RESEARCH REPORT - No. 1605 FY 2016-2017

Title of research project	Establishment and evaluation of a novel toxicity evaluation system for chemical substances based on the expression changes of plasma microRNAs
Research project no.	1605
Research period	FY 2016–2017
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【Abstract】

This study consists of the following four main themes,

[#1] <Micro RNA-mediated Th2 bias in the pathogenesis of methimazole-induced acute liver injury in mice>. MicroRNA (miRNA) is a class of small non-coding RNAs containing approximately 20 nucleotides that negatively regulate target gene expression. Little is known about the role of individual miRNAs and their targets in immune- and inflammation-related responses in chemical-induced liver injury. In the present study, involvement of miRNAs in the T helper (Th) 2-type immune response was investigated using a mouse model of methimazole (MTZ)-induced liver injury. Co-administration of L-buthionine-*S,R*-sulfoximine (BSO) and MTZ induced acute hepatocellular necrosis and elevated plasma levels of alanine aminotransferase (ALT) in female Balb/c mice. The hepatic mRNA expression of Th2 promotive factors was significantly increased concomitantly with plasma ALT levels. In contrast, the hepatic mRNA expression of Th2 suppressive factors was significantly decreased during the early phase of liver injury. Comprehensive profiling of hepatic miRNA expression was analyzed before the onset of MTZ-induced liver injury. Using *in silico* prediction of miRNAs that possibly regulate Th2-related genes and subsequent quantification, we identified up-regulation of expression of miR-29b-1-5p and miR-449a-5p. Among targets of these miRNAs, down-regulation of Th2 suppressive transcription factors, such as SRY-related HMG-box 4 (SOX4) and lymphoid enhancer factor-1 (LEF1), were observed from the early phase of liver injury. In conclusion, negative regulation of the expression of SOX4 by miR-29b-1-5p and that of LEF1 by miR-449a-5p is suggested to play an important role in the development of Th2 bias in MTZ-induced liver injury.

[#2] <Identification of specific microRNA biomarkers in early stages of hepatocellular injury, cholestasis, and steatosis in rats> Recently, studies on circulating microRNAs (miRNAs) as potential biomarkers of liver injury have received increasing attention. It has been demonstrated that miR-122 and miR-192, which are liver enriched, could be potential biomarkers of DILI; however, these miRNAs cannot discern types of injuries. In the present study, we comprehensively analyzed time-dependent profiles of plasma miRNA in rats with drug- or chemical-induced hepatocellular injury, cholestasis, and steatosis with high-throughput NGS (next generation sequencing). To enable the comparison of miRNA expression levels between DILI models with different severity and peak time of injuries, the stages of injury were defined

as early, middle and late, according to cluster patterns of miRNA expression profiles. Through differential analysis, we characterized miRNAs that were specifically up- or down-regulated in each liver injury model. Several miRNAs were dramatically changed earlier than traditional biomarkers such as ALT and AST. For example, in an APAP-induced hepatocellular injury model, plasma let-7b-5p was up-regulated as early as 3 h after dosing, whereas a significant change in ALT level was observed at 12 h. We then focused on the liver injuty type-specific miRNAs in plasma that were up-regulated at the early stage of injury. RT-qPCR analysis validated that let-7b-5p and miR-1-3p for hepatocellular injury, miR-143-3p and miR-218a-5p for cholestasis, and miR-320-3p for steatosis models showed significant increases in the early stage of the injuries. The present study suggests the usefulness of miRNAs as specific biomarkers for the early detection of liver injury.

[#3] <Identification of specific microRNA biomarkers in early stages of renal injury in rats> In this study, we comprehensively analyzed time-dependent profiles of plasma miRNA in rats with drug- or chemical-induced renal injury, such as tubular injuty and glomerular injury, with high-throughput NGS (next generation sequencing). MiRNAs expression levels between two renal injury models with different severity and peak time of injuries were analyzed in cluster patterns of miRNA expression profiles. Through differential analyses, we characterized miRNAs that were specifically up- or down-regulated in each renal injury model. The three miRNAs (miR-22-3p, let-7b-5p, and miR-192-5p) were specifically up-regulated in the rat plasma with tubular injury. Interestingly, these three miRNAs were specifically down-regulated in the rat plasma with glomerular injury. As a common biomarker for the both renal injury, the three miRNAs (miR-21-5p, miR-16-5p and let-7f-5p) were suggested. We are now doing further validation study on these miRNAs. The present study suggests the miRNAs candidates as injury type-specific biomarkers.

[#4] < Establish and evaluate miRNA biomarkers considering immune- and inflammatory-related factors. Study for the association with idiosyncratic hepatic injury> Previously, we have reported animal models of compounds that cause so-called idiosyncratic liver injury. From our research background and results, we believe it is possible to separate miRNAs that change specifically due to the characteristics (drug efficacy etc.) of compounds and miRNAs that change due to liver injury. However, most of the animal models had established in mice. It was elucidated that the rats are species with strong drug resistance. Therefore, currently many conditions are under investigation of suitable modeling conditions for hepatic injury in many compounds. What we have finished so far is only the combination of ticlopidine, a liver disorder drug and clopidogrel as a control. In the schedule, we will add 5 additional sets of compounds and plan to confirm the reliable miRNAs derived from hepatic injury.