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Institutionen för molekylär medicin och kirurgi

Studies on the mechanisms of action and physiological relevance of SOCS proteins

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ABSTRACT

Understanding systemic biological pathways and the key cellular mechanisms that dictate disease states, drug response, and altered cellular function in metabolic disorders is a significant challenge. Research in the last 20 years have made it clear that tissue communication, through the actions of endocrine, paracrine or autocrine factors play a significant role in pathogenesis of complex multiorgan diseases such as the metabolic syndrome. The actions of these factors are governed both at the site of production and through mechanisms that regulate the sensitivity of target tissues.

The Suppressors of Cytokine Signaling (SOCS) proteins act as negative regulators of the main cytokine and growth factor signaling pathways in multiple tissues and as such have important physiological functions. The molecular basis for SOCS actions as well as their role in the pathogenesis of metabolic diseases is just starting to be understood. All SOCS proteins (SOCS1 to 7 and CIS) are characterized by the presence of structural motifs called SOCS box and a SH2 domain. SOCS are thought to act as substrate recognition subunits of multimeric Cullin/RING E3 ubiquitin ligase complexes. It has been proposed that the SH2 domain mediates the recognition of tyrosine phosphorylated signaling proteins to be targeted for ubiquitination and subsequently, proteasomal degradation, thereby inhibiting cytokine signaling.

In this thesis we investigated the mechanisms of action of SOCS2 and SOCS6 in the inhibition of cellular signaling and the physiological consequence of their actions. We demonstrated that both SOCS2 and SOCS6 assemble a canonical ECS (Elongin/Cullin/SOCS) complex through the interaction of SOCS box with Elongin B and C, cullin 5 and Rbx2. We also demonstrated that SOCS2 and SOCS6 exert E3-ligase activity towards the growth hormone (GH) receptor and cKIT proteins, respectively. Our structural and binding studies confirmed the existence of substrate binding motifs mainly in the SH2 domains and the N-terminal domain of both SOCSs. The C-terminus harbours the cullin 5 recognition domain that controls both E3 ligase activity of the complex as well as the SOCS stability. We proposed that extended target recognition domain in the SOCS proteins may serve to broaden their specificity toward various targets and hence their ability to regulate various signaling pathways. On the other hand, the existence of a degradation signal within the Elongin C interacting domain of SOCS proteins may serve to secure their timely actions avoiding competition from SOCS that are not engaged in active E3 ubiquitin ligase complexes.

The patho-physiological role of SOCS2 was studied in SOCS2 knockout ($SOCS2^{-/-}$) mice. In line with the in vitro studies (paper I), we observed an increased GH sensitivity in $SOCS2^{-/-}$ mice, demonstrated by low plasma GH/IGF1 ratio. In the liver, this enhanced sensitivity was manifested through increased VLDL secretion and reduced hepatic triglycerides levels. $SOCS2^{-/-}$ showed reduced hepatic steatosis upon high fat feeding as compared to wild type littermates but also exhibited increased adiposity and fat deposition in the skeletal muscles accompanied by profound systemic insulin resistance. We also demonstrated the involvement of SOCS2 in the regulation of inflammatory pathways. $SOCS2^{-/-}$ mice showed an exacerbated response to a high fat diet, with increased expression of inflammatory cytokines such as IL-6, RANTES, IL1β both in the liver and adipose tissue. We also identified possible mechanisms to explain these phenomena by demonstrating that macrophages isolated from $SOCS2^{-/-}$ mice showed higher phagocytic activity and higher LPS-induced NF-κB activity; indicative of SOCS2 negative regulation of TLR4 signals.

Given the short half-life of SOCS proteins in the cells, the regulation of SOCS gene transcription is an important mechanism to control their function. The nuclear receptor LXR has regulatory functions on hepatic lipid metabolism that overlap with those controlled by the GH. Therefore, in order to understand the molecular basis for possible crosstalks between these two pathways, we studies how LXR ligands regulate the GH receptor signaling in liver. We showed that LXR agonist downregulates STAT5b protein levels and suppresses GH receptor activity in hepatocytes through a mechanism involving SREBP1. The regulation by SREBP1 occurs through the modulation of STAT5b protein stability and results in reduced expression of GH target genes such as SOCS2. These results provide a plausible explanation for the hepatosteatosis observed upon LXR agonist treatment in vivo.

In conclusion, through structural, *in vivo* and *in vitro* studies, we provide mechanistic and functional data on SOCS2 and SOCS6; information that may lead to a better understanding of the distinct physiological functions of these proteins. Given our demonstration on the key role of ubiquitination on SOCS functions, future mechanistic studies of SOCS2 and SOCS6 function should focus on the identification of ubiquitination targets of these proteins. Are the physiological functions ascribed to these proteins a result of the degradation of a few target proteins or do they have many targets? If the later is the case, how is SOCS target-specificity structurally determined and how is their activity regulated in time and cellular location? The demonstration that SOCS2 regulate both GH receptor and TLR4 signaling offers system where these questions can be addressed. At physiological levels, the $SOCS2^{-/-}$ mice constitute a novel model system for the study of the metabolic syndrome with unique features that are relevant to the human disease. In the $SOCS2^{-/-}$ mice, insulin resistance and production of inflammatory cytokine are exacerbated by high fat feeding and associated with obesity and deposition of triacylglycerides in the muscle. In these conditions reduced accumulation of TG in liver is observed. This model presents opportunities for future investigations aiming to distinguish between steatotic versus inflammatory causes for insulin resistance.

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