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COMMENTARY

STAT3 and REDD1: an unconventional story of gene repression

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The non-canonical functions of the transcription factor STAT3 have been poorly studied in comparison to its canonical mechanisms of gene expression activation. Here, Köhler et al. put the spotlight on a novel unconventional repressing mechanism of STAT3 over the REDD1 gene, named *DDIT4*. These findings are crucial to expand the knowledge of the stress-induced short-lived REDD1 protein that inactivates mTOR and the consequences of this fine-tuned regulation in the context of pathological conditions such as cancer or neurodegenerative diseases.

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STAT3 canonical and non-canonical functions in health and disease

Janus kinases (JAKs) are activated upon cytokine stimulation and phosphorylate signal transducers and activators of transcription (STATs) at tyrosine (Y) residues which results in dimerisation and translocation of STAT to the nucleus. Once in the nucleus, these transcription factors activate gene expression. Specifically, STAT3 is mainly known as an activator of gene transcription that requires the phosphorylation of its Y

Abbreviations
AD, Alzheimer's disease; APRE, acute phase response element; *DDIT4*, DNA-damage-inducible transcript 4; HD, Huntington's disease; iNOS, inducible nitric oxide synthase; JAK, Janus kinase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; mTOR, mechanistic target of rapamycin; mTORC1, mechanistic target of rapamycin complex 1; NFκB, nuclear factor kappa B; OXPHOS, oxidative phosphorylation; PD, Parkinson's disease; REDD1, regulated in development and DNA damage response 1; SOCS3, suppressor of cytokine signaling 3; STAT, signal transducer and activator of transcription; TAZ, transcriptional coactivator with PDZ-binding motif; TF, transcription factor; TSC1/TSC2, tuberous sclerosis complex 1/2; YAP, yes-associated protein.

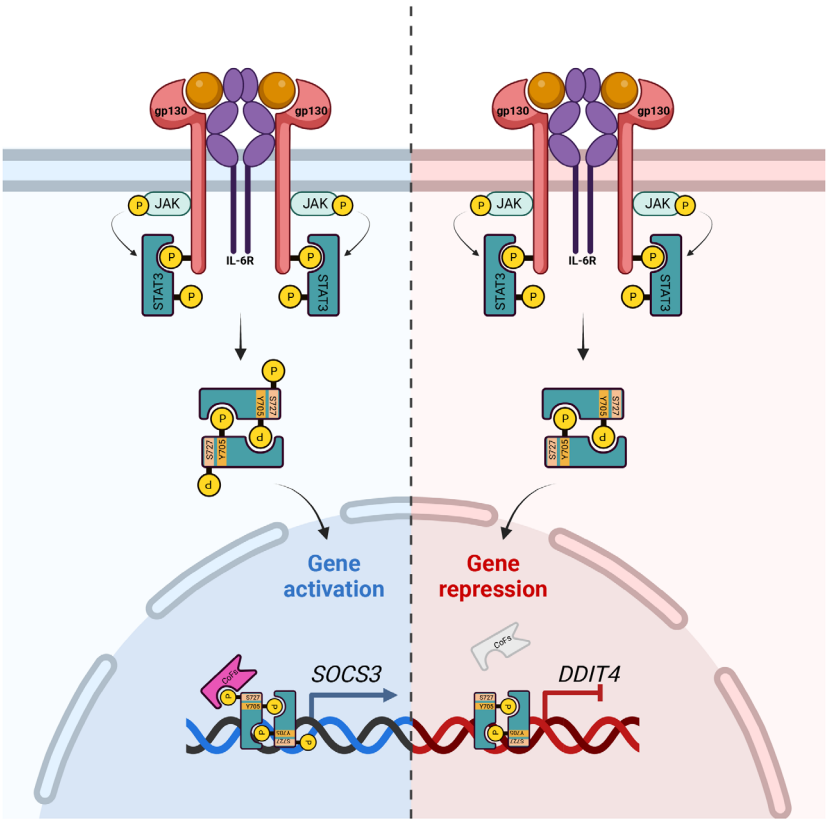

Fig. 1. The JAK/STAT3 pathway canonical function as a coactivator (left) and the novel mechanism as a transcriptional repressor over DDIT4 described by Köhler et al. (right). The canonical function of STAT3 requires phosphorylation of Y705 via JAKs and phosphorylation of serine 727 (S727) to dimerise and translocate into the nucleus and bind specific regions within the promoter of the target gene. It then requires the recruitment of specific activating cofactors (CoFs) to show maximal activation of transcription. As reported by Köhler et al., the phosphorylation of Y705 is also necessary for its function as a repressor of DDIT4 expression. Moreover, one possibility is that STAT3 could bind non-canonically to the promoter of DDIT4 via its N-terminal domain, which is necessary for the reduction of REDD1 expression. Nonetheless, it can also be that STAT3 interacts and blocks other transcription factors via its N-terminal domain. Created in Biorender.com.

residue 705 via JAKs to dimerise and translocate into the nucleus, where it binds to acute phase response elements (APREs) within the promoters of its target genes, such as suppressor of cytokine signaling 3 (SOCS3) (Fig. 1) (reviewed in Ref. [1]).

However, STAT3 has also non-canonical functions that are numerous and diverse but much less studied. In line with this, STAT3 has been shown to regulate the mitochondrial OXPHOS via direct interactions with components of the system [2]. Moreover, STAT3 negatively regulates the expression of some genes, such as inducible nitric oxide synthase (iNOS) by directly binding to nuclear factor kappa B (NFκB) [3]. Köhler et al. not only show that STAT3 can repress the expression of REDD1 but also elegantly dissect the elements of this novel gene repression function [4]. They identified the phosphorylation of residue Y705 and the N-terminal domain of STAT3 as essential for REDD1 gene repression (Fig. 1).

The importance of this novel non-canonical regulation of STAT3 upon REDD1 gene expression resides in the fact that REDD1 protein inactivates mechanistic target of rapamycin kinase (mTOR) kinase activities, a crucial hub that orchestrates many cellular responses to extracellular signals, such as nutrient levels, cytokine release or stress.

STAT3 is upregulated in carcinogenic processes [2]. As pointed out by Köhler et al., the link of this novel mechanism of REDD1 expression via STAT3 in cancer appears to be obvious since the upregulation of STAT3 may explain the described downregulation of REDD1 in such tumorigenic processes.

Interestingly, in neurodegenerative processes, the exact status of JAK/STAT3 signalling is still not fully understood. Some studies describe a reduction of STAT3 expression and Y705 phosphorylation in hippocampal neurons of AD patients, and STAT3 inversely correlates with Aβ levels in this context (reviewed in Ref. [1]). On the other hand, abnormal activation of STAT3 was also

associated with AD, due to its role in the induction of astrocyte reactivity [5]. Therefore, the relevance of this new non-canonical regulation of REDD1 via STAT3 is not yet elucidated. Some studies have pointed to the specific inhibition of STAT3 signalling as a therapeutic target in neurodegeneration [6], whereas others suggest diametrically opposite approaches (i.e. to activate JAK/STAT3 signalling) [1]. In addition, other regulations may apply to possibly explain this apparent contradiction in the mechanism discovered by Köhler et al. For instance, STAT3 phosphorylation at Y705 is upregulated in several tauopathies, pathologies composed by an accumulation of hyperphosphorylated protein Tau, which include Alzheimer's Disease (AD) or frontotemporal dementia. Indeed, overexpressing human P301L mutant tau (P301L-hTau) increased the phosphorylated level of STAT3 at Y705 by JAK2. Notably, STAT3 could not translocate into the nucleus; rather, it was located mainly in the cytoplasm via P301L-htau acetylation of STAT1, which causes it to bind to STAT3 and sequester it in the cytoplasm, thus disabling it from gene regulatory functions [7]. In addition, even though the role of STAT3 in astrocytes and neuroinflammation has been extensively described, less work has been done on the regulation of STAT3 in neurons. For instance, inactivation of the JAK2/STAT3 axis causes cholinergic neuronal dysfunction, and it is linked to memory impairment in AD. This suggests a differential regulation of the STAT3 phosphorylation in distinct cell types [1].

All in all, the specific contribution of the non-canonical regulation of STAT3 upon *DDIT4* transcription needs to be addressed in other scenarios such as tumorigenesis or neurological disorders to unravel its physiopathological importance.

REDD1 role in physiological and pathological conditions

Regulated in development and DNA damage response 1 (REDD1), also known as RTP801, is a stress-responsive protein expressed at low levels in many tissues, including neurons and astrocytes. It is encoded by the stress-responsive gene DNA-damage-inducible transcript 4 (*DDIT4*), whose transcription increases in cases of hypoxia [8], DNA damage [9] and exposure to dopaminergic neurotoxins such as 6-hydroxydopamine, MPTP or rotenone [10]. Others manifest threats to cell integrity as increased reactive oxygen species production [8] or sustained ER stress [11] notably increases *DDIT4* expression. Nonetheless, REDD1 gene expression is upregulated when cells are exposed to mild stresses related to the metabolic status, for instance, under fasting [12] or glucose deprivation [13] conditions. Overall, this suggests that REDD1 is not only a stress-regulated protein but also a key regulator of organism homeostasis.

REDD1 inhibits mTOR via the tuberous sclerosis complex 1/2 (TSC1/TSC2) [14]. Even though it was first proposed that REDD1 interacts with 14-3-3 protein to exert its function, crystallographic and functional analysis of the protein revealed that mTORC1 inhibition via REDD1 is not dependent on 14-3-3 [13]. Nonetheless, the exact mechanism by which REDD1 acts has not been yet fully elucidated.

REDD1 is also an interesting target from a pathogenic point of view. Expression of REDD1 is altered in a vast range of pathologies, from inflammatory diseases to cancer and neurodegenerative disorders. For instance, we have described that REDD1 is upregulated in post-mortem brains of patients with Parkinson's (PD) [9], Huntington's (HD) [15] and AD [16], and it has been linked to increased neuroinflammation and cognitive deficits in both HD [17] and AD [16] mice models. Interestingly, selective knockdown of REDD1 in hippocampal neurons with shRNA-containing AAV particles ameliorates cognitive deficits as well as diminishes astrocytes- and microglia-mediated neuroinflammation in a murine model of HD [16] and AD [15].

Regarding cancer, it has been proposed that REDD1 constitutive and sustained expression may benefit tumour cell survival and metastasis in hypoxic conditions as well as resistance to chemotherapy or ionising radiation via decreased energy consumption [18].

REDD1 is thereby an interesting target regarding the control of cell growth, proliferation and survival via the mTOR signalling pathway, arguably one of the central cascades in cell biology. Remarkably, REDD1 stands out among other negative mTOR regulators due to its ability to efficiently shut down protein synthesis – a high energy-consuming process under the control of mTORC1 – quite early during acute metabolic stress.

To the best of our knowledge, only one publication has shown a similar regulation on REDD1 expression hitherto. YAP/TAZ oncogenic transcriptional co-activators were shown to act as transcriptional co-repressors of *DDIT4*, thereby promoting cell growth and survival [19]. Therefore, the non-canonical role of STAT3 in the negative regulation of REDD1 appears not to be uncommon, and other non-canonical regulations of the *DDIT4* gene – whether reducing or not REDD1 transcription – are also perfectly feasible.

Concluding remarks

The work presented by Köhler et al. opens a new paradigm regarding the expression of REDD1 and its

modulation. Given the pivotal role of REDD1 in neurodegenerative diseases as well as carcinogenesis, it is a piece of worthwhile information that points to a specific regulatory target. This opens several research lines linking the specific contribution of the STAT3/REDD1 axis in the regulation of pathogenic mechanisms in neurodegenerative diseases, among others. Hence, this novel mechanism paves the way for further studies aimed at the discovery of novel regulatory pathways for STAT3/REDD1 inhibition as potential disease-modifying treatments.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

PG-S and CM wrote, reviewed and edited the manuscript.

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