Genotype-dependent glycosylation affects histidine-rich glycoprotein activation by plasmin

Yang ZOU [1], Matti PRONKER [1,2], Albert J. R. HECK [1,2], Karli R. REIDING [1,2]

[1] Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands, [2] Netherlands Proteomics Center, Padualaan 8, 3584 CH, Utrecht, The Netherlands

y.zou2@uu.nl

Histidine-rich glycoprotein (HRG) is an abundant plasma glycoprotein with 3 reported *N*-glycosylation sites, which integrates many biological processes, such as antiangiogenic activity[1,2], immune complex clearance[3], and pathogen clearance[4]. Importantly, the protein is known to have 5 genetic variants with minor allele frequencies of more than 0.1, meaning they exist with substantial frequency in the human population[2]. Among them, Pro204Ser can induce a new *N*-glycosylation site at Asn202[3]. Considerable research has been performed into the biological activity of HRG, while research on its glycosylation is rare[5]. To close this knowledge gap, we used C18-based nanoLC-MS/MS to investigate the glycosylation characteristics of HRG from human plasma, recombinant Chinese hamster ovary (CHO) cell lines and recombinant HEK293 cell lines with targeted mutations. Within endogenous plasma HRG, every *N*-glycosylation site proved dominant with N4H5S2 (**Figure 1a-d**). For the recombinant HRGs, on the other hand, glycans showed with different antennarities, sialylation and core-fucosylation, as well as the appearance of high-mannose glycans and antennary fucosylation. Furthermore, we discovered a previously unreported *O*-glycosylation site, Thr256, which showed an approximate 90% glycan occupancy in all HRG types (**Figure 1e**).To investigate the relevance of HRG glycosylation characteristics and its biological function, we set up an assay to study the plasmin cleavage of HRG under various conditions. In doing so, we showed that the sialylation of the new *O*-glycan, as well as the mutation dependent *N*-glycosylation, influence the plasmin cleavage of HRG significantly (**Figure 1f-g**).

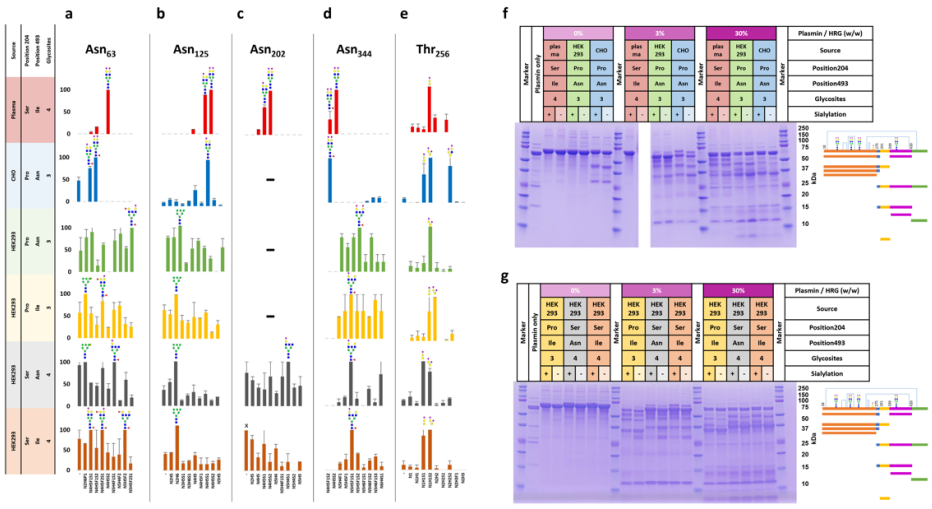

Figure1. Main N-glycan and O-glycan profile distribution of HRG from different sources and reducing gels of HRG proteins treated with plasmin.

Bibliographic references:

1.A. Thulin, M. Ringvall, A. Dimberg, et al. (2009), Mol. Cancer. Res. (7) 1792-1802.
2.A. K. Olsson, H. Larsson, J. Dixelius, et al. (2004), Cancer. Res. (64) 599-605.
3.N. N. Gorgani, C. R. Parish, S. B. Easterbrook Smith, J. G. Altin (1997), Biochemistry. (36) 6653-6662.
4.J. K. H. Poon, M. D. Hulett, C. R. Parish (2010), Blood. (115) 2473-2482.
5.J. K. H Poon, K. K. Patel, D. S. Davis, C. R. Parish, M. D. Hulett (2011), Blood. (117) 2093-2101.