● PERSPECTIVE

The paradigm of protein acetylation in Parkinson's disease

Acetylation is a post-translational modification that is regulated by two antagonistic enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs transfer the acetyl group from acetyl-CoA to lysine residues of proteins while HDACs remove it (Yakhine-Diop et al., 2018b). The impairment of HAT or HDAC activity elicits changes in the protein acetylation status which disturb several cellular processes, among others, gene expression, autophagy *etc.*, leading finally to cell death. Both enzymes are associated with Parkinson's disease (PD) pathogenesis. In dopaminergic cells, neurotoxins provoke apoptotic cell death by increasing histone acetylation levels. While paraquat (Song et al., 2011) and rotenone (Feng et al., 2015) reduce HDAC activity, dieldrin (Song et al., 2010) enhances HAT activity. However *in vivo*, paraquat-induced upregulation of α-synuclein triggers histone hypoacetylation. Therefore, in PD models, proteins are hypoacetylated or hyperacetylated in response to a stimulus. All these controversies become a paradigm given that a HDAC inhibitor (Collins et al., 2015) as well as a HAT inhibitor can be cytoprotective (Yakhine-Diop et al., 2018a). Most of the studies that link acetylation to PD generally focus on the level of histone acetylation. Recently, a comparative study has been performed in two groups of fibroblasts from PD patients with or without the G2019S leucine-rich repeat kinase 2 (*LRRK2*) mutation. This is the first time that, by western-blotting, a smear of acetylated proteins as a whole reveals the difference between idiopathic (WT *LRRK2*) PD (IPD) and genetic (G2019S *LRRK2*) PD (GPD) under basal conditions. Those changes were more evident with higher molecular weight (MW) proteins (**Figure 1**) displaying a hypoacetylation in IPD and a hyperacetylation in GPD cells (Yakhine-Diop et al., 2018a). With peptides subjected to LC-MS, we also observed that hypoacetylated peptides were more abundant in IPD cells than in GPD (Yakhine-Diop et al., 2018b).

HDACs behavior in PD models: HDAC enzymes are eighteen proteins divided in four classes for their homology and structure. Class I (HDAC1, 2, 3, 8), class II (HDAC4, 5, 6, 7, 9, 10) and class IV (HDAC11) HDACs are nuclear and/or cytosolic proteins. Their deacetylase activity is Zn^{2+}-dependent and can be unspecifically inhibited by trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA) (Choong et al., 2016) or Valproic acid (VPA) (Gonzalez-Polo et al., 2015). The last class is the group of sirtuins (SIRTs) known as class III HDACs with 7 proteins and are NAD^+-dependent (Yakhine-Diop et al., 2018a). The role of HDAC proteins in PD-related neurotoxins is inconclusive. In two different studies, it has been reported that 1-methyl-4-phenylpyridinium iodide (MPP^+) can either decreases (Park et al., 2016) or increases (Choong et al., 2016) class I and II HDACs. In our study, an exhaustive characterization of HATs and HDACs (class I, II and III) enzymes drives us to the conclusion that hypoacetylated proteins in IPD cells are due to the consequences of accumulated damaged mitochondria. Indeed, the inhibition of mitochondrial complex I generally decreases the oxidation of NADH to NAD^+ (Yakhine-Diop et al., 2018a) and consequently reduces the activity of class III HDACs (Schwab et al., 2017). HDAC activity was determined by colorimetric assay and HDAC protein levels by western-blotting. The overall cellular HDAC activity consists of class I, II, III and IV HDACs, and its reduction was not significant in GPD cells (Yakhine-Diop et al., 2018a). In addition to the aforementioned hypoacetylation in IPD cells, total HDAC activity has been found remarkably decreased (**Figure 1**). Such a phenotype was unexpected, but the fact was SIRT inhibition triggered an increase activity of class I and II HDACs in IPD cells (Yakhine-Diop et al., 2018a). The most relevant were HDAC2, HDAC3 and HDAC4 proteins (**Figure 1**). Though the unspecific inhibitor of class I and II HDACs, TSA, reestablished the reduced histone 3 acetylation level at lysine 17, its effect was harmful (Yakhine-Diop et al., 2018a). Thus, a specific inhibition of class I and II HADCs could be protective as previously reported (Collins et al., 2015; Choong et al., 2016). However, in our unpublished data, VPA induced cell death in both IPD and GPD cells. VPA is a non-selective inhibitor of class I and IIa (HDAC4, 5, 7 and 9) HDACs (Gonzalez-Polo et al., 2015). Toxic effect of TSA or VPA is probably due to the expression of HDAC6 (class IIb HDAC) protein, which has already been reduced in GPD and IPD cells. The diminution of HDAC6 increases the level of acetylated tubulin at lysine 40 (**Figure 1**). Moreover, the only difference in class I HDAC expression occurring between IPD and GPD was HDAC3 protein (**Figure 1**) and might be involved in the hypoacetylation of nuclear proteins (histone 3 and 4). We then think a selective inhibitor of HDAC2, HDAC3 or HDAC4 (**Figure 1**) would be better to restore the acetylation level of proteins in IPD cells and/or to reduce cell death in both PD models.

SIRT critical role in PD models: Sirtuins (SIRT1, 2, 3, 4, 5, 6, and 7) are mitochondrial, nuclear and/or cytosolic proteins. Generally, SIRT activity is lower than other HDAC activity but essential for cell survival. Besides, cells are more susceptible to class III HDACs inhibition by nicotinamide in a dose-dependent manner (Yakhine-Diop et al., 2018a). In rotenone-treated neuroblastoma cells, SIRT1 protein is reduced which induces p53 expression and p53-mediated cell death (Feng et al., 2015). SIRT1 activity is modulated by its phosphorylation at serine 47 and it is enhanced in GPD cells and not in IPD cells (**Figure 1**) (Yakhine-Diop et al., 2018a). SIRT1 is implicated in autophagy regulation because of its deacetylase activity on autophagy-related (ATG) proteins. Its deacetylase activity also controls mitochondrial biogenesis and mitophagy, a selective mitochondrial degradation. In GPD fibroblasts and neuroblastoma cells, mitochondrial SIRT proteins (SIRT3 and SIRT5) (**Figure 1**), as well as the outer mitochondrial membrane protein TOM20 and subunit IV of cytochrome c oxidase (COXIV) were reduced due to mitochondrial clearance (Yakhine-Diop et al., 2018a). However, Schwab et al. attribute this mitochondrial content reduction to a mitochondrial trafficking deficit in GPD iPSC-derived dopaminergic neurons (Schwab et al., 2017). Back to our data, specific SIRT1 inhibition (EX-527 or gene silencing) provokes mitochondrial fragmentation, prevents mitophagy induction and increases the percentage of propidium iodide-positive cells in both IPD and GPD. In contrast to GPD, mitophagy is downregulated in IPD cells (**Figure 1**). Mitochondrial markers except SIRT3 are accumulated in IPD cells and can be cleared by the mitochondrial uncoupler, carbonyl cyanide 3-chlorophenylhydrazone (CCCP) (Yakhine-Diop et al., 2018a). The inhibition of SIRT activity in GPD iPSC (Schwab et al., 2017) could be alleviated by mitochondrial turnover through mitophagy, which compensates the reduction of total HDAC activity in GPD fibroblasts (**Figure 2**) (Yakhine-Diop et al., 2018a). To corroborate our hypothesis, it will be interesting to supplement IPD cells with NAD^+ to activate class III HDACs, at least SIRT1, for monitoring mitophagy flux.

It still remains unclear why proteins are hyperacetylated in GPD cells (Yakhine-Diop et al., 2018a). HAT activity was slightly increased and its inhibition by anacardic acid has not exhibited any aberrant effect in GPD cells. In contrast, anacardic acid protects against MPP^+-induced cell death and IPD-associated cell death (Yakhine-Diop et al., 2018a). MPP^+ is an inhibitor of mitochondrial complex I (Yakhine-Diop et al., 2017) and upregulates class I HDAC proteins (Choong et al., 2016). Otherwise, Park et al. (2016) reported a decrease of HDAC1 and HDAC2 with MPP^+ treatment. Despite all these discrepancies, the inhibition of HATs (Park et al., 2016; Yakhine-Diop et al., 2018a) reshapes the imbalance between total HAT and HDAC activities observed in IPD cells or induced by MPP^+ exposure. SIRT downregulation are responsible for this imbalance that subjects IPD cells to a striking cell death (**Figure 1**) in conjunction with an accumulation of damaged mitochondria and a reactive oxygen species generation. IPD represents the majority of PD cases and their pathological characteristics are somehow well determined: altered mitochondria, loss of mitochondrial membrane potential, increasing reactive oxygen species and reduced autophagy degradation. Whether there is a decrease or an increase in class I and II HDACs in IPD, it is clear that HAT inhibitors exert beneficial effects and could be an efficient therapy. To date, there are a lot of gaps in the mechanism of GPD that need to be elucidated unless its acetylation phenotypes resemble those of IPD.

Yakhine-Diop SMS was supported by Isabel Gemio Foundation. Uribe-Carretero E was supported by a FPU predoctoral fellowship FPU16/00684 from Ministerio de Educación, Cultura y Deporte, Spain. Niso-Santano M was funded by "Ramon y Cajal Program (RYC-2016-20883) Spain. González-Polo RA was supported by a "Contrato destinado a la retención y atracción del talento investigador, TA13009"

Phenotypes observed in PD models (**in bold changes between IPD and GPD**)		Idiopathic PD (IPD)	Genetic PD (GPD)
Histone acetyltransferases (HATs)	PCAF	↑	↑
	TIP60	↓	=
Histone deacetylases (HDACs) (Class)	**HDAC activity**	↓	=
	HDAC1 (I)	=	=
	HDAC2 (I)	↑	↑
	HDAC3 (I)	↑	=
	HDAC4 (IIa)	↑	↑
	HDAC6 (IIb)	↓	↓
	SIRT1 (III)	↓	↑
	SIRT3 (III)	↓	↓
	SIRT5 (III)	↑	↓
Acetylated proteins	**High MW (100-250)**	↓	↑
	Low MW (25-100)	↑	=
	Histone 3	↓	=
	Histone 4	↓	=
	Tubulin	↑	↑
Mitophagy flux	Induction	↑	↑
	Degradation	↓↓↓	↑
ROS generation		↑↑↑	↑
Cell death		↑↑↑	↑

Figure 1 Phenotypes related to acetylation in PD models.
Resume the significant changes occurring in fibroblasts from PD patients (Yakhine-Diop et al., 2018a). Blue and red arrows display, respectively, the increase or the decrease of the different phenotypes observed in IPD and GPD cells respect to the control cells. GPD: Genetic Parkinson's disease; IPD: idiopathic Parkinson's disease; PD: Parkinson's disease.

from Junta de Extremadura. JM.F. received research support from the Instituto de Salud Carlos III, CIBERNED (CB06/05/004) and Instituto de Salud Carlos III, FIS, (PI15/00034). This work was also supported by "Fondo Europeo de Desarrollo Regional" (FEDER) from the European Union. The authors also thank FUNDESALUD for helpful assistance.

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***Received:** October 29, 2018*
***Accepted:** December 15, 2018*

***doi:** 10.4103/1673-5374.250575*

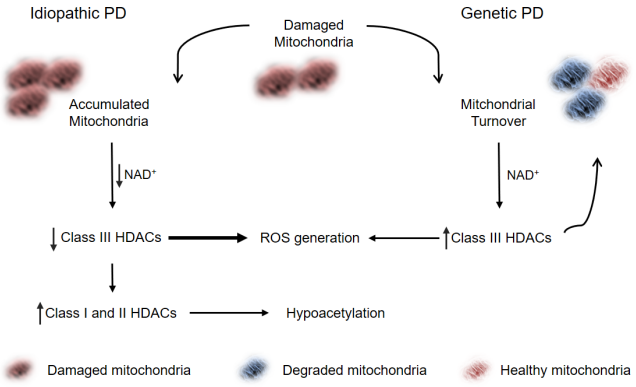

Figure 2 Compensatory mechanism of class III HDACs in GPD.
Accumulation of damaged mitochondria decreases the activity of class III HDAC (NAD^+-dependent) in IPD, which increases the activity of class I and II HDACs leading to the hypoacetylation of proteins and ROS generation. In GPD, the degradation of damaged mitochondria maintains the activity of class III HDAC and the mitochondrial turnover to compensate total HDAC activity. GPD: Genetic Parkinson's disease; HDAC: histone deacetylase; IPD: idiopathic Parkinson's disease; ROS: reactive oxygen species.

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