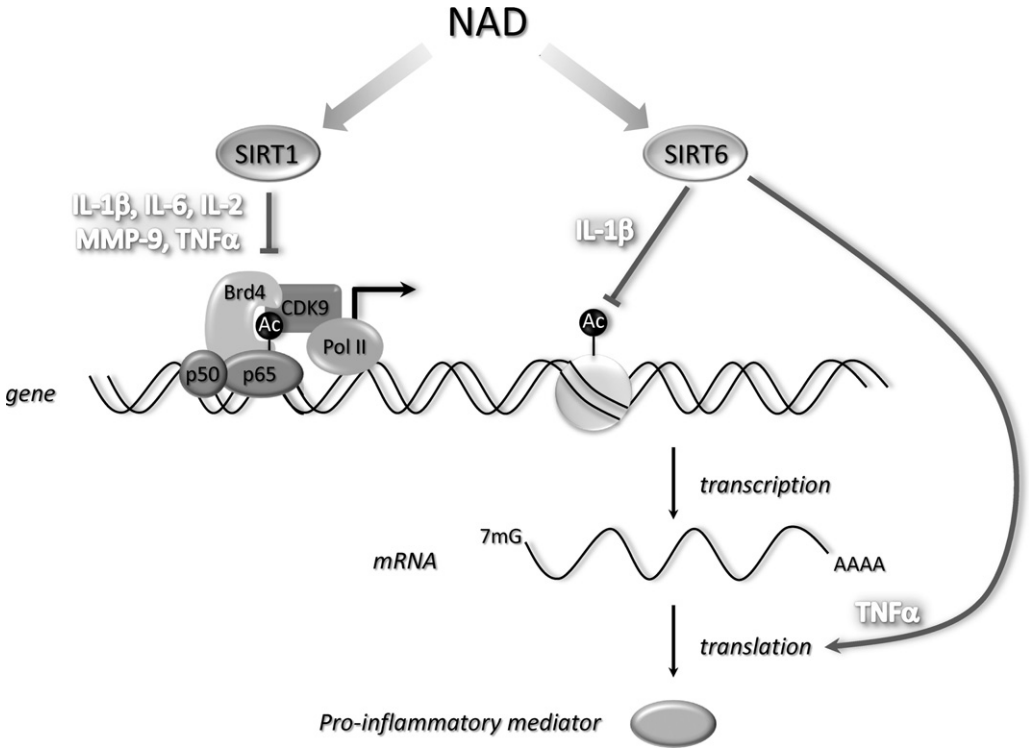

Fig. 2. Putative model of how two NAD-dependent deacetylases, SIRT1 and SIRT6, can modulate the expression of pro-inflammatory mediators, by concerted or opposing mechanisms. SIRT1 deacetylation of p65 lysine 310 can inhibit the recruitment of the bromodomain-containing coactivator Brd4. Lack of Brd4 recruitment is thought to impair the binding of CDK9 and the recruitment and phosphorylation of RNA polymerase II (PolII), leading to reduced transcription of several pro-inflammatory mediators such as interleukin (IL)-1β, -2, -6, TNFα and MMP9. An additional mechanism could reduce IL-1β transcription through SIRT6-dependent deacetylation of histone 3 lysine 9 in nucleosomes. Whether this mechanism exclusively regulates this cytokine expression remains to be established. Finally, SirT6 could also have a positive effect on the translation of TNFα mRNA and, possibly, other selected transcripts. Note that SIRT6 activity is either exclusively positive (e.g. TNFα) or negative (e.g. IL-1β) for a given pro-inflammatory gene expression.

2.3. SIRT1-dependent deacetylation of FoxP3 leads to its degradation

Tregs are $CD4^+$ T cells endowed with suppressive capacities, whose most specific and widely accepted marker is the transcription factor FoxP3. High and stable levels of FoxP3 expression have been proposed to be required for adequate Treg function [41]. The acetylated form of FoxP3 displays improved repressive functions and is a poor substrate for polyubiquitin-mediated degradation [42]. SIRT1 has been shown to bind and decrease FoxP3 protein levels, possibly through its deacetylation. Accordingly, nicotinamide mediated SIRT1 inhibition increases both $FoxP3^+$ protein levels and the relative proportion of FoxP3+ cells, resulting in an overall increased Treg suppressive function [42]. In line with these results, Yang and coworkers reported that resveratrol, a natural SIRT1 activator [43], depletes $CD4^+$ $CD25^+$ Tregs in tumor-bearing mice [44]. As previously discussed, SIRT1 appears to limit the abundance and activity of a well described transcription factor affecting T cell functions. Notably however, and in marked contrast to the previously described observations, the antagonizing role of SIRT1 on the function and development of Tregs appears to promote, rather than inhibit, an inflammatory response.

2.4. SIRT1 role in granulopoiesis

A recent report reveals that SIRT1 is not only implicated in immune cell activation but might also play an important role in their development. Indeed, Nampt-mediated NAD synthesis has been shown to trigger neutrophil differentiation from bone-marrow precursors in a SIRT1-dependent manner [45]. This pathway is affected in individuals suffering from congenital neutropenia, whose treatment consists in daily injections of granulocyte colony stimulating factor (G-CSF). Skokowa and colleagues have shown that G-CSF induces both Nampt and SIRT1 expression, leading to an increased expression of the transcription factors C-EBPα and β. C-EBP activity increases expression of both G-CSF itself and of its receptor, thus leading to a positive autoregulatory loop. This study demonstrates that administration of nicotinamide, the major NAD precursor in vivo, leads to an increased NAD synthesis, C-EBPα and β expression and consequently granulopoiesis. Since C-EBPβ is known to be regulated by acetylation [46], it is tempting to assume that it could be added to the growing list of transcription factors representing novel SIRT1 substrates.

3. SIRT6

Similarly to SIRT1, SIRT6 appears to be mainly localized in the nucleus, predominantly associated to chromatin [47]. In 2006 the description of SIRT6 knockout mice drew much attention due to the severe premature aging observed in these mice [47]. These mice appear normal at birth but undergo an accelerated degenerative process and die at approximately three weeks of age. They show impaired IGF1 and glucose serum levels, a decrease in bone mineral density and, at the immune level, a dramatic and systemic increase in lymphocyte apoptosis. Using these same mice, Kawahara et al. later demonstrated that lymphopenia could be rescued by p65 heterozygosity, arguing again for a sirtuin-dependent regulation of NFkB-driven gene expression [48].

3.1. SIRT6 inhibits NFkB dependent transcription by affecting chromatin structure

Kawahara et al. focused on the possible link between SIRT6 and NFkB to explain the accelerated aging phenotype of SIRT6$^{-/-}$ mice. These authors were able to demonstrate that although SIRT6 directly binds to p65, it actually interferes with its transcriptional activity by deacetylating H3 lysine 9 (H3K9) on the promoter of selected NFkB target genes rather than directly modulating p65 activity [48]. Thus, while SIRT1 inhibits NFkB activity by direct posttranslational modification of p65, SIRT6 acts by decreasing promoter accessibility to p65 (Fig. 2). Notably, a compensatory role for SIRT6 has been recently demonstrated in SIRT1-deficient macrophages [29]. In these cells, SIRT6 was found to be strongly associated with selected NFkB promoters following TNFα stimulation, thus attenuating the increased NFkB activity caused by the loss of SIRT1. Accordingly, siRNA-mediated inhibition of SIRT6 protein expression further increased the expression of selected NF-kB genes (such as IL-1β in SIRT1 KO mice). Even though the nature of this compensatory mechanism was not uncovered, these observations confirm that both SIRT1 and SIRT6, although acting at distinct levels, represent negative regulators of NFkB activity (Fig. 2).

3.2. A potential role for SIRT6 in post-transcriptional regulation of pro-inflammatory cytokines

In contrast to the anti-inflammatory role generally attributed to sirtuins, we and others have recently reported a possible positive regulatory role for SIRT6 in the induction of pro-inflammatory cytokine expression, in both innate and adaptive immune cells [49,50]. In these studies, reduction of intracellular NAD levels, obtained by inhibiting the enzymatic activity of Nampt, led to reduced secretion of selected cytokines (such as TNFα, IL-6 and/or IFNγ) by immune cells, while leaving other pro-inflammatory mediators (such as CCL5/Rantes) unaffected [49,50]. Surprisingly, several sirtuin inhibitors could reproduce these effects, suggesting therefore that the NAD-sirtuin axis could also represent a positive regulatory loop required for adequate cytokine secretion. Using different approaches, both studies led to the identification of SIRT6 as the sirtuin member able to positively regulate TNFα and IFNγ synthesis. Notably, over-expression of an enzymatically active (but not a catalytically inactive) form of SIRT6 led to supraoptimal production of TNFα by upregulating the translational efficiency of TNFα mRNA [49]. In agreement with this observation, dowregulation of intracellular NAD levels and sirtuin inhibitors reduced TNFα protein synthesis with no significant effect on TNFα mRNA accumulation in response to microbial stimulation. Collectively these data suggest a complex regulatory role for SIRT6 in controlling an inflammatory response, since this sirtuin member could inhibit selected NFkB target genes at the transcriptional level, while increasing the translation of other cytokine mRNAs by a yet to be defined mechanism (Fig. 2). Note however that TNFα gene transcription does not appear to be affected by siRNA-mediated SIRT6 downregulation [29], suggesting that SIRT6 may in fact specifically promote TNFα translation, while inhibiting IL-1β gene transcription in the same cells. Collectively, these observations suggest that sirtuins, and in particular SIRT6, may affect the cytokine profile of activated immune cells in a subtle fashion, by acting at distinct steps of the synthetic process.

4. SIRT7

Finally, a function in neutrophil development has also been proposed for the nuclear sirtuin, SIRT7. Vakhrusheva and cow-orkers have generated a SIRT7 knockout mouse and have shown that SIRT7 disruption predisposes the mice to heart hypertrophy together with increasing cardiac inflammation [51]. These authors observed an increased infiltration of immune cells (especially granulocytes) in SIRT7$^{-/-}$ hearts, which correlated with constitutively higher levels of in situ pro- and anti-inflammatory cytokine production, in particular in aged mice. SIRT7 has been shown to display a nucleolar localization and to affect RNA polymerase-I transcription [52]. Although this sirtuin member appears to be highly expressed by cells of the immune system (see data available at http://biogps.gnf.org), further work will be required to evaluate whether SIRT7 is a "bona fide" immune regulator, or whether it affects an inflammatory response indirectly by controlling immune cell survival and/or ribosomal RNA synthesis capacities.

5. Pharmacological perspectives

5.1. Modulation of sirtuin activity through NAD metabolism

Due to their highly dynamic nature and sensitivity to small molecule modulators, enzymes mediating post-translational modifications represent ideal targets for pharmacological intervention. Sirtuins appear as particularly suitable for in vivo modulation. Their strict NAD-dependency connects these enzymes to other enzymatic pathways [53], offering numerous possibilities for affecting their enzymatic activity. Several observations indicate, for example, that increased intracellular NAD levels often lead to increased enzymatic activity of selected sirtuin members [54]. This is particularly true for SIRT1, whose activity has been clearly shown to be positively regulated by protocols leading to increased intracellular NAD levels. Forced expression of Nampt in fibroblasts has been shown to increase intracellular NAD levels, causing an enhanced transcriptional repression by SIRT1 [55]. A similar direct relationship between Nampt, intracellular NAD levels and SIRT1 activity has been recently confirmed in several cell types including skeletal myoblasts [56], vascular smooth muscle cells [7,57], and chondrocytes [58]. Of interest, the nuclear form of Nicotinamide Mononucleotide Adenylyl Transferase (NMNAT-1), the enzyme catalyzing the last step in NAD biosynthesis from nicotinamide in mammals, has been found to bind to SIRT1 and to be recruited to selected gene promoters [59]. This close association strongly suggests a mechanism whereby neo-synthesized NAD is made directly available to SIRT1, thus providing a link between cell metabolism and the regulation of gene transcription.

The possibility to affect sirtuin activity through NAD metabolism has recently led Imai to propose the use of "nutriceuticals" as a novel type of sirtuin activators [8]. Although administration of NAD could represent the most straightforward approach to increase intracellular NAD concentrations, the widespread expression of NAD-degrading ectoenzymes represent a likely mechanism affecting exogenously administered NAD bioavailability before it could reach the initially targeted cells. Moreover, systemic administration of NAD could also affect lymphocyte numbers through the process of NAD-Induced Cell Death (NICD) [60]. As previously discussed, exogenous nicotinamide, a vitamin B3 component representing the major NAD precursor in mammalian cells and displaying low toxicity in vivo [45], may represent an ideal alternative to affect intracellular NAD levels. Accordingly, SIRT1 was successfully activated in humans following oral administration of nicotinamide [45], suggesting that this strategy could prove useful in defined clinical settings. Notably however, nicotinamide can both activate and inhibit sirtuins (by respectively promoting NAD synthesis and acting as an end-product inhibitor of the deacetylation reaction), and it may therefore be difficult to predict which of these activities will prevail in vivo. Administration of nicotinamide mononucleotide (NMN), the product of the Nampt catalyzed reaction, may represent a useful and clinically relevant alternative to modulate sirtuin

activity in vivo [61]. NMN can be viewed as a rather "neutral" molecule, lacking the inhibitory properties of nicotinamide and being unable to activate NAD-consuming reactions directly. Even though NMN could be potentially degraded by the ectoenzyme CD38 [62], it has been shown to effectively increase intracellular NAD levels in vitro, while displaying minimal toxicity when administered to animals [63] Further in vivo studies are warranted to better define its pharmacokinetic properties, tissue selectivity and overall effect on sirtuin activity.

Inhibition of NAD-consuming enzymes represents an alternative approach to modulate sirtuin activity in vivo. PARP1 (the major intracellular NAD-consuming enzyme) and SIRT1, for example, are two long known rivals that share part of their substrates and whose activity depends upon NAD availability, although with distinct sensitivities [64]. PARP1 overactivation inhibits SIRT1 activity by depleting NAD and increasing nicotinamide levels [53], and PARP inhibitors have been shown to contribute to maintain high intracellular NAD levels [20] resulting in increased SIRT1 activity [20,65]. Notably, SIRT1 has also been shown to directly interact with PARP1 and inactivate it in a deacetylase-dependent manner [66], further illustrating the competing activities of these two NAD-consuming enzymes. In any event, these preliminary observations offer new pharmacological opportunities to activate sirtuins by both promoting NAD biosynthesis and by switching off competing NAD-consuming enzymes.

5.2. Small molecule modulators of sirtuin activity

The potential capacity of sirtuins to promote longevity and to increase cell resistance to stress, has led to the blossoming of numerous research activities aiming at identifying sirtuin-activating drugs. This topic has been recently reviewed (see as an example [67]) and will not be further discussed herein. It is noteworthy however that although numerous sirtuin-activating compounds have been described, their mode of action, remains often controversial. Resveratrol, the leading and most used sirtuin-activating drug [43] has been shown to affect several non-sirtuin related enzymatic activities in the cell [68], complicating the interpretation of most in vivo effects of this compound. Moreover, its sirtuin-enhancing capacities, originally established with an in vitro assay using an artificial substrate, have also been recently questioned [69]. Nonetheless, screening of several chemical libraries has led to the identification of several sirtuin-activator candidates, some of which display the expected functional properties of a bona fide sirtuin activity promoting agent. In particular, Nayagam and colleagues [70] have recently demonstrated the in vitro anti-inflammatory properties of SIRT1-activating compounds. In vivo administration of a sirtuin activator, has also been shown to decrease inflammation while concomitantly improving insulin sensitivity in naturally obese rats [27]. Finally, Smith and colleagues [71] have recently demonstrated the ability of two structurally distinct SIRT1 activators to recapitulate many of the signaling pathways generally controlled by SIRT1. In particular, these compounds have been shown to inhibit NFkB transcriptional activity and TNFα secretion in vitro, further confirming the potential therapeutic relevance of sirtuin activators in an inflammatory setting.

Sirtuin inhibitors have also been extensively studied, mostly in the setting of cancer therapy, as shown by [72]. It is noteworthy however that nicotinamide, the prototypic sirtuin inhibitor, displays anti-inflammatory properties both in vitro and in vivo [49,73]. Although nicotinamide may represent a general inhibitor for all NAD-consuming reactions, we and others have been able to confirm the potential beneficial role of sirtuin inhibitors as anti-inflammatory compounds both in vitro and in vivo [49,50], although these observations need to be further extended to more clinically relevant settings.

6. Conclusions

Based on the brief review of the literature presented herein, it is tempting to conclude that sirtuins do represent clinically relevant targets for the development of novel anti-inflammatory compounds. Several complicating factors that may limit such development need however to be considered. Although numerous reports concur to identify SIRT1 as a negative regulator of NFkB activity, it is worth noting that SIRT1 does not merely switch off NFkB activity, but rather plays a subtle role in selectively modulating the expression of NFkB-dependent genes. This is particularly well illustrated by the finding that SIRT1 KO mice do not display the extremely inflammatory phenotype of mice expressing a constitutively active form of the p65 NFkB subunit [74], and by the finding that the acetylation status of the lysine 310 of p65 affects the expression of some, but not all not all NFkB target genes [35]. As a consequence, the possibility that the K310 deacetylated forms of p65 could be redirected to other target gene promoters cannot be presently excluded. As an example, in cells expressing the murine p65 mutant S276A, that is unable to recruit histone acetylases, the induction of TNFα and IL-6 is lost but the activation of the COX-2 gene is preserved [75], arguing in favor of different roles for the Lys310-acetylated and Lys310-deacetylated forms of p65. Further work is therefore required to precisely identify the target genes that may be preferentially inhibited by sirtuin activators in vivo. SIRT1 appears to negatively affect several transcription factors expressed by immune cells. In particular, the capacity of SIRT1 to negatively regulate the activity of both NFkB and FoxP3 appears as intriguing. When expressed by T cells, NFkB and FoxP3 regulate an immune response in opposite fashion, NFkB being considered as a positive regulator of T cell responses while FoxP3-expressing cells represent regulatory cells endowed with suppressive activity toward multiple immune effectors. This observation suggests that sirtuin inhibitors may act in a cell context dependent fashion, and although SIRT1 KO animals display an autoimmune-prone phenotype (in keeping with an overall anti-inflammatory role for SIRT1 in vivo), caution should be taken in generalizing the in vivo properties of sirtuin modulating compounds based on in vitro observations performed using purified cell subpopulations. The potential opposing roles of SIRT1 and SIRT6 on selected target genes is also a potential confounding factor illustrating the complex regulatory role of this enzyme family on the immune response. As previously discussed, although both SIRT1 and SIRT6 appear to negatively regulate the transcriptional activity of several NFkB genes, SIRT6 has also been shown to positively control the translational efficiency of TNFα mRNA. During an inflammatory reaction, TNFα is synthesized very early and several studies suggest that this cytokine orchestrates the release of other cytokines, potentially explaining the high beneficial impact of TNFα blocking reagents in the treatment of several inflammatory disorders [74]. Notably, mice expressing a constitutively active p65 form die a few weeks after birth from excessive inflammation, unless TNFα signaling is disrupted [74], We therefore believe that understanding the respective role of SIRT1 and SIRT6 in controlling TNFα production is warranted before a sirtuin-based anti-inflammatory therapy can be envisioned.

Low grade, chronic inflammation is associated with aging and linked to pathological conditions, such as type 2 diabetes and cancer. Noteworthy, sirtuin activity has been shown to play a role in related processes such as cellular and organismal lifespan extension [6,7], glucose homeostasis [63] and cellular differentiation [42,58]. The work reviewed herein suggests that some of the pathological processes linked to metabolic disorders or aging may

in fact be functionally linked to the immunomodulatory function of this family of metabolic sensors.

In conclusion, depending on the family member examined and on the experimental conditions, sirtuins display either pro- or anti-inflammatory properties. Although these activities can be modified by specific activators/inhibitors, a better understanding of the role of each member of the sirtuin family and the development of drugs able to specifically activate/inhibit individual sirtuins isoforms is warranted before clinically relevant applications of such compounds could be envisioned.

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