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論文内容の要旨

Autosomal trisomy is a numerical chromosomal anomaly with an extra single copy of an autosomal chromosome. It is associated with general developmental failure followed by processes of neurodegeneration. Moreover, variable cognitive impairment and behavioural phenotypes are markedly presented, although relationship between learning difficulty and autosomal trisomic conditions is still unclear.

The most common and important autosomal trisomy at live birth in human is trisomy 21, 3 copies of human chromosome 21. It attributes to over 95% of Down syndrome. Many efforts had been made to identify loci and genes on human chromosome 21 in association with the neurodevelopmental pathology through such methods as gene cloning, locus mapping, animal modelling and chromosome sequencing. However, only very few critical genomic regions and genes in the human chromosome 21 can be linked with neurological manifestations in Down syndrome. Thus, it is quite unlikely that neurological manifestations in Down syndrome are due to only a few numbers of specific genomic regions and genes through autosomal trisomy. Instead, currently prevalent working hypothesis of autosomal trisomy is the global (namely genome-wide) dosage imbalance of increased gene products in the extra chromosome.

A direct link of the cognitive conditions of Down syndrome to the global gene dosage imbalance is complexly difficult because of its multiply underlying genetic causes and the large number of genes affected by the aneuploidy itself. Taking an advantage of technological advancements of genomic approaches, however, a large-scale analysis of transcriptomic and proteomic profiling have been conducted for understanding the gene dosage imbalance of autosomal trisomy. In most of those studies, unfortunately, neural tissues and neurogenesis-related cells have been never used: Indeed, the studies were carried out mostly on adult materials, even amniocytes, placental or fibroblast cells. Moreover, it is known that many of the pathological traits of autosomal trisomy are likely to be linked to the alteration of neural pathways during an early stage of embryonic development. In particular, abnormalities in brain developments contributing to the cognition phenotypes in autosomal trisomy are likely to arise during embryogenesis. Thus, it is critically important to conduct genome-wide investigation at early stages during neurogenesis and neural differentiation.

The aim of the present thesis is to clarify the genomic features of autosomal trisomy by examining the working hypothesis that autosomal trisomy causes the global dosage imbalance of increased gene products in the extra chromosome. To attain the aim, I conducted genome-wide analyses of transcriptomic and proteomic profiling for understanding the genomic features of gene dosage imbalance of autosomal trisomy,

using mouse embryonic stem cell lines containing either extra human or mouse autosomal chromosome(s). Establishing these chromosomally engineered embryonic stem cell lines of mouse by chromosome transfer technologies, I used the aneuploid neurons to which the engineered embryonic stem cells were differentiated *in vitro*. In particular, I focused upon chromosome 21 trisomy in this study because of its apparent connection to Down syndrome.

For a large scale analysis of gene expression profiling, I employed microarray and two-dimensional electrophoresis for transcriptomic and proteomic examinations, respectively. The profiling data obtained was subjected to data mining by bioinformatics analysis that I conducted, in order to study molecular mechanisms of autosomal imbalance and its genomic implications in early development of the nervous system.

Once I obtained candidate genes associated with autosomal trisomy from the data mining, I further carried out quantitative and functional analyses of those genes by embryoid body formation assay and RNA interference *in vitro* and teratoma and chimeric models of mouse *in vivo*. These analyses were conducted to identify possible roles of those genes in the autosomal trisomy at early stages of neuron differentiation and brain developments.

In the present study, I obtained the following results:
First, my transcriptomic analysis of microarray demonstrated that for the trisomic genes (on chromosome 21) in the differentiating aneuploid neurons, there were three categorical gene classes of up-regulated, no changed and down-regulated gene expressions: (1) 30 genes showed the 1.5-fold increased level of expression (primary gene dosage effect), which occupied 54%, (2) 14 genes indicated no changes (dosage compensation), which corresponded to 25%, and (3) 12 genes manifested 1.5-fold decreased level (reverse gene dosage effect), which was 21%. Thus, I found that the significant imbalance of gene dosage of autosomal trisomy was taking place in the genome-wide fashion at early stages of the developing neurons and brains.

Second, I focused upon the genes of primary dosage effect, namely up-regulated genes, because they occupied a largest proportion in the trisomic genes examined. Then, I found that 118 disomic genes (on all the autosomal chromosomes except chromosome 21) manifested significantly positive correlation with the trisomic genes (on chromosome 21) whereas 47 other disomic gens showed significantly negative correlation. Thus, I found a total of 165 genes that are located on all the autosomal chromosomes other than chromosome 21 and that are possibly influenced by the secondary or trans-acting effects of the genes on additional chromosome 21. Moreover,

those 165 disomic genes are rather evenly distributed over the entire genome, implying that there exists genome-wide background association with the trisomic genes of primary gene dosage effect.

Third, from my gene ontology analysis, I showed that the trisomic genes of primary gene dosage effect are mainly involved in the molecular function of proteins such as translational regulators and nuclear transcriptional factors. When I conducted further a comparative proteomic analysis, I made the observations indicating a possibility that the post-transcriptional and translational machineries are working as a underlying mechanism of gene products manifested during the neurogenesis in autosomal trisomy. This is also good indication for supporting genome-wide association of the trisomic genes of primary gene dosage.

Fourth and the last, I successfully identified a few of gene sets that show clear association with the enhanced apoptosis during early neural differentiation *in vitro* and in aneuploid neurons *in vitro* as well as fetal brains *in vivo*. Those gene set may be the candidate genes that are responsible for possible link of the cognitive conditions of Down syndrome to the genome-wide gene dosage imbalance.

In conclusion, I found that the gene dosage imbalance of autosomal trisomy globally (genome-widely) affects gene expression and protein expression of the disomic genes early in neurogenesis. The autosomal imbalance is associated with general neuronal loss possibly through underlying of neural apoptosis during neural differentiation. The understanding of the molecular mechanisms of the neural development in autosomal trisomy relies on the integration of multilevel molecular data such as transcriptomic, proteomic, and metabolomic data. From these reasons, I concluded that massive data mining and its further systematic analysis by bioinformatics approaches are essential for understanding the genomic implication of the gene dosage imbalance in autosomal trisomy.

博士論文の審査結果の要旨

Human chromosome 21 trisomy, which attributes to over 95% of Down syndrome, is a numerical chromosomal anomaly with an extra single copy of chromosome 21. It is associated with variable cognitive impairment and behavioural phenotypes as well as general developmental failure followed by processes of neurodegeneration. However, relationship between those neurological defects and chromosome 21 trisomic condition is still unclear.

The aim of this thesis is to clarify the genomic features of the chromosome 21 trisomy by examining the working hypothesis that the 21 trisomy causes the global dosage imbalance due to increased gene products in the extra chromosome. To attain the aim, this thesis conducted genome-wide analyses of transcriptomic and proteomic profiling for understanding the genomic features of gene dosage imbalance of 21 trisomy, using differentiating neurons from mouse embryonic stem cell lines containing either extra human chromosome 21 or its mouse counterpart.

In this study, the following four main results were obtained:

icroarray analysis for trisomic genes on chromosome 21 in the differentiating aneuploid neurons demonstrated that there are three categorical gene classes of up-regulated, no changed and down-regulated gene expressions, and that the significant imbalance of gene dosage of 21 trisomy is taking place at early stages of the developing neurons and brains.

2) Further microarray analysis identified a total of 165 genes that are located on all autosomal chromosomes other than chromosome 21 and that are possibly influenced by the secondary or trans-acting effects of the genes on additional chromosome 21. Moreover, those 165 disomic genes are rather evenly distributed over the entire genome, implying that there exists genome-wide background association with the trisomic genes of primary gene dosage effect.

3) Gene ontology analysis and comparative proteomics analysis showed that the 21 trisomic genes of primary gene dosage effect are mainly involved in the molecular function of proteins such as translational regulators and nuclear transcriptional factors.

4) This study successfully identified a few of gene sets that show clear association with the enhanced apoptosis during early differentiation of aneuploid neurons *in vitro* as well as fetal brains *in vivo*. Those gene set may be the candidate genes that are responsible for possible link of the cognitive conditions of Down syndrome to the genome-wide gene dosage imbalance.

All these findings that the gene dosage imbalance of chromosome 21 trisomy genome-widely affects gene expression and protein expression of many disomic genes

during early neurogenesis contributed to understanding relationship between the neurological defects and chromosome 21 trisomic conditions, and would be indispensable infrastructure for feature studies.