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ABSTRACT

Carbohydrate structure is one of the important macro-biomolecules. Carbohydrate chains are synthesized through the coordinated action of glycosyltransferases, taking a step-by-step approach. The carbohydrate chains of glycolipids and glycoproteins at the cell surface change dramatically in a variety of biological phenomena, such as embryogenesis, development, differentiation, immunoresponses, and cancer metastasis. The Lewis x (Le^x) epitope is one of such carbohydrate. Le^x is defined as Galβ1,4 (Fuc α1,3) GlcNAc, which is synthesized by transferring a Fuc to the GlcNAc residue of the type 2 chain, Galβ1,4GlcNAc-R, with a α1,3-linkage. This fucose-transfer is catalyzed by α1,3FucT. So far, the human genes encoding five α1,3FucTs (hFucTIII, IV, V, VI and VII or FUT3, 4, 5, 6 and 7) have been cloned. CD15 is one of the differentiation markers of the cells, and the CD15-epitope has been determined as the Le^x carbohydrate structure. Several immunohistochemical studies have detected CD15 antigens in certain neuronal cells and glial cells in the central nervous system (CNS) of humans and rodent. The expression of CD15 antigen in the CNS is developmentally regulated, and considered to play an important role in neuronal development. In our previous study, mFucTIX was identified as the most likely candidate for the enzyme synthesizing the Le^x structure (CD15-epitope) in the mouse CNS.

I therefore cloned a human fucosyltransferase gene, named hFucTIX, orthologous to the mouse *FucTIX* gene. I screened the human stomach cDNA library with a mFucTIX cDNA probe encompassing the full-length open reading frame (ORF), and obtained several clones encoding the *hFucTIX* gene. The deduced amino acid

sequence of hFucTIX, consisting of 359 amino acid residues, indicated a type II membrane protein and was very highly conserved with mFucTIX. The Namalwa cells stably expressing the *hFucTIX* gene were established, used for flow cytometry analysis, and assaying of α1,3FucT activity. The hFucTIX transcripts were abundantly expressed in brain and stomach, and interestingly were detected in spleen and peripheral blood leukocytes. I also performed FISH analysis, and the *hFucTIX* gene was shown to be located in the 6q16, long arm of human chromosome 6. The phylogenetic tree indicated that the FucTIX first diverged in vertebrate evolution and the rate of nucleotide substitution indicates that FucTIX seems to be under strong selective constraint. To know the detailed phylogenetic relationship of this gene family, I obtained novel *FucT* gene sequences from chicken and xenopus cDNA or genomic DNA. Those were counterparts of FucTIX, named xFucTIX and cFucTIX, and I also found two novel FucTs, named XFTI and CFTII. Then I reconstructed the phylogenetic tree of the α1,3FucT gene family. XFucTIX and cFucTIX were clustered with the Fuc-TIX subfamily. XFTI and CFTII were clustered with the Fuc-TIV, and Fuc-TVII, respectively. These novel sequences also improved the bootstrap values of the phylogenetic tree.

The dramatic changes of glycoconjugates observed during embryogenesis and the differentiation of cultured embryonal carcinoma (EC) cells suggest that cell surface glycoconjugates play a vital role in embryogenic development. A glycoconjugate having a Le^x determinant (recognized by anti-SSEA-1 antibody) was found to be maximally expressed at the morula stage and to decline greatly after that stage. Since compaction was inhibited by multivalent Le^x oligosaccharide, this structure may play a role in this process, the very first overt morphogenic change during embryogenesis. I

therefore studied fucosyltransferase mRNA levels in mouse early embryo using competitive PCR-methods. I determined that the FucTIX is responsible for the Le^x synthesis in the mouse early embryo.

The past few years have seen rapid advances in sequencing the genomic DNA of human, *Caenorhabditis elegans*, and so on. As a result, a large number of novel glycosyltransferase genes have been discovered from those genome sequences. How did they increase their family members during the genome evolution? In vertebrate genomes it is often found that the homologues of a group of genes often form another cluster on a different chromosome. It seems to be sure that the two genome duplication events occurred, one close to the origin of the vertebrates and the second close to the origin of the gnathostomes. As a result, the MHC paralogous regions were detected on chromosomes 1, 6, 9, and 19. Genes of the Hox cluster are also mapped to chromosomes 2, 7, 12, and 17. Therefore, glycosyltransferase genes may also show "homologous clusters" such as the MHC region and Hox cluster. If so, they might have arisen as the results of three separate duplication events like MHC and Hox clusters.

I thus conducted molecular evolutionary analyses on 19 glycosyltransferase gene families. It is the first attempt that so many glycosyltransferase genes were analyzed through molecular evolutionary methods.

(I) FucT: I constructed the phylogenic tree of α1,2FucT family. The topology of tree was not compatible with the established mammalian phylogenic tree. To confirm their phylogenetic relationship, I constructed phylogenetic network. As a result, I found that the gene conversion occurred in the α1,2FucT gene family. I also reconstructed the phylogenetic tree of α1,3FucTs including novel FucT sequence, xFucTIX, cFucTIX, XFTI, and CFTII. The α1,6FucT was distinct from other FucT, such as molecular

weight, and the substrate specificity.

(II) GalT: β1,3GalTs, and β1,4GalTs. The β1,3GalT family shares some motif with β1,3GnTs. The tree topology indicates that β3GalTs and β3GnTs diverged before Drosophila and vertebrate speciation, and they increased their family members by gene duplications. The phylogenetic tree of β1,4GalT family indicated that β1,4GalT increased gene numbers before divergence of vertebrates and invertebrates.

(III) GlcNAcT includes the Mgat gene family. No sequence homology was found among these Mgats, in spite of their enzymatic similarity.

(IV) β1,6GlcNAcT. This family contains two distinct groups based on their enzyme activity. One is C2GnT group, and the other is IGnT group. But they share their sequence homology, so I constructed the phylogenetic tree. As expected, IGnT first diverged from C2GnTs by gene duplication.

(V) I performed the phylogenetic analysis of polypeptide: GalNAcT. This gene seems to the oldest glycosyltransferase within this study, for they diverged before Metazoans and Proteostome divergence.

(VI) Sialyltransferases: The sialic acids are typically found at the outermost ends of N-glycans and O-glycans, and glycosphingolipids. Sialyltransferases were divided into three groups, depends on their enzyme activity and sequence homology.

These phylogenetic analyses revealed that the glycosyltransferase genes increased their membres by gene duplication. I estimated the numbers of ancestral genes and duplication events. Although I failed to find simple and clear explanation between the chromosomal locations and the topology of phylogenetic trees. I found novel candidates of gene cluster region, 3, 11, 18, and 22.

Finally, I calculated the numbers of synonymous (d_S) and nonsynonymous

(d_N) nucleotide substitutions for each glycosyltransferase genes, and estimated the evolutionary rates. Comparison of evolutionary rates revealed that the glycosyltransferase tend to evolve slowly than other genes. FucTs indicated somewhat higher evolutionary rates than the others. However, FucTIX conspicuously showed a very slow evolutionary rate.

論文の審査結果の要旨

糖鎖は糖転移酵素によって逐次合成・伸長される。糖鎖の結合様式や構造は非常に多様で複雑である。また、糖転移酵素遺伝子自体も非常に多様性に富んでおり、同じような結合様式を触媒する酵素群がファミリーを形成していることが明らかにされてきた。また糖鎖は、細胞表面において、発生、分化、免疫応答や細胞の癌化など、様々な生物学的状況に応じて劇的に変化することが知られている。さて、この学位論文で課題としているα-1,3フコース転移酵素遺伝子は、フコースをGalβ1-4GlcNAcβ-RのGlcNAc残基にα-1,3結合で転移する酵素をコードする。

金子さんは、まず（1）マウスcDNAをプローブにして、ヒトのライブラリーとのハイブリダイゼーションによって、新たなヒトのα-1,3フコース転移酵素（Fuc-TIX）遺伝子をクローニングした。そして、（2）非同義置換数の解析から、ヒトとマウスFuc-TIX遺伝子間の相同性は非常に高く、ヒストンなどと同じレベルでアミノ酸が非常に強く保存されていることを明らかにした。従って彼女は、（3）Fuc-TIXが機能的制約を受けて、その配列が進化的に保存されていると考察した。さらに、彼女は、（4）この新規のFuc-TIX遺伝子と、既知のFuc-TIX遺伝子群のいずれが初期胚におけるSSEA-1糖鎖合成に関わるか明らかにするため、マウス受精卵における両遺伝子の発現をcompetitve RT-PCR法を用いて確認した。その結果、（5）新規のFuc-TIX遺伝子が初期胚におけるSSEA-1抗原合成を担っている可能性を示唆した。また、彼女は、（6）ニワトリとアフリカツメガエルよりPCR法を用いてクローニングを行い、これらの種から新規にFuc-TIX遺伝子を得た。そして、（7）これらの新規遺伝子とデータベースから得られら多くの相同遺伝子をもとに系統樹を作成し、進化速度の一定性を仮定して遺伝子の出現年代を推定した。その結果、彼女は、（8）フコース転移酵素遺伝子ファミリーは魚類の出現以降に遺伝子重複により、その数を増やしたことを示した。

また、金子さんは、（9）糖転移酵素遺伝子群の進化を考察する目的で、Fuc-TIX遺伝子を含む19種類の糖転移酵素遺伝子ファミリーに関して分子進化学的解析を行った。その結果、（10）糖転移酵素遺伝子群は、おそらく動物と植物の分岐以降には現存する遺伝子ファミリーに別れて存在しており、その後、脊椎動物の出現以降に、遺伝子重複もしくは染色体の倍加に伴ってファミリー内の遺伝子数を増やしてきた、という結論を得た。糖転移酵素は同じ活性を担うファミリー内でも基質特異性などが微妙に異なり、糖鎖の多様性が糖転移酵素の多様性によっていることを反映している。また、同義・非同義置換数の解析の結果、彼女は、（11）遺伝子ファミリー内で進化速度が遅く保存されている遺伝子と、進化速度が早い遺伝子が混在していることを明らかにした。そして、彼女は、（12）遺伝子数が増加した結果、進化速度が早くなる糖転移酵素遺伝子が現れ、糖転移酵素遺伝子の活性の多様性が増加してきたと、考察した。

以上のように、金子さんは、Fuc-TIX遺伝子のクローニング、配列解析そして分子進化学的解析を行うことにより、この遺伝子の進化的ダイナミックスを初めて示した。特に、進化の特定の時点で遺伝子重複を繰り返すことによって、遺伝子数とその多様性を増やしてきたという考察は興味がある。これらの結果は、Fuc-TIX遺伝子の機能の解明にも貢献すると思われる。従って、審査員一同は、金子さんの学位論文は、独創性、内容ともに総合研究大学院大学の基準を満たすと評価し、これを合格とした。