Glycoside cluster effects on antibody recognition of MUC1 glycopeptides
(抗体によるMUC1糖ペプチド認識機構における糖鎖クラスター効果)

MUC1, a type of mucin, present on the epithelial cell surface which is stretched throughout the human body which plays a major role in immune response, cell differentiation and other biological functions. Alteration in the O-glycosylation leads to tumorigenesis and metastasis.

Antibodies reacting with human epithelial cell membrane MUC1 glycoprotein having aberrant glycoforms are highly potential diagnostic and therapeutic reagents in various cancers and interstitial lung diseases. However, the essential epitope structures for the anti-MUC1 antibodies have remained unclear. There is little systematic approach to decipher the effects of the O-glycosylation states at neighbouring Ser/Thr residues in the context of the disease-relevant epitope regions in MUC1 extracellular tandem repeating domain for the antibody recognition. In the present study, we established a comprehensive approach for the characterization of anti-MUC1 antibodies by combining microarray-based epitope profiling and NMR-based conformational analysis of synthetic MUC1 glycopeptides. Epitope mapping analysis using a microarray displaying 23 synthetic MUC1 glycopeptides revealed that anti-KL6/MUC1 monoclonal antibody (anti-KL6 mAb) has an absolute binding specificity with an essential epitope, Pro-Asp-Thr[Neu5Acα(2→3)Galβ(1→3)GalNAcα1→]-Arg-Pro-Ala-Pro, in an ultimately glycoform-specific manner when compared with other well-studied anti-MUC1 mAbs, DF3 and SM3 directing the same Pro-Asp-Thr-Arg (PDTR) motif in the tandem repeats. Multiple O-glycosylations at the neighbouring Ser/Thr residues did not disturb this specific recognition by anti-KL6 mAb even when modified with sterically hindered core 2 type pentasaccharide moieties (SC2). To our surprise, both DF3 and SM3 abrogated drastically the binding ability with putative MUC1 fragments having an immunodominant PDTR motif when other glycosylation sites are occupied by Tn (GalNAcα1→) or T antigen [Galβ(1→3)GalNAcα1→]. However, the modification at the adjacent two Ser residues with O-glycans containing ST antigen [Neu5Acα(2→3)Galβ(1→3)GalNAcα1→] resulted exceptionally in a substantial enhancement of the affinity of DF3 with the PDTR region. These results demonstrated for the first time that the O-glycosylation states around the immunodominant PDTR motif influence strongly the binding potency and profile of DF3 and SM3. NMR studies on the synthetic MUC1 fragments elicited the molecular mechanisms that the multiple O-glycosylations at the adjacent Ser/Thr residues induces significant conformational alterations at the PDTR motif in a glycoform-dependent manner. Strikingly, it was uncovered that an extended peptide backbone conformation at the glycosylated PDTR region can be disrupted and converted into a turn-like structure by the multiple modifications at other Ser/Thr residues with Tn antigen, one of the most frequently observed carbohydrate antigens associated with many human carcinomas. On the contrary, the multiple modifications with ST antigen did not affect the original conformation of the immunodominant PDTR region. This is the reason why the binding ability of DF3 directing this epitope region can be recovered by the multiple O-glycosylations at the neighbouring Ser/Thr residues with ST or SC2. These results clearly indicate that DF3 and SM3 simply showed an enhanced affinity with an
extended peptide backbone structure of the PDTR motif forced by the first -glycosylation with GalNAc residue. It is important to note that the interaction of DF3 or SM3 with this rigid and compact epitope region is highly sensitive to the conformational effects by the multiple O-glycosylations at the neighbouring Ser/Thr residues, namely the glycoside cluster effects on the antibody recognition. Anti-KL6 mAb was proved to be only anti-MUC1 monoclonal antibody that can recognise a unique glycopeptidic neo-epitope elaborated through the sitespecific posttranslational modification with ST antigen independent from the glycoside cluster effects due to the O-glycosylation states at the adjacent Ser/Thr residues within the MUC1 tandem repeats.