New modifiers of insulin signalling identified by interaction screens with ASNA-1 in *C. elegans*

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofie/medicine doktorsexamen framläggs till offentligt förvar i Salens namn eller beteckning, Sal B, 9tr, Norrlands universitetssjukhus (NUS), måndagen den 10 december, kl. 09:00. Avhandlingen kommer att förvaras på engelska.

Fakultetsopponent: Professor Richard W. Padgett, Walksman Institute, Department of Molecular Biology and Biochemistry, Rutgers University, New Jersey, USA.
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Abstract

Background: Insulin is a hormone released by the pancreatic beta cells in response to elevated levels of nutrients in the blood. Insulin triggers the uptake of glucose, fatty acids and amino acids into the liver, adipose tissue and muscles. Genes regulating insulin signalling are thus of vital importance for metabolic homeostasis and for preventing the development of diabetes. This thesis aims to identify new modifiers of insulin signalling, while carrying out functional studies of a homolog to human arsenite translocating ATPase, ASNA1. ASN1 activates the insulin signalling pathway and promotes insulin secretion in mammalian cell lines and in Caenorhabditis elegans. A second aim is to better understand how ASN1 and its interactors regulate sensitivity to the chemotherapeutic drug, cisplatin. Results: Regulators of insulin/IGF signalling (IIS) in C. elegans were identified based on the Larval arrest arrest aspect of the asna-1 depletion phenotype. Sixty-five genes were selected by virtue of their predicted interaction with ASNA-1 and screened for asna-1 like larval arrest upon inactivation of the genes. mrps-2, mrps-10, mrpl-43 encoding mitochondrial ribosomal protein subunits, and enpl-1 encoding an ER chaperone, GRP94 homolog were identified as the genes which when inactivated caused larval arrest without any associated feeding defects. IIS was weaker and insulin secretion was defective in these knockdown animals. ENPL-1 and ASNA-1 proteins interacted with one another both ex vivo and in vitro. ASNA-1 protein and mRNA levels were greatly reduced in enpl-1 mutants and enpl-1(-);asna-1(-) double-mutant worms displayed synthetic lethality. Overexpression of the insulins INS-4 and DAF-28 caused partial rescue of the germline phenotype of enpl-1 mutants, indicating that the phenotype of enpl-1 mutants was due at least in part to insufficient insulin levels. Studies of enpl-1 mutants also helped to understand the role of asna-1 in cisplatin sensitivity. The unfolded protein response (UPR) was induced in asna-1 and enpl-1 knockdown animals. enpl-1 mutants displayed higher sensitivity to cisplatin, when compared to asna-1 mutants and this correlated to higher UPR in enpl-1 knockdown animals. Pharmacological induction of the UPR in intrinsically cisplatin resistant wildtype worms also resulted in increased cisplatin sensitivity. This suggests that manipulation of ENPL-1 levels or of the UPR could enhance the anti-tumoral effects of cisplatin based cancer therapy. With a yeast two-hybrid screen 27 putative physical interactors of ASNA-1 were identified. Among these candidates was smn-1, which encodes survival of motor neuron protein homolog. RNAi knockdown of smn-1 caused a larval arrest phenotype similar to asna-1 depleted animals and smn-1 positively regulated IIS, like asna-1. Defects in IIS may be at the level of insulin release because neuropeptide secretion was impaired upon smn-1 knockdown. Further in vitro binding studies showed that SMN1 and ASNA-1 interacted and inactivation of smn-1 in asna-1 mutants resulted in decreased viability. This implies that SMN1 is another modifier of ASNA-1 and also a new component in IIS. Conclusion: With a directed RNAi screen and a yeast two hybrid screen several interactors of ASNA-1 that are also IIS modifiers were identified. ENPL-1 and SMN1 are both involved in insulin release. We also found that induction of the UPR in enpl-1 and asna-1 mutants is a possible mechanism for increased sensitivity to cisplatin.

Keywords

Insulin, C. elegans, ASNA1, Cisplatin, GRP94, unfolded protein response, SMN1