Abstract of Doctoral Dissertation

Degree requested: Doctor of Life Science

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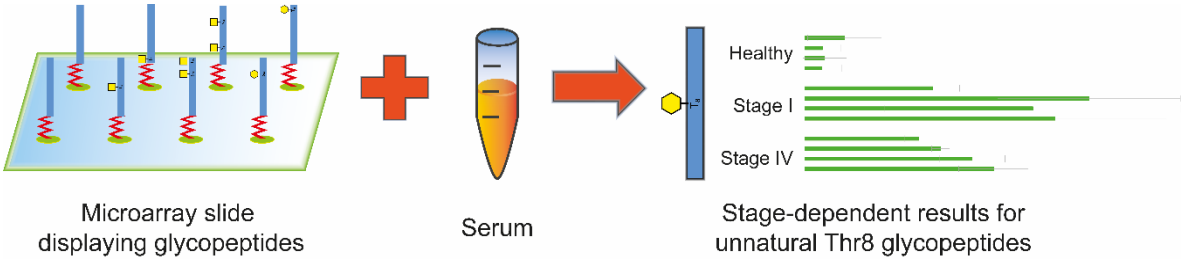
Title of Doctoral Dissertation

Unnatural MUC1 based glycopeptides in early stage breast cancer biomarkers discovery
(非天然型 MUC1 糖ペプチドによる乳がん早期バイオマーカーの探索)

Mucin 1 (MUC1) is a highly glycosylated *O*-glycoprotein that experiences alterations in cancer cells and assists the tumor progression. In many human carcinomas and, in particular, in breast cancer MUC1 is overexpressed and aberrantly glycosylated. As a consequence, previously covered antigens are now exposed and accessible to the immune system. This results in the production of antibodies towards these neoepitopes, providing an exploitable divergence between healthy individuals and cancer patients antibody profiles. In order to properly differentiate and develop specific diagnostic tools for early breast cancer detection, we require of the appropriate chemical probes.

In this thesis, we have focused on the MUC1 tumor-associated carbohydrate Tn antigen (*α*-*O*-GalNAc-Ser/Thr) because of its tumor high specificity, well-defined chemical structure and starting point role for more complex tumor antigens. Previous studies report the use of autoantibodies as potential cancer biosensors. With the intention of develop a more effective and robust sensing device we considered the substitution of GalNAc monosaccharides by stable glycomimic units. To investigate our hypothesis, two different glycopeptide libraries presenting the natural Tn antigen or the sp^2-iminosugar-derived unnatural analog were produced through microwave assisted solid-phase peptide synthesis. The whole glycopeptide collection was then evaluated with anti-MUC1 (SM3, VU-3C6, and VU-11E2) monoclonal antibodies (mAbs) in a microarray platform. The most promising candidates were tested with healthy, stage I and stage IV breast cancer sera with the aim of discovering serological autoantibodies as stage-dependent breast cancer diagnostic biomarkers.



Despite the variability between mAbs, the suitability of the glycopeptides bearing the unnatural sp^2-iminosugar-based Tn antigen mimic to detect anti-MUC1 antibodies was demonstrated. The present results also revealed that the glycopeptide mimic-antibody interactions are glycosylation pattern-specific and underlined the crucial contribution of the PDTR epitope embedded in the glycopeptide backbone to mAbs binding. Furthermore, stage I breast cancer serum experiments clearly showed autoantibodies binding with a specific sp^2-iminosugar glycopeptide with almost no interaction with healthy serum, results which will promote further studies on their function as early cancer biomarkers.