



CIS is a negative regulator of IL-15-mediated signals in NK cells

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The cytokine-inducible SH2-containing protein (CIS, encoded by *Cisb*) is a member of the suppressors of cytokine signaling (SOCS), a family of intracellular proteins that have been shown to regulate the activity of a variety of cytokine and growth factor receptors (1). Similar to the other members, CIS presents a central SH2-domain able to interact with phosphotyrosine residues and a SOX box motif that recruits the ubiquitin-transferase system (u-ts) and directs targeted proteins to proteasomal degradation.

CIS has also been shown to interact with the cytoplasmic domains and suppress the signals of different receptors including the IL-2R (2). In the study recently published in *nature immunology* (3), Delconte and colleagues analyze the role of CIS in regulating the IL-15 signaling pathway in NK cells. This pathway includes signals that are transmitted through the IL-2R β (CD122) and γ c (CD132) subunits that, upon engagement, associate and activate JAK1 and JAK3 (*Figure 1*). It follows STAT5 activation that induces the transcription of genes involved in the survival, proliferation and function of NK cells (4). The authors deepened the regulatory role of CIS in mouse NK cells using a *Cisb*-deleted model (*Cisb*^{-/-}). They show that CIS is expressed early during NK cells differentiation and that saturating high concentrations of IL-15 increased its expression at both mRNA and protein level. *Cisb*^{-/-} NK cells showed upregulation of IL-2R β expression and hyper-responsiveness to IL-15, which resulted in increased survival, proliferation, cytotoxicity and IFN- γ production as compared to *Cisb*^{+/+} NK cells (*Figure 1*). Moreover, in *Cisb*^{-/-} NK cells cytokine stimulation modulated more than 1,000 genes, including

those coding for granzymes, and increased phosphorylation of 69 kinases including JAK-1, JAK-3, STAT5 and kinases mainly involved in regulating cellular proliferation such as CDK1/2, Prkr and Aurora kinases. By using a recombinant trimeric complex formed by a human CIS-SH2 construct coupled to elongins B and C, the authors show that CIS, similar to SOCS-1 and SOCS-3, binds to JAK-1 and JAK3. CIS, which lacks the kinase inhibitor region (KIR), interact with JAK1 via the SH2 domain, targets JAK1 to proteasomal degradation and inhibits its enzymatic activity (*Figure 1*), although with an efficacy 100 fold lower than SOCS1. It has been previously published that exposure of mouse NK cells to high concentrations of IL-15 results in the activation of the evolutionarily conserved serine-threonine kinase mTOR (mammalian target of rapamycin complex) a crucial metabolic checkpoint regulating translation and glycolytic pathway in both homeostatic and pro-inflammatory conditions (5). Although CIS and mTOR share analogy in the mechanisms of induction, the two pathways appear to be disconnected. Indeed, in *Cisb*^{-/-} NK cells IL-15 does not increase the phosphorylation of AKT, that represents a crucial event during mTOR activation (6). In line with this observation, *Cisb*^{-/-} NK cells show normal mitochondrial respiration and glycolysis, functions that are regulated by the AKT activity.

Cisb^{-/-} mice apparently are healthy and display normal NK and T lymphocytes frequency and phenotype (3). According to an increased activity of CIS-deficient immune cells, *Cisb*^{-/-} mice show fewer lung metastases than *Cisb*^{+/+} when intravenous injected with melanoma, prostate or breast cancer tumor cell lines, a protective effect that is

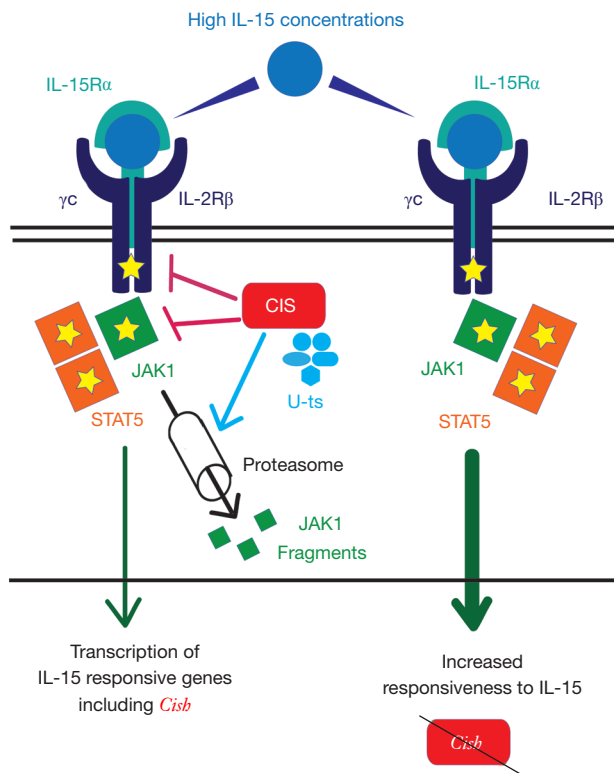


Figure 1 Regulatory role of CIS in IL-15 stimulated NK cells. The IL-15 binding to the receptor activates JAK1 (JAK3) and STAT5 leading to the transcription of genes regulating survival, proliferation and effector functions of NK cells. A high IL-15 concentration leads to the transcription of *Cish* whose product binds IL-2R β and JAKs. CIS inhibits JAK1 tyrosine phosphorylation and drives JAK1 to proteasomal degradation thanks to the recruitment of the ubiquitin-transferase system (u-ts). In *Cish*^{-/-} mice NK cells show increased responsiveness to IL-15. CIS, cytokine-inducible SH2-containing protein.

mainly dependent on the effector function of NK cells. Finally, even fewer metastases were detected in *Cish*^{-/-} mice treated with a combination of anti-PD-1 and anti-CTLA-4 mAbs (7-9). Since CIS is induced by high concentrations of IL-15, the authors suggest that, in the mouse model used, NK cells might receive such intense stimulus from the inflammatory tumor microenvironment. In particular, immune cells colonizing tumor tissues, such as dendritic cells or macrophages, might “trans-present” high concentrations of IL-15 to NK cells.

Overall, the study demonstrates that CIS represents a negative feedback mechanism activated not only by IL-2 (2) but also by IL-15 switching off NK cells effector functions

including cytotoxicity against tumor cells (4,10,11). In this context, a recent paper showed that in CD8⁺ T cells CIS inhibits the TCR signaling by physically interacting with the TCR intermediate PLC- γ 1 and targeting it for proteasomal degradation (12). The Authors also propose CIS as a novel immune checkpoint and highlight the potential benefit of therapies combining CIS inhibition and low doses of IL-15 to improve anti-tumor NK cell function in patients.

Importantly however, the information on CIS is still scarce and we cannot underestimate the possible side effects of unleashing the function of lymphocytes with high cytolytic and proliferative capacity. Moreover, a condition sine qua non will be to inactivate CIS in selected and specific target cells. It can't be disregarded previous observations showing that CIS expression is not restricted to lymphocytes but is present in other normal tissues including kidney, lung, liver, stomach and heart thus suggesting that it may represent a wide type of regulatory molecule (1,13). Moreover, CIS was shown to interact and regulate highly pleiotropic receptors including the growth hormone (1). Although *Cish*^{-/-} mice were healthy, fertile and with no evidence of phenotypic or immunological abnormalities, further studies are needed to exclude that CIS targeting might result in dangerous dysregulation or breaking of crucial feedback mechanisms that tune important cellular functions in both hematopoietic and non-hematopoietic tissues. At this regard, a recent study shows that aged *Cish*^{-/-} mice develop an inflammatory lung condition associated with altered IL-4-STAT6 and IL-2-STAT5 signaling in CD4⁺ T cells (14). Moreover CIS and SOCS1-7 would play a key role in regulation of cellular homeostasis and inflammation and are now regarded as tumor suppressors both in hematological and solid malignances (1). Indeed, a link has been demonstrated between reduced levels/function of SOCS proteins (due to gene mutation/deletion or epigenetic mechanisms) and cancer development and progression (1). The role of some proteins belonging to this family could be even more complex. Indeed, SOCS-2, which is closely related to CIS (1), appears to play a dual opposite role, being able to control both growth factor receptors and other SOCS proteins depending on its concentration and cellular context. Last but not least, it is important to note that an untargeted CIS neutralization might strength not only the immune-stimulatory effects of IL-15 but also its pro-angiogenic property (15-17).

In conclusion, the results by Delconte (and other research groups) contribute to our knowledge on CIS

properties. However, data must be validated in the human system and further studies are required to demonstrate the absence of long-term systemic side effects of CIS inactivation and to translate the results into the clinical practice.

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Footnote

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