The importance of saccharides in GAS1 function

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GAS1 (Growth Arrest-Specific protein 1) is a monomeric soluble protein, anchored to the plasma membrane, which carries out different functions, highlighting its role as coreceptor of Sonic Hedgehog ligand (SHH) (Wierbowski, BM et al. (2020) Dev. Cell 55 (4), 450-468). In animal cells, this signaling pathway plays an essential role in embryogenesis and stem cells homeostasis in adult tissues. The alteration or absence of GAS1 has been related to the appearance and severity of a disease called holoprosencephaly, characterized by an incomplete division of the forebrain (Seppala M et al. (2007) J. Clin. Invest. 117 (6), 1575-1584). One of its characteristics is the presence of a glycosylation at the residue Asn117. This modification has been revealed to be very important for GAS1 function, but, quite surprisingly, its role has not been studied in detail yet. So, the main purpose of our investigation is to characterize the glycosylation pattern of this protein and examine how different glycans introduced by host expression systems can affect its function. With this purpose, we have cloned and produced the soluble domain of GAS1 in the yeast recombinant expression system *Pichia pastoris* and in mammalian cells. The proteins were structurally characterized, and functional experiments were performed to evaluate how the different glycosylation patterns affect its function. We tested its ability to bind cholesterol, which is key for its function (Huang P et al (2022) Dev. Cell 57 (5), 670-685)); and their ability to extract the ligand SHH from HEK293 membranes. We were able to determine that some of the differences observed between both recombinant proteins were very probably being caused by the different glycans added by the two host expression systems employed. More experiments are still required to confirm and quantify these already preliminary, though very promising, observations.