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N-Glycosylation type and structure analysis of HIV-1 and Anti-HIV-1 broadly neutralizing antibodies (bnabs) by sequential Exoglycosidase cleavage and Hilic Chromatography

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Oligosaccharides in glycoproteins influence many aspects of protein function, e.g. half-life, potency, immunogenicity, efficacy etc. Regulatory agencies require demonstration of consistency in glycosylation of the manufactured lots for human therapy. Therefore, characterization of carbohydrate is necessary for therapeutic and preventative glycoproteins. In this poster, N-glycans of an HIV-1-envelope (Env) protein vaccine candidate and five HIV-1 broadly neutralizing antibodies (bNAbs) have been characterized by means of Endoglycosidase deglycosylation and followed by exoglycosidase sequencing of oligosaccharides to give structural information on the sequence of monosaccharides and type of linkage within the oligosaccharide chain. From the data of sequential treatment of the panel of eight exoglycosidases, and the known specificity of the exoglycosidases, the type, order and linkage of monosaccharide within the N-glycan chain were deduced. The N-glycan species were separated by hydrophilic interaction chromatography (HILIC) and the glycan peaks were identified. Given the facts that the HIV-1-envelope (Env) trimer protein is covered by a glycan shield of 84 (3x28) N-linked oligosaccharides, and the five HIV-1 bNAbs have N-glycosylation sites in either Fc region or in both Fab and Fc region, which is challenging for glycan analysis. The successful utilization of endoglycosidase (PNGaseF) and exoglycosidase panels give unique characteristic N-glycan profiles for the HIV-1 trimer vaccine and the five bNAbs. The results (Figure 1) demonstrate (1) predominant high-mannose species in the HIV-1; (2) neutral complex species in 10E8; (3) neutral and afucosylated sialidated complex in N6 and VRC07; (4) G0f is the predominant glycoform in VRC01 along with the other neutral and fucosylated/afucosylated sialidated complex species; (5) fucosylated/sialidated and neutral complex glycoforms were found for CAP256. Although mass spectrometric methods can provide accurate masses of molecular or fragment ions, they are unable to distinguish between isomeric monosaccharide residues (e.g. all neutral hexoses have the same mass of 162 Da). In such case, exoglycosidase with a known highly specific sequential degradation can provide fast and accurate oligosaccharide linkage identification for HIV-1 vaccine and bNAbs development.

Biography

Dr Yanhong Yang has her expertise in protein characterization utilizing LC, LC/MS, and electrophoresis techniques. She had been working in the biopharmaceutical industry for over ten years focusing on recombinant protein characterization including protein carbohydrate analysis, primary and secondary structure characterization (Amino Acid sequencing, PTM, disulfide bond linkage etc.). She also has experience in analytical project representative role for Phase 1 to Phase 3 project development. In November 2016, Dr Yang joined Vaccine Product Production in Vaccine Research Center of NIH. She has been working on HIV Trimer and HIV neutralizing mAbs from research candidate to Phase 1 clinical trial, focusing on analytical assay development for product quality attribute monitoring, including aggregates, product purity, charge heterogeneity and N-glycosylation etc.

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