Identification of juvenility-associated genes in the mouse hepatocytes and cardiomyocytes.

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学位記番号	博士甲第884
学位授与の要件	学位規則第４条第１項
学位授与年月日	令和３年３月９日
学位論文題目	Identification of juvenility-associated genes in the mousehepatocytes and cardiomyocytes. （マウス肝臓細胞と心臓細胞における若年性遺伝子の同定）
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別紙様式３（課程博士・論文博士共用）

論　文　内　容　要　旨

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Background:
Young individuals possess distinct properties that adults do not. The juvenile animals show higher activities for growth, healing, learning and plasticity than adults. The machinery for establishing these juvenile properties is not fully understood. To better understand the molecular constituents for the above properties, we performed a comprehensive transcriptome analysis of differently aged mouse hepatocytes and cardiomyocytes by high-throughput sequencing. To assess the physiological relevance of these juvenility-associated genes (JAGs), we investigate their associations to human genetic diseases.

Purpose:
To identify genes that constitute to the physiological properties of juvenile cells.

Method:
To identify the specific gene sets we called as juvenility-associated genes (JAGs) which constitute to the juvenile properties of young organs, we performed comprehensive transcriptome by high-throughput sequencing in the differently aged mouse hepatocytes and cardiomyocytes. Hepatocytes and cardiomyocytes were isolated from mice at postnatal day 1 and 7 (P1 and P7) considered as juvenile phase while postnatal days 56 (P56) as adult phase. By observing at these 2 points (juvenile phase vs adult phase), we aimed at delineating consistent alterations to capture the consistently relevant genes for the juvenile properties. We performed gene ontology (GO) analysis to obtain an overview for the biological process and molecular functions of the hepatocyte JAGs, cardiomyocytes JAGs and common JAGs.

Result and discussion:
Among all the 29,720 transcripts, 3,306 genes exhibited the expression levels higher than the cut off and the fold changes higher than 2.0 both at P1 and P7 comparing to P56 hepatocytes. We here identified hepatocyte JAGs (hepato-JAGs) that are the functional building blocks

（備考）１．論文内容要旨は、研究の目的・方法・結果・考察・結論の順に記載し、２千字程度でタイプ等を用いて印字すること。
２．※印の欄には記入しないこと。

for juvenile properties in the liver. The GSEA showed significant enrichment of the hepato-JAGs into the functions such as cell and cellular component sizes, cell shape, protein metabolism and the Ras signalling. Numerous extracellular matrix (ECM) genes were expressed highly in the juvenile hepatocytes. From GO analysis with the genes expressed in each P1, P7 and P56, we showed transition of maturation status in the postnatal hepatocytes. Transcriptome analysis in the postnatal hepatocytes clarifies the maturation dynamics and the genes characterizing each maturation stage. Cardiomyocyte JAGs (cardio-JAGs) were identified from all the 29,720 transcripts based on the expression levels and fold changes comparing to adult cells. From GO analysis, we found cardio-JAGs exhibit significant associations with keywords such as alternative splicing, cell cycle and phosphorylation and the cellular components such as chromosome and ECM. The gene set enrichment analysis (GSEA) showed significant association with functions such as “cell size”, “cell cycle”, “Wnt signaling” and “mesenchyme development”. Comprehensive GO analysis revealed that the P1-associated genes were linked with translation-related functions and enriched in mitochondrion. The P7-associated genes were linked with RNA processing-related terms while P56-associated genes were linked with mitochondrial functions such as “mitochondrion” and “electron transport chain. This comprehensive gene expression profiling reveals the functional maturation dynamics and the pivotal genes characterizing each maturation stages in the postnatal cardiomyocytes. We next identified 846 common JAGS, the overlapping of hepato-JAGs and cardio-JAGs as genes universally relevant to establish the juvenile properties. The GO analysis revealed terms such as “cell cycle”, “nucleus” and “chromosome”, while unexpected terms “alternative splicing”, “ubiquitination”, “acetylation” and “extracellular matrix” were significantly enriched in the common JAGs. Thus, these analyses identified common JAGs underlie the juvenile properties of the young animals. We next tested the associations of mutation in the common JAGs with pediatric diseases. We found the rate for the successful annotation with a disease were higher in the common JAGs than all genes, indicating the common JAGs enriched the physiologically indispensable genes. Numerous growth-associated genes were included in common JAGs and this implies that common JAGs had dense connections to the childhood-onset diseases, including growth disorders and progeria syndromes.

Conclusion:

We presented juvenility-associated genes (JAGs) as a specific set of genes that form the physiological properties of the juvenile cells which has capacities for growth and maturation. These JAGs possess functions such as alternative splicing, phosphorylation and ECM, setting these functions as the important bricks that constitute juvenile properties of young cells. The JAGs have dense connections with childhood-onset genetic diseases, indicating their physiological relevance and their potential as the new therapeutic target for genetic diseases.

別紙様式８（課程博士・論文博士共用）

学位論文審査の結果の要旨

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小児の生理的特性の解明により、小児難病の治療法について知見を得られる可能性がある。本研究ではマウスで生後１日、７日、56 日の肝細胞と心筋細胞のトランスクリプトーム解析によって幼若性関連遺伝子群(JAGs)を見出す試みを行った。

１）肝細胞と心筋細胞では、それぞれに小児期に強く発現する特異的な遺伝子群が異なっていた。

２）gene ontology(GO)により幼若な肝細胞ではリン酸化タンパク質、細胞周期、有糸分裂、選択的プライシング、細胞質、膜、核、細胞外エクソソーム、ECM が、心筋細胞では選択的プライシング、リン酸化、染色体、ECM などといったキーワードが示された。

３）P1, P7, P56 でそれぞれに発現が強くなる遺伝子群があり、GO によって P1 では翻訳に、P7 では ECM に、P56 では酸化還元に関連した遺伝子群が示された。

４）肝細胞と心筋細胞で共通な JAGs(common JAGs)の GO 解析を行うと細胞周期、核、染色体の他に、選択的スプライシング、ユビキチン化、アセチル化、ECM といったキーワードが多く示された。

５）全疾患関連変異遺伝子の割合はすべての遺伝子におけるよりも、common JAGs の方が高くなっており、抽出された遺伝子では特に小児の遺伝的疾患が強く示されていた。

よって JAGs が幼若期の明確な特徴が示すこと、また、これらが小児難病治療法開発の標的となりうることが示唆された。

（５９８文字）