Medical Glycoscience

Strategy for pioneering the post-genome era in Japan: glycoproteomics

Glycoproteomics and Medical Glycoscience

The Genome Project which started in 1980s achieved its goal to identify human genome sequences faster than expected. After that, proteomic researches have started utilizing genome data as their basis. The technological development of analysis made these researches possible. It is however difficult to understand the whole picture of the biological system just by analyzing proteins. A protein shows its function after various modifications, and the modification associated with sugar chains is thought to be most important and to be difficult to analyze. The focus of research is now changing from genome to proteome and further to glycome. We believe that research activity will be recognized for its potential for human use. We inform researchers worldwide who study lifescience the significance of glycoproteomics, and expand and promote industrial technologies required for the development of domestic glycoscience. As a start, we will focus on finding the biomarkers of practical use by applying the fundamental technologies we have gained so far. In the course of identifying the biomarker, we expect to find drug seeds. We hope that our research will eventually lead to the well-being of mankind.

Director
Research Center for Medical Glycoscience
Hisashi Narimatsu

Establishment of Glyco-innovation and Industrial Technology (GLIT)

In the lifescience field, research subjects are shifting from genes to proteins with the development of medical and pharmaceutical applications. Actually, more than a half of proteins of eukaryotes possess sugar chains, and as a form of glycoproteins, they are known to have various functions in living organisms. For example, the functions of sugar chains are deeply involved in cancer, cellular differentiation, development, infectious diseases, brain, reproduction, receptor proteins, etc. Domestic organizations including research institutes such as AIST, universities and companies retain world leading knowledge and technologies in the areas of glycogene resources, structural and functional analyses and sugar chain synthesis. However, the system which provides the knowledge and technologies efficiently for the utilization in the related researches as well as for industrial applications is not well developed and organized. Therefore, by sharing the technologies, resources and information with researchers from a variety of sections, and also by assisting human resource development through education, we think we can create an intellectual creation cycle which contributes to acceleration of industrial application, especially to the development of novel diagnoses methods and drug discovery. To this end, we have established GLIT: Glyco-innovation and Industrial Technology, which is comprised of members not only from universities and research institutes but also from companies, medical agencies, and governments.

Research Center for Medical Glycoscience
Yoh-ichi Shimma

Biological Function of Glycans

● Search for glycan biomarker of hepatocellular carcinoma

Chronic Hepatitis(CH), liver cirrhosis(LC), hepatocellular carcinoma (HCC)

Hepatitis C-type virus (HCV) transmission were caused by administration of blood products, whose patients with CH have been certified to take aids for curative treatment since April 1, 2008. Based on results from epidemiological studies, HCV infection is known to cause CH, resulting in LC and HCC that are the significant sequelae at 20～30 years after HCV infection. The iatrogenic HCV transmission had occurred in the patients in 1980s', whose risk of LC and HCC comes higher day by day. Regarding

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current aspects of HCV associated disease, committee for strategic hepatitis treatment of Ministry of Health, Labour and Welfare proposed to develop detection markers for LC and HCC with high sensitivity as soon as possible. The one reason is that present detection markers for either evaluation of LC or early detection of HCC have not been good enough to serve this purpose.

New pathological knowledge of the hepatic disease and novel techniques for medical care innovate medical intervention.

New knowledge and novel techniques for medical care have altered basic approaches of medical diagnosis and intervention for not only for liver cancer but also esophageal and gastric cancer. Inflammatory reaction and fibrosis in LC and CH are also exemplary. Though fibrosis used to be considered an evidence for wound healing in general, fibrotic change associated with HCV infection is currently deemed worse prognostic marker for the liver disease. Moreover, it is pointed out that detection and treatment against fibrosis would play an important role to cure this disease.

Additionally, novel techniques such as radiofrequency ablation (RFA) have altered not only the treatment approach but also diagnostic criteria for HCC. Generally, HCC arises from LC at 7~8 % and CH at 3~4 % per year, small foci of which used to be difficult for surgical pathologist to diagnose because of the weak atypism on the histological appearance. RFA therapy is as effective as surgical resection for less than 2 cm diameter tumor in spite of less invasiveness than surgery, emergence of which drastically changed such situation. As a consequence, such lesion is now classified in well differentiated HCC, and exclusively treated by RFA therapy.

Future vision on medical care for liver disease

By intensive cooperation, we attempt to explore suitable biomarkers for clinical utilities on diagnosis and treatment of CH, LC, and HCC in Research Center for Medical Glycoscience of AIST. As lectin micro-array method can sensitively and comprehensively evaluate alterations of carbohydrate structures of proteins derived from hepatocytes, it is expected to improve estimation methods for not only LC but also early detection of HCC. Furthermore, through the studies to determine carbohydrate structures on alpha-fetoprotein (AFP) from HCC and placenta as a surrogate cancer antigen, we have established techniques to distinguish cancer associated AFP from placental AFP based on the differential glycosylation pattern. Taken such new technologies together, we are exploring further specific carbohydrate biomarkers that are used clinically in liver disease.

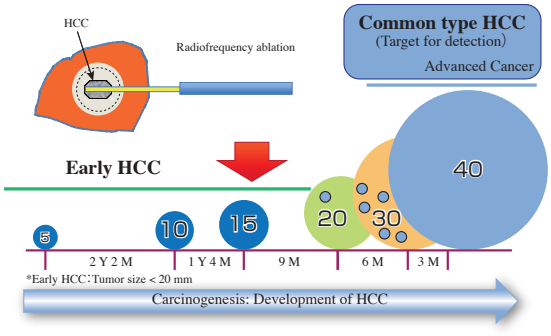


Fig.1 Radiofrequency ablation and Hepatocellular Carcinoma: Novel techniques such as radiofrequency ablation (RIA) have altered not only the treatment approach but also diagnostic criteria for HCC.

New biomarkers in our research goals

New biomarkers are expected to reduce overall burden of medical care by decreasing frequency of liver biopsy, and to cut health care costs by decreasing frequency of computed tomography (CT) scan and Fibroscan test. Additionally, new biomarkers are expected to be useful in foreign countries as BRICs where the prevalence of HCV infection is as high as, or higher than in Japan. We predict the improved sanitation of the societies and long-term follow up of HCV infected patients in these countries due to the economical development, where outcome of HCV is going to be noticed as social problems. Our biomarkers that are developed must be effective to help for overcoming the disease in these countries as well as in Japan.

Research Center for Medical Glycoscience
Yuzuru Ikehara

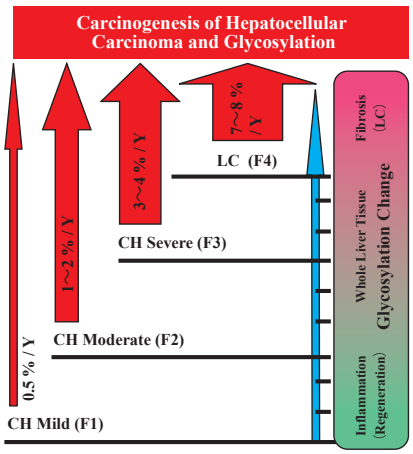


Fig.2 Liver Fibrosis and Hepatocellular Carcinoma (HCC) risk: HCC arises from Liver Cirrhosis (LC) at 7~8 % and Chronic Hepatitis (CH) at 3~4 % per year. Glycosylation pattern of liver tissues changes with development of fibrosis.

Glycoengineering of FGF proteins

What are FGF proteins?

FGF stands for fibroblast growth factor protein family that is composed of 22 members in the human body. The FGF proteins are expressed by and act on various types of the cells in our body. They modulate a variety of physiological processes through regulating cellular proliferation, differentiation and other activities. Thus, smart usage of FGF proteins would enable treatment of many pathological conditions and utilization of biological functions outside our body.

Why do we need to put glycans on proteins?

Many proteins in our body are naturally modified with various glycans. The glycans mediate molecular interations and protect proteins from degradation. Thus, artificially modifying recombinant proteins with glycans is a promising approach to gain biologically active proteins equipped with these functions.

Modification of FGF with heparan sulfate.

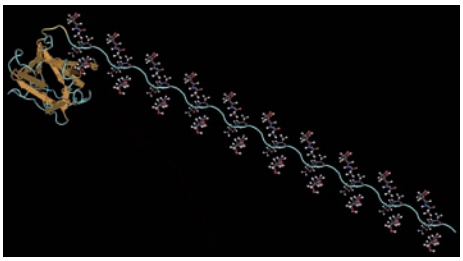
Heparan sulfate is a class of long sugar chain composed of repetitive units, resides on cell surfaces and in extracellular matrices, and is involved in cellular communication. By the aide of heparan sulfate, FGF proteins are stabilized and transduce their signals through FGF receptors on cell surface. We developed a technology to modify a FGF protein itself with heparan sulfate sugar chains by utilizing glycosynthesis ability of animal cells. The modified FGF, PG-FGF1, demonstrates augmented activity as well as improved stability, and is expected to be applicable to various clinical purposes (Table).

FGF1 protein modified with heparan sulfate (PG-FGF1) has many advantages over natural FGF1 as a therapeutic agent.

		PG-FGF1	FGF1 (aFGF)	FGF2 (bFGF)	FGF10 (KGF2)	PDGF
target tissue	epithelium	+	+	–	+	–
	dermis	+	+	+	–	+
stability	protease resistance	+	–	–	–	–
	heat resistance	+	–	–	–	–
	acid resistance	+	–	–	–	–
	alkaline resistance	+	–	–	–	–
activation in wound fluid		+	–	–	–	–
adsorption loss		low	high	high	high	high
effective concentration		low	high	high	na	na
total cost		low	high	high	na	na

Modification of FGF with O-glycans

The *O*-linked (mucin type) glycans, rather short sugar chains, modify proteins in clusters to form a shape resembling that of a brush. They affect interaction with water molecules as well as with other molecules. We developed a technology to modify a FGF protein with cluster *O*-glycans by utilizing glycosynthesis ability of animal cells (Figure). The *O*-glycan modified FGF showed elongated half life in circulating blood. Furthermore, elimination of the terminal sialic acids of the *O*-glycans made its half life even shorter than that of natural FGF.



Proposed structure of a FGF protein modified with cluster O-glycans.
[Drawn by using structures of FGF1 and a mucin peptide generated by Cn3D software (http://www.ncbi.nlm.nih.gov)]

Perspectives

The FGF proteins modified with glycans demonstrate high specificity, augmented activity, enhanced stability and controlled half life. These features would make them safer and more effective therapeutic agents than the natural FGF proteins in clinical applications. A technology for expressing such glycoproteins with uniform glycan structures in large amounts will be the subject of future research and development.

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Biological function analysis of mice lacking a glycogene which synthesizes polylactosamine chains

Glycans that regulate immunity

Over the past few years, we have been isolating and characterizing many glycogenes including glycosyltransferases. We are attempting to elucidate the function of carbohydrate chains (glycoconjugates). We analyzed glycogene (especially glycosyltransferase)-deficient mice to investigate *in vivo* function of carbohydrate chains. In order to generate many strains of glycogene-deficient mice, we selected the target genes which are thought to be disease-related and biologically important.

Here, I would like to present our research findings on the phenotype of polylactosamine synthase (β1,3-*N*-acetylglucosaminyltransferase 2, β3GnT2)-knockout mice which is one of the strains of knockout mice we have created. Polylactosamine containing the repeating units of *N*-acetyllactosamine (LacNAc) (Gal[galactose]β1-4GlcNAc[*N*-acetylglucosamine]β1-3)$_n$, is a fundamental structure of carbohydrates carried on glycoproteins (*N*-, and *O*-glycans) and glycolipids (Fig. 1). Polylactosamines are further modified by the addition of different carbohydrate antigens such as Lewis antigens and other blood group antigens. We investigated *in vivo* function of polylactosamines using β3GnT2-knockout mice. We first analyzed immunological responses in the knockout mice. The results from flow cytometry analysis, LEL lectin-blot analysis and radioisotope metabolic labeling analysis, showed that the amount of polylactosamine chains on *N*-glycans was greatly reduced in the tissues of the knockout mice (Fig. 1). Furthermore, we screened polylactosamine-carrying molecules of wild-type mice by lectin microarray analysis, and found that polylactosamine was present on CD28 and CD19, both known as immune co-stimulatory molecules in T cell receptor signal transduction (T cells) and B cell receptor signal transduction (B cells), respectively (Fig. 2). Polylactosamine levels on these molecules were reduced in the knockout mice. Knockout T cells were more sensitive to the induction of immune signals such as intracellular calcium flux, on stimulation with anti-CD3ε/CD28 antibodies, and proliferated more vigorously than wild-type T cells. Knockout B cells also showed hyperproliferation on BCR stimulation. These results indicated that polylactosamine chains on glycoproteins are important regulatory factors, presumably suppressing excessive responses during lymphocyte activation.

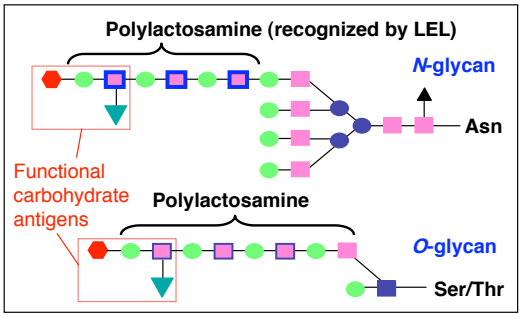

Fig.1 Examples of carbohydrate structure containing polylactosamine
galactose
N-acetylglucosamine
GlcNAc transfered by β3GnT2

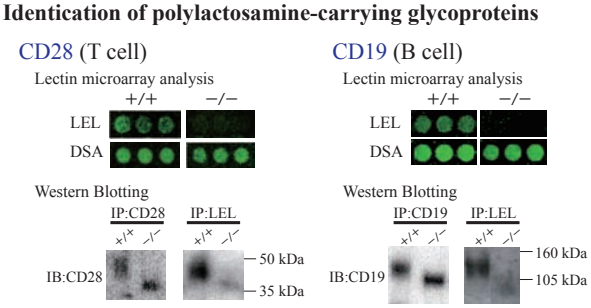

Fig.2 Glycan analyses of immunoprecipitated glycoproteins by lectin microarraying (above). We analyzed receptor proteins on cell surfaces. The LEL signals which indicate polylactosamine chains were decreased on immunoprecipitated glycoproteins. **Western blot analyses of immunoprecipitated glycoproteins (below).** This mobility shift of CD28 and CD19 was observed in knockout mice. These results are consistent with the reduction of molecular weight of the decreased polylactosamines. +/+: wild-type mice, -/-: homozygous null mice, IP: immunoprecipitation, IB: Immuno-blot.

Future prospects

Through the analyses of phenotypes of glycogene-deficient mice along with the identification of structural alterations of glycans and glycan-carrying molecules, we believe that these studies will contribute to a better understanding of the regulatory mechanisms of glycoconjugates. In addition, it is expected that such analysis of molecular mechanism using glycogene-deficient mice enables us to gain further insights into the biological function of carbohydrate chains (glycoconjugates). We will continue investigating the *in vivo* functions of glycoconjugates using glycogene-deficient mice.

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Research Center for Medical Glycoscience
Akira Togayachi

Structural Analysis of Glycoproteins

● Structural and quantitative analysis of glycoproteins using mass spectrometry

Using glycogenes that have been massively cloned from human genome, we have created a glycan library and a multistage tandem mass spectral (MS^n) database of glycans. Structural analysis of glycans by a simple sequencing is quite difficult due to their structural complexities such as positional isomers, stereo-isomers and branching structures. In spite of the structural complexity, as each glycan has its own characteristic spectral pattern in the MS^n spectra, we can analyze the glycan structures by a method similar to finger print matching of their MS^n spectral patterns. We built a prototype of the glycan analysis system using the MS^n spectral database of glycans in 2006, and we are working toward its practical use[1]. Furthermore, this MS^n database of glycans will be opened to the public soon through RIO-DB (http://riodb.ibase.aist.go.jp/riohomee.html).

Applying the analytical techniques for glycan structures, we are searching for glycan biomarkers which may be useful in early detection of cancers and in selection of the best medical treatment for particular patients. A great challenge for glycan biomarker discovery is "enrichment". Cancer biomarkers secreted from cancerous tissues to serum are expected to be exiguous (typically ng/mL order in concentration). Therefore, structural analysis of glycans of the cancer biomarkers can not be performed unless the biomarkers are enriched in some way. We are aiming to discover novel glycan biomarkers by developing a simple method for enrichment of mucins which are the major constituents of epithelial mucus and have long been implicated in health and in disease.

In addition to mucins, other serum glycoproteins carrying cancer-specific glycans are thought to be good biomarkers. If their core proteins are originated from particular tissue cells, they must have higher tissue specificity. Therefore, we are now trying to discover such biomarker target, *i.e.,* tissue-specific protein having cancer-specific glycans and detectable label in serum, under the close cooperation amongst outside medical institutions and the research teams of Research Center for Medical Glycoscience. Potential glycoproteins are captured from the culture media of a series of cancer cell lines with specific probe lectins or anti-glycan antibody-immobilized column, and their core proteins are identified comprehensively by liquid chromatography / mass spectrometry (LC/MS)-based proteomic approach called "IGOT method"[2]. Using stable isotope-labeling, the change of their label can be analyzed quantitatively. Many biomarker candidates have been identified from the media of hepatocellular carcinoma (liver cancer) and other cancer cells. Now their validation studies are underway and the discovery of novel cancer serobiomarkers is expected.

Research Center for Medical Glycoscience
Akihiko Kameyama, Hiroyuki Kaji

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● Glycan profiling by means of lectin microarray

Development of lectin microarray as a high sensitive glycan profiler

Lectin microarray system is an emerging technique for analyzing glycan structures based on the glycan profiling concept utilizing a group of glycan-discriminating proteins, lectins. This technology mainly comprises of a lectin array slide and a microarray scanner, both of which we originally developed in collaboration with Moritex Co during the NEDO SG project (2002-2005). The former includes spots of over 40 plant lectins with different binding specificities for multiplex detection of glycan-lectin interactions, through which detailed features of glycan modifications and branching patterns will be obtained (Fig.1). For the latter, we adopted an evanescent-field fluorescent-assisted detection principle, whereby no glass washing process is required [1]. In general, the glycan-lectin interaction is relatively weak in comparison with antigen-antibody interactions for example. Thus, some glycans once bound to a lectin on the array may dissociate during the washing process. This should result in significant reduction in the signal intensity.

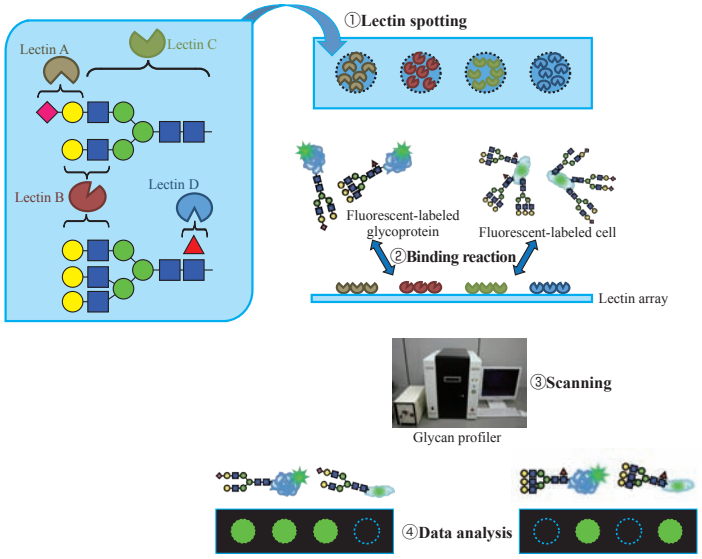

Fig. 1 Lectin array is a practical approach to profile glycans expressed on proteins and cells by means of lectins, as decoder molecules, each which shows different sugar-binding specificity. Thus, distinct sugar-binding patterns will be obtained for different cells and glycoproteins.

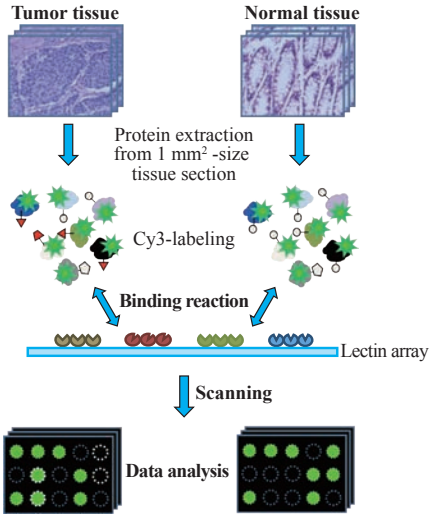

Fig.2 As a result of comparison, it is clear that some lectin signals (circled with *broken lines*) show significant changes, which are attributed to those in glycan structures (shown in red).

While most of the conventional microarray scanners need the washing process, our scanner is unique in getting rid of this problem. Furthermore, our continuous improvement in the array platform achieved an analysis of a glycoprotein structure with the highest sensitivity in 2008 (only 10 picogram of protein is required for assay) [2].

Discrimination of stem cells on the basis of differential cell glycomics

It is said that over a hundred of glycosyltransferases cooperatively act to synthesize/maturize glycans. Glycosylation pattern should reflect alteration of gene expression levels of individual glycosyltransferase during cell differentiation and proliferation. Therefore, cell separation becomes possible by means of differential profiling targeting cell surface glycans (Fig.1). Due to extremely high sensitivity and accuracy, the developed glycan profiling system is considered to best fit the purpose of "stem cell reader", which contributes to regenerative medicine in terms of quality control of stem cells, *e.g.,* before transplantation. In this regard, we have already constructed systematic manipulation protocols including preparation of fluorescent-labeled glycoproteins from only ten-thousands of cells and data-mining procedures, such as for gain-merging and max-normalization[3]. Now, we can discriminate mouse embryonic stem cells from their differentiated forms with retinoic acid. Our group also developed a skilled technique for direct analysis of live cells[4]. Since this method does not require undesirable cell fragmentation, but internally labels the cells, it represents a novel, direct procedure for profiling cell surface glycans.

Lectin microarray should accelerate glycan-related biomarker discovery

There have been enormous advances in the findings of glycosylation alterations in the process of oncogenesis. Therefore, novel tumor-specific glycoproteins accompanying substantial structural changes in glycan moieties will become potential biomarkers with higher specificity than those established previously. For this reason, we developed an extremely feasible methodology enabling differential glycan analysis targeting restricted areas of tissue sections using an ultra-sensitive lectin microarray[5]. In fact, the developed method is sufficient to detect glycoproteins derived from approximately 1,000 cells derived from tissue sections (1.0 mm^2 and 5 μm in thickness). With this system, tumor-related glycan alterations can be clearly detected as signal differences in appropriate lectins on the array (Fig.2). Obviously, the developed technology is straightforward and comprehensive, and thus should accelerate discovery of a series of novel disease-associated glyco-biomarkers under the concept of glycoproteomics.

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Glycan synthesis

● Human glycoprotein production in yeast

Therapeutic glycoproteins and production host

Protein therapeutics, such as antibody therapeutics and cytokine administration, are now known as the largest class of new candidates developed by the pharmaceutical companies. Although most of these glycoproteins are produced in mammalian cells, there is concern for inadequate bovine serum supply and the risk of bovine spongiform encephalopathy. We are constructing a mammalian-type glycoprotein expression system in yeast because manufacturing costs are cheaper than mammalian cells and yeast is virus-free.

Strategy of human glycoprotein production in yeast

Yeasts have a drawback of inability to attach mammalian-type sugar chain for the production of therapeutic glycoproteins for human use. We have cloned and analyzed yeast-specific glycosyltransferases genes for more than ten years. Based on the knowledges, we disrupted the genes involved in hypermannosylated modification. Next, we introduced the genes responsible for the sugar-nucleotide synthesis, the transport of sugar-nucleotide from cytosol to Golgi lumen, and the transfer and hydrolysis of sugars. In the above, the introduced genes are not limited to mammalian ones if the encoded enzymes share the same substrate specificity. We have already reported the production of the human antibody and lysosomal enzyme for enzyme replacement therapy of lysosomal diseases. In the case of the lysosomal enzymes, *in vitro* glycosidase digestion technique was combined to the *in vivo* expression system in yeast, because uncovering of mannose-6-phosphate residues in yeast is so complicated.

In contrast, the engineering of *O*-glycosylation has not been attempted in yeast because *O*-mannosylation is one of the specific modifications in yeast and is vital for yeast cells. We have also succeeded in making both *O*-GalNAc peptide and mucin-type glycoprotein in yeast by introducing three responsible genes encoding *Bacillus* UDP-GlcNAc 4-epimerase, human UDP-Gal/GalNAc transporter 2, human ppGalNAc-T1 and *Drosophila* β-1,3 GalT (Fig. 1). Combined usage of a compound inhibiting yeast protein *O*-mannosyltransferase (PMT) suppressed yeast-specific *O*-mannosyl modification, and increased mucin-type glycoprotein production. We also succeeded in the secretion of human podoplanin (aggrus), which is known as a platelet-aggregating factor on cancer cell, with core 1 structure in the yeast strain. After *in vitro* sialylation, the podoplanin induced platelet aggregation. Interestingly, substitution of ppGalNAc-T1 for ppGalNAc-T3 caused loss of platelet aggregation activity of the podoplanin whereas sialylated core1 structure was also detected by lectin microarray. We have already reported that a sialylated core 1 structure at Thr52 in PLAG domain of podoplanin is essential for platelet aggregation, and our results indicated ppGalNAc-T1 recognized the Thr52 of it and transferred GalNAc residue in the yeast.

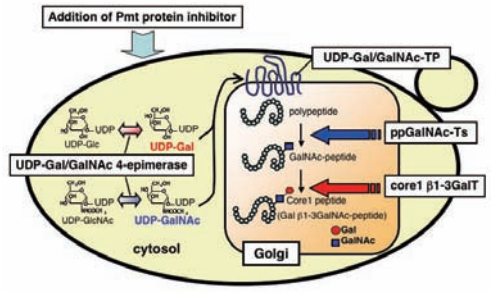

Fig. 1 Strategy for production of mucin-type glycoprotein in yeast.
This strategy consists of "shut-down" of the genes related to yeast-specific sugar modification and "knock-in" of the genes for humanized glycoprotein production.

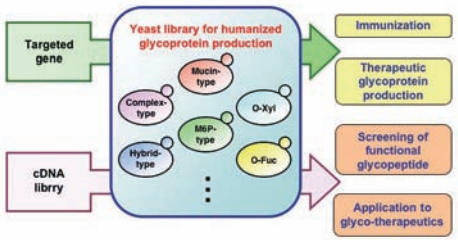

Fig. 2 Application of yeast library for functional analysis of humanized glycoprotein

Future plans

Based on the development of yeast system to produce mammalian *N*- and *O*-glycosylated sugar chains, it is reasonable to say that the production of therapeutic glycoproteins and glycopeptides by yeast has now become a good candidate in any manufacturing process. This yeast system has potential for both functional analysis of mammalian-type glycan and production of mammalian-type glycoprotein for pharmaceutical use. We expect that expression of the targeted gene or cDNA library in our system leads to finding more functional glycopeptides (Fig. 2) and glycoproteins for development of therapeutics.

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Development of effective production methods for therapeutic glycoproteins in yeast using a novel mutagenesis technique

Problems with glycoprotein production in yeast

Although therapeutic glycoproteins containing a mammalian-type *N*-linked oligosaccharides have been produced by disrupting genes responsible for the biosynthesis of the outer chain of mannan, this approach led to a growth defect as well as decreased protein productivity in these yeast strains. The production of glycoproteins using yeast cells has been attempted by many groups. However, the decreased protein productivity in engineered yeast strains was an obstacle to the development of efficient glycoprotein production in yeast. For economic reasons and for effective production of such glycoproteins in yeast, development of appropriate strains is highly desirable. We applied to yeast a novel mutagenesis technique, based on the disparity theory of evolution, which was developed by Neo-Morgan Laboratory Inc., for producing glycoproteins with mammalian-type *N*-linked oligosaccharide.

Development of yeast strains for effective production of humanized glycoprotein

With this novel mutagenesis technique, it is now possible to induce extensive non-lethal mutations in the yeast genome. This technology could increase the error threshold without losing genetic information, and hence could produce a large number of advantageous mutants. The DNA polymerase δ variant, with defective proofreading function, encoded by the *pol3-01* gene of the yeast *Saccharomyces cerevisiae* is known to act as a strong mutator. Here, we constructed a yeast expression plasmid designated YEplac195-*pol3-01*, containing the *pol3-01* gene and capable of introducing multiple mutations due to dominant negative expression of the *pol3-01* gene. We used

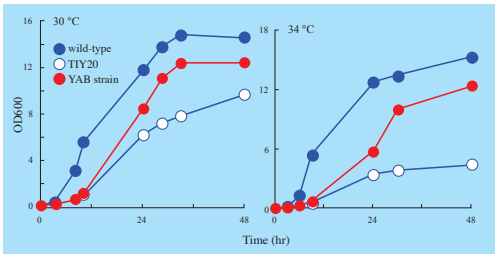

Fig.1 Growth curves. Yeast cells, as indicated in the plot, were grown at 30 °C and at 34 °C. At the indicated time points, OD600 of each culture was measured to monitor cell growth.

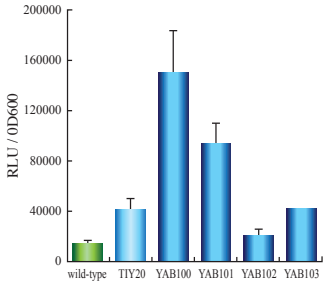

Fig.2 Luciferase activity in culture medium. Luciferase activity (in RLU) in each culture was normalized to optical density at 600 nm, and expressed as RLU/OD600.

this plasmid to transform the *S. cerevisiae* TIY20 strain, which is deficient in the outer chain of yeast mannan type *N*-glycan due to the disruption of three genes *(och1Δ mnn1Δ mnn4)* [1]. TIY20 manifests a temperature-sensitivity and cannot grow at 37.5 °C. The volume of the YEplac195-pol3-01 transformant cultures was increased stepwise in order to promote cell division. This was necessary to accumulate mutations in the yeast genome. We selected 4 colonies (designated YAB100, YAB101, YAB102 and YAB103) which grew at 37.5 °C. The growth kinetics of these strains at 30 °C and 34 °C are shown in Figure. 1. At 30 °C or 34 °C, all YAB strains steadily proliferated, whereas TIY20 was greatly inhibited. These observations suggested that the YAB strains not only suppressed the ts-phenotype but also overcame the growth defect. We also investigated the structure of the *N*-linked oligosaccharides in the W303-1B (wild-type), TIY20 and YAB strains. While those from the W303-1B strain revealed several peaks corresponding to $Man_8GlcNAc_2$ to $Man_{14}GlcNAc_2$, the oligosaccharides from the TIY20 and YAB strains showed only one peak corresponding to $Man_8GlcNAc_2$. Furthermore, we analyzed how efficiently the YAB strains would secrete a foreign protein, namely the reporter protein CLuc, a natural secretary luciferase from *Cypridina noctiluca*. Surprisingly, CLuc secretion was approximately 10 times better in one of these mutants (YAB100) than the wild-type strain (Fig. 2)[2]. This suggests that this strain developed here is suitable for the production of humanized glycoprotein.

Future prospects

We succeeded in isolating useful yeast strains for the production of glycoproteins by using a novel technique that accelerates the evolution of microorganisms. Using the isolated yeast strains, we will develop a new yeast strain which can produce human glycoproteins containing complex-type and hybrid-type *N*-linked oligosaccharides, and aim to provide useful human glycoproteins for therapy.

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Collaborators

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References

[1] S. Takamatsu *et al.: Glycoconj. J.*, 20(6), 385-397(2004).
[2] U.S. Patent Application No. 11/713,540 BREEDING METHOD FOR YEAST, YEAST AND PRODUCTION METHOD FOR GLYCOPROTEIN OR BETA-GLUCAN

Glycoinformatics, Database

● Computational structural analysis for saccharides : fragmentation and interaction analyses

Empirical and/or theoretical rules for the fragmentation of oligosaccharides would be of considerable assistance in the development of novel tools for the structural analysis of oligosaccharides for use in glycomics. We focused on the sodiated saccharides, and fragmentation mechanisms were simulated according to proposed reaction mechanisms in order to find the general rules of glycan fragmentation. Several computational methods, such as molecular mechanics calculations, semi-empirical calculations, and electron orbital calculations, were used to interpret and analyze the experimentally observed fragmentations. We explore, by theoretical calculations, the reasons why sodium-adduct ions of oligosaccharides produce certain characteristic fragment patterns.

We have developed a web-based tool named SGCAL (Structural Glycomics CALculations). SGCAL (Fig. 1) is capable of building a 3D structure from oligosaccharide sequence information and of visualizing the calculated results and the experimental mass spectra, thereby supporting investigations on correlations between the structure of oligosaccharides and their fragmentation patterns[1]. All collision-induced dissociation (CID) spectra were obtained from sodium adduct ions by using a mass spectrometer, and the observed fragment ions were recorded as lists of peaks in SGCAL.

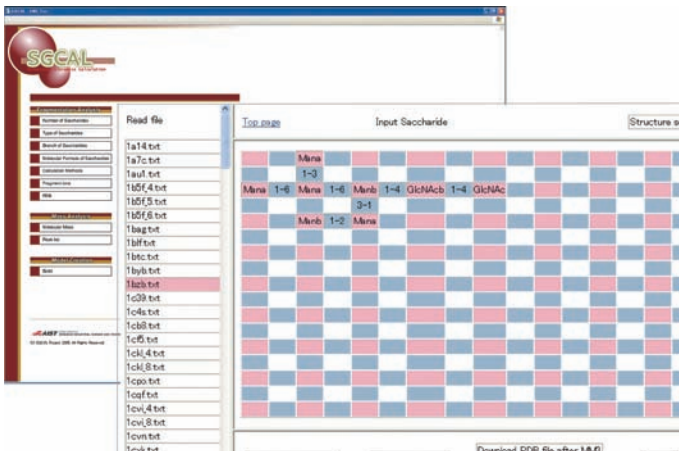

Fig.1 Fragmentation analysis for oligosaccharides (SGCAL: http://sgcal.cbrc.jp)

Theoretical calculations were performed for the oligosaccharides and the results were compared with those obtained experimentally to provide information on structure-reactivity relationships[2,3]. SGCAL also has a mass-analysis function for searching through calculated results and experimental data: when mass values with an allowable mass range are entered into a search box, the mass-analysis function will retrieve lists of parent and fragment ions that meet the search criteria.

We develop algorithms/software to analyze the recognition mechanism of biological macromolecular complexes and simulate protein-protein docking, molecular dynamics, quantum mechanics calculations on the complex molecules using Grid computing environment (Fig. 2). Theoretical calculations using computational chemistry-based methods can be useful in the analysis of experimentally obtained data. Theoretical rules for the fragmentation and recognition mechanism of saccharides can be a novel tool for the structural analysis of oligo- and poly-saccharides in glycomics.

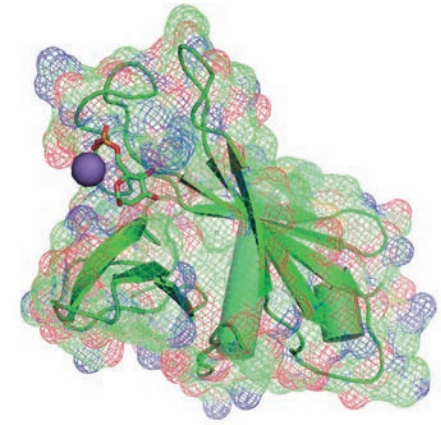

Fig. 2 Docking simulation for lectin-saccharide

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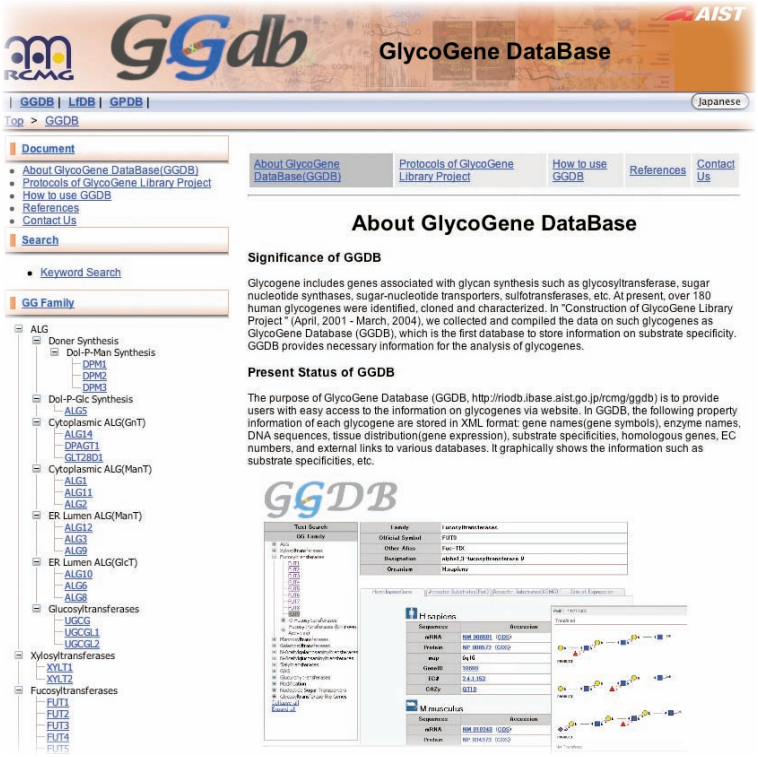
[3] H. Suzuki *et al.*: *Int. J. Mass Spectrom.*, In press (2008).

● Integration of glycodatabases

There are various sugar chain related databases in Japan. For example, KEGG Glycan stores the information on sugar chain structures reported in journals. GlycoEpitope has various information of antibodies which recognizes sugar chain epitopes including antigenic sugar chain structures. GALAXY determines a sugar chain structure from the results of HPLC analysis. LipidBank compiles glycolipid structures and their biological activities in conjunction with the structural analysis data of glycans. AIST published the following databases on the Web which were constructed based on the results of the NEDO project. Glyco-Gene database (GGDB) stores the compiled information on human glycogenes. Lectin database (LfDB) presents general information of lectins along with the interaction data between the lectins and standard sugar chains. Glycoprotein database (GlycoProtDB) exposes the actual glycosylation sites of glycoproteins identified by IGOT method, and mass spectral database (GMDB) shows images of fragmentation pattern of sugar chains resulted from mass spectral analysis.

Each database has its own way of use so that users need to learn how to use each database to retrieve necessary information individually, which is time consuming and inconvenient. Research Center for Medical Glycoscience of AIST is now constructing an integrated glycodatabase in which users can search information stored into the databases with a single query using a cross-searching service, and is also developing a system which allows access to other databases used in the field of lifescience. It is also important to provide information on glycoscience, especially those related to sugar chains such as cancer, immunology, infectious diseases, with researchers of other fields in order to bring more understanding to glycoscience.

To this end, we have been constructing the integrated glycodatabase (JCGGDB) as a part of the lifescience integrated database project directed by the coalition of 4 ministries. We are planning to expand and improve the database which covers a wide range of information, from basic to specialized information, and to create a user friendly interface, in the near future.



GlycoGene DataBase (http://riodb.ibase.aist.go.jp/rcmg/ggdb/)

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