学 位 論 文 審 査 の 要 旨
Doctoral Dissertation Evaluation Review

博士の専攻分野の名称　博士（生命科学）
Degree requested: Doctor of (Life Science)

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学 位 論 文 題 名
Title of Doctoral Dissertation

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)

博士学位論文審査等の結果について（報告）
Results of Evaluation of the Doctoral Dissertation (Report)

Recently, studies on glycoprotein are the driving force towards early detection regarding various cancers and other inflammatory diseases; hence it is considered as an effective diagnostic tool in recent years. However, there is a hurdle which could be a reason for pseudo detection for some diseases. Hence finding the exact differentiation between the glycoforms is a crucial and effective challenge for researchers.

In this study, based on the previous data available in the area of MUC1 mucin, 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 that displayed 23 synthetic MUC1 glycopeptides revealed that anti-KL6/MUC1 monoclonal antibody (anti-KL6 mAb) has 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 the other well-studied anti-MUC1 mAbs DF3 and SM3, which are directed against the same Pro-Asp-Thr-Arg (PDTR) motif in the tandem repeats.

Later it was also understood that multiple *O*-glycosylations at the neighboring Ser/Thr residues did not disturb this specific recognition by anti-KL6 mAb, even when modified by sterically hindered core 2-type pentasaccharide moieties (SC2). To our surprise, both DF3 and SM3 exhibited a drastic decrease in binding ability with putative MUC1 fragments with an immunodominant PDTR motif when other glycosylation sites were occupied by Tn antigen (GalNAcα1→) or T antigen [Galβ(1→3)GalNAcα1→]. However, modification at the two adjacent Ser residues by *O*-glycans that contained ST antigen [Neu5Acα(2→3)Galβ(1→3)GalNAcα1→] resulted, exceptionally, in a substantial enhancement of the affinity of DF3 for the PDTR region.

Overall experiments in my study demonstrated for the first time that the *O*-glycosylation states around the immunodominant PDTR motif strongly influence the binding potency and profile of DF3 and SM3. NMR studies of the synthetic MUC1 fragments discovered the molecular mechanisms by which multiple *O*-glycosylations at the adjacent Ser/Thr residues induce significant conformational alterations in the PDTR motif in a glycoform-dependent manner. Anti-KL6 mAb was proved to be the only anti-MUC1 mAb that can recognise a unique glycopeptidic neo-epitope generated *via* site-specific posttranslational modification by ST antigen independently from *O*-glycosylation states at the adjacent Ser/Thr residues within the MUC1 tandem repeats.

Finally in conclusion the author has a new finding that refines the study for becoming a new or a much accurate diagnostic tool for many cancers and other various related diseases. Therefore, we acknowledge that the author is qualified to be granted the Doctorate of (Life Science) from Hokkaido University.