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Where Sin3a meets STAT3: balancing STAT3-mediated transcriptional activation and repression

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Significance statement: STAT3 is able to mediate epigenetic silencing of tumor suppressor genes (TSG). However, little is known about the molecular mechanisms involved, except that this action is mediated by DNA methylation and requires STAT3 acetylation. Here, Gambi and collaborators confirm that oncogene-driven constitutive STAT3 acetylation is responsible for TSG silencing. Further, they identify the Sin3a transcriptional repressor complex as an obligatory partner of STAT3 on the promoters of the repressed genes, shading light on the mechanisms involved in STAT3-mediated transcriptional repression. Importantly, this STAT3-Sin3a axis emerges as a potential selective therapeutic target to specifically hit STAT3-dependent tumors.

Signal Transducer and Activator of Transcription (STAT) 3 plays a multitude of different functions downstream of cytokines and growth factors receptors and of oncogenes. Failure of tightly controlling its activation, leading to aberrant constitutive activity, is a hallmark of pathological conditions including inflammatory and auto-immune diseases and cancer (1), and indeed addiction to STAT3 activity is a frequent feature of many different tumors. Despite the general agreement on STAT3 being an oncogene, its specific role in tumors is far from straightforward. Indeed, while in most cases STAT3 activities contribute in multiple ways to tumor transformation, growth and progression, this factor can also act as a tumor suppressor, inducing apoptosis or reducing motility and Epithelial to Mesenchymal Transition (1), supporting the idea that STAT3 activities and functions are strongly context- and cell type-specific. Likewise, while the best characterized nuclear activities of STAT3 relevant to cancer are related to transcriptional induction, it has often been observed that more genes are up-regulated rather than down-regulated as a consequence of its inactivation in cancer cells, suggesting that the ability to repress transcription of specific target genes plays a relevant role in STAT3 pro-oncogenic activities. Indeed, constitutively active STAT3 was shown to be essential to trigger and maintain the silencing of several tumor suppressor genes (TSGs) in NPM-ALK-+ T cell anaplastic large cells lymphomas (ALCL), breast cancer and melanoma (2, 3).

The multiple and sometimes contradictory roles played by STAT3 are likely the result of a number of factors, not least the numerous post-translational modifications that regulate both its canonical nuclear activities and its non-canonical functions in the cytoplasm, the mitochondria and the Endoplasmic Reticulum (4). While phosphorylation on Tyrosine 705 (Y-P) is required for STAT3 dimerization, nuclear localization, DNA binding and transcriptional activities, Serine-727 phosphorylation (S-P) enhances STAT3 trans-activating ability but more importantly is required for its non-canonical functions in both the mitochondria and the ER, modulating OXPHOS activities, ER Calcium signaling and apoptotic responses (5, 6). As to the other PTMs, Lysine methylation and Cysteine oxidation or glutathionylation reduce STAT3 trans-activating power, while acetylation, which has been observed on Lysine residues within both the N-terminal and the TAD domains mainly downstream of the CBP/p300 Histone Acetyl Transferase (4), is associated to enhanced transcriptional activity because of dimer stabilization and enhanced Tyr-phosphorylation, nuclear localization and interaction with CBP/p300.

Indeed, like all other STATs, STAT3 trans-activating potential mainly relies on the ability of recruiting to promoters the Histone Acetyl Transferase (HAT) CBP/p300, triggering localized histone hyperacetylation. On the other hand, STAT3-mediated transcriptional repression of TSGs was shown to require its acetylation on the K867 residue and to correlate with DNMT1 interaction and promoter hypermethylation (3). However, why would STAT3 complex with DNMT1 on the regulatory regions of tumor suppressor genes and trigger epigenetic silencing, while recruiting CBP/p300 leading to activation of cell-cycle and anti-apoptotic genes promoters, is so far still a mystery.

The findings described by Gambi and colleagues in this issue of Cancer Research partially shed light on the mechanisms involved (7). A conundrum in type I IFN signaling was its well-known ability to trigger anti-viral and antiproliferative responses via the activation of the ISGF3 transcriptional complex (STAT1: STAT2:IRF9) while failing to induce STAT3-mediated transcription despite its canonical robust activation. These data suggested the ability to uncouple the phosphorylation and DNA binding activity of STAT3 from its trans-activating power. Previous work by Icardi and co-workers in Jan Tavernier's group (8) led to the identification of Sin3a, the central component of the Sin3a co-repressor complex, as a direct partner of acetylated STAT3. Sin3a and STAT3 co-localized at promoters of STAT3 target genes upon both type I IFN and LIF treatment, involved STAT3 Y-P as well as K-acetylation. Despite the involvement of the DNA Binding Domain of STAT3, devoid of known acetylated residues, the interaction was greatly enhanced by acetylation on the N-terminal K87 residue, and promoted STAT3 deacetylation and segregation from the nucleus. Importantly, these effects were tightly cell-specific, as Sin3a did not affect LIF or IL-6-mediated induction of acute phase response genes in hepatoma cells. This work identified Sin3a as an important regulator of STAT3 transcriptional activity, able both of inhibiting its trans-activating potential downstream of type I IFN, curbing its pro-proliferative functions, and of modulating the amplitude of STAT3-mediated transcriptional induction downstream of its canonical stimuli such as LIF or IL-6. In the present issue, Gambi and colleagues show that Sin3a is also the main mediator of the epigenetic silencing of TSGs triggered by STAT3 in both ALCL and triple negative breast cancer (7). Indeed, oncogene-induced constitutively acetylated STAT3 and Sin3a bind as a complex to TSG promoters, and inhibition of STAT3 acetylation (and S-P) by resveratrol decreases this binding while reactivating the expression of a number of TSGs in ALCL. On the other hand, also Sin3a silencing increases TSGs expression, impairing cell growth both *in vitro* and *in vivo* and enhancing spontaneous

apoptosis. These findings bridge the previously reported ability of type I IFN to repress STAT3-mediated transcription (8) with the ability of constitutively acetylated STAT3 to trigger the epigenetic repression of TSGs (3). Both activities indeed require Sin3a and Sin3a-STAT3 interactions at gene promoters. Sin3a prominent role in STAT3-dependent transcriptional repression is compatible with the reported DNA methylation on the repressed gene promoters, and with the recruitment of DNMT1, HDAC1 and MeCP2, which are all known interactors of the Sin3a co-repressor complex.

Several questions remain still open. In particular, the exact interplay of signaling events regulating STAT3 acetylation levels is poorly understood, along with the identification of the Lysine residues implicated. Indeed, in the present paper the authors show that STAT3 K685 acetylation levels correlate with STAT3-mediated TSGs repression and Sin3a interaction. However, other Lysine residues are likely involved, since either acetyl-deficient or acetyl-mimicking mutations of K685 failed to affect Sin3a binding. One candidate is the K87 residue within the N-terminal domain, which was shown to enhance STAT3-Sin3a interactions (8). However, there are about 47 conserved Lysines in the STAT3 sequence. These will have to be characterized to assess their acetylation and how it is regulated downstream of different signals in order to shed complete light on the pathway. Although the mechanism of action of resveratrol, which reduces both S-P and K-acetylation on STAT3, is poorly understood, MAP kinases, PI3K and mTOR, all activated downstream of NPM-ALK, have been variably implicated in STAT3 S-P and K-A. In particular mTOR, a known resveratrol target, could represent the key molecule involved in coordinately orchestrating STAT3 S-P and K-A levels.

Likewise, unknown are the molecular events determining whether the STAT3-Sin3a complex bound to genes' regulatory regions leads to i) moderated transcriptional activation upon stimulation with the canonical physiological STAT3 activators IL-6 and LIF, ii) impaired trans-activating power following type I IFNs treatment, or iii) active transcriptional repression downstream of oncogenic activation. Each of the named stimuli can activate distinct signaling pathways, likely leading to even subtly different PTM combinations on STAT3 and to the concomitant activation of alternative transcription factors and co-factors, which in turn can determine the final transcriptional output. In this vein both STAT1 and STAT3, which exhibit opposite functions, have been shown to cross-regulate their activities downstream of type II IFNs and IL-6, respectively, contributing to shape cytokine signaling specificity and suggesting that the abundance and activation status of these two factors may significantly qualitatively and quantitatively alter cytokine responses (9, 10). Indeed, the

ability of either factor to compensate for each other's absence suggests that the cellular and signaling context leading to their activation determines whether genes are activated, not activated or even repressed. It is tempting to speculate that these effects could be at least partially mediated by STAT3 acetylation patterns and the degree of interaction with Sin3a downstream of different signals.

In conclusion, the schematic dogma establishing that there are two forms of STAT3, either unphosphorylated and inactive or phosphorylated and active, has since long been disproven. STAT3 comes in many flavors, which modulate its functions in different cell compartments. Even only concentrating on its nuclear functions, the specific distribution of its many PTMs can subtly regulate specific functions determining the subsets of genes that are in each case activated, not activated or repressed. Considering the many physiological and pathological functions of STAT3, one may predict that a generalized, good-for-all inhibitor, which many laboratories and pharmaceutical companies are striving to develop, may reveal toxic and/or not sufficiently specific. Understanding the molecular bases regulating the multifaceted functions of STAT3 and its PTMs may offer opportunities to try and develop inhibitors of specific functions, either in the cytoplasm or in the nucleus. Accordingly, the work here described suggests that decoy molecules able to disrupt the Sin3a-STAT3 interaction could allow to specifically reactivate TSGs in those tumors that exhibit addiction to STAT3 activity.

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