In planta engineering of KDNylated glycoproteins

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The 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) is a seldom studied sugar residue in the family of sialic acids [1]. Unique characteristics of KDN are resistance to sialidase, termination of polysialic acid chain length, and its presence as free sugar in certain tumors. In contrast to Neu5Ac, the biological function and immunogenic profiles of KDN carrying glycoconjugates are largely unknown. The KDN and Neu5Ac biosynthetic pathways overlap at different levels, making it highly challenging to produce KDN in mammalian cells. To address this challenge, we considered advantage of plants lacking sialic acid biosynthesis pathway in engineering approaches.

For in planta engineering a donor substrate for KDNylation, two proteins involved in the KDN biosynthetic pathway are co-expressed, the human N-acetyl neuraminic acid synthetase (hNANS) synthesizing KDN and the CMP-sialic acid synthase 2 from Zebra fish (ZfCMAS2) to activate the KDN to CMP-KDN [2]. Quantitative analysis showed a measurable amount of free KDN and CMP-KDN, indicating the ability of plants in synthesizing CMP-KDN. To synthesize KDNylated glycoproteins, we co-expressed a reporter glycoprotein along with 2 proteins required for CMP-KDN synthesis and 3 proteins involved in the mammalian sialylation pathway.

The mass spectrometry analyses of reporter highlights the ability of plants to synthesize KDNylated proteins. However, they also point to the need for optimization of the engineering strategies to obtain quantitatively KDNylated proteins for further functional studies.

Bibliographic references:

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