Research Center for Medical Sciences Core Research Facilities

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General Summary

The Research Center for Medical Sciences of The Jikei University School of Medicine has been reorganized, and in April 2019 the Division of Molecular Cell Biology and the Division of Molecular Genetics of the Core Research Facilities for Basic Science were integrated and are now known as the Core Research Facilities. This integration has consolidated the facilitation of on-campus research support.
1. Annual Registration System
This system is intended to supply research benches and other equipment to researchers of the university to perform experiments. Once registered, researchers can freely use the various devices in our institution. This system also provides, if necessary, technical advice and guidance on specific fine-morphological or biochemical approaches to a registrant's experiment. In 2019, 168 researchers registered at our annual registration system, and we provided research support 244 times for electron microscopy and 1 time for laboratory experiments.
2. System for Providing Research Services
Advances in research technologies and equipment enable us to perform more precise and accurate observations of specimens in medical sciences. For researchers who cannot perform experiments owing to limits of time and funds, our staff can prepare samples for scanning electron microscopy and transmission electron microscopy, record images, or perform high-performance liquid chromatography and mass spectrometry. The service fee is minimal because services are limited to the university.

Research Activities

Possibility of nicotinamide phosphoribosyltransferase suppression as a molecular target
Although brain tumors, particularly gliomas, are intractable and resist many first-line treatments, candidate target molecules have recently been identified by analyzing what is known about genes and proteins. Nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme in the nicotinamide adenine dinucleotide (NAD^+) biosynthetic salvage pathway which converts nicotinamide to nicotinamide mononucleotide. The converted nicotinamide mononucleotide is further metabolized to NAD^+ and serves as a coenzyme of various types of dehydrogenation. Previous research suggests that increases in NAMPT transcription and expression correlate with the growth or clinical grade of glioma. Therefore, because NAMPT modulation can be directly applicable as an adjuvant

remedy for radiotherapy or chemotherapy, we established cell lines that suppressed NAMPT expression with short hairpin interfering RNA. As a result, NAMPT inhibition alone suppressed cell growth and increased radiosensitivity, but the effects were transient. Furthermore, inhibition did not alter the sensitivity of glioma to the antineoplastic agent temozolomide. This involved other salvage pathways. We are creating a system to enhance and prolong the effect of NAMPT suppression.

Human hepatocyte chimeric mice and an animal model of hepatitis virus infection
We have established human hepatocyte chimeric mice with an efficient method that we had developed and also used chimeric mice to create an animal model of hepatitis B or C virus infection. We are intensely performing research on the efficacy of novel antiviral agents, the mechanism of progression to chronic infection, and ultrastructural alterations of intrahepatocellular organelle after viral eradication.

Single nucleotide polymorphisms, and resistant-associated variants in the treatment of chronic hepatitis C virus infection
Direct-acting antiviral agents are the first-line treatment for chronic hepatitis C virus infection. We are investigating the association of single nucleotide polymorphisms of the genes with the blood drug concentration, treatment response, and direct-acting antiviral agent-induced liver damage. Resistant-associated variants are also being investigated in detail.

The association between serum microRNA expression levels and treatment outcome/prognosis in hepatocellular carcinoma
We measure serum microRNA expression levels in an intrahepatic feeding artery, proper hepatic artery, and peripheral vein when we perform transcatheter arterial chemoembolization (TACE) for patients with hepatocellular carcinoma (HCC), and are investigating the association between serum microRNA expression levels and treatment outcome/prognosis in patients who have HCC and were treated with TACE/radiofrequency ablation (RFA).

Comprehensive gene expression profiling analysis of microRNA/messenger RNA
We are profiling and analyzing the expression of microRNA/messenger RNA in the liver tissue of hepatitis B virus (HBV)-infected human hepatocyte chimeric mice. We have found the novel interaction between microRNA and messenger RNA in HBV replication and lifecycle. We are also investigating the association between serum microRNA expression level and treatment outcome/prognosis in HCC patients who were treated with TACE/RFA.

A new method for measuring cholesterol efflux capacity using liquid chromatography-ultrahigh-resolution mass spectrometry with stable isotope-labeled cholesterol
The incidence of cardiovascular events correlates inversely with cholesterol efflux capacity (CEC) more than with the high-density lipoprotein cholesterol level. The measurement of CEC is used to qualify cardiovascular disease risk and is conventionally per-

formed with radioisotope-labeled cholesterol. So, we established a CEC measurement technique using stable isotope-labeled cholesterol as an alternative, and we compared our method with radioisotope- and fluorescence-labeled cholesterol methods using cells and patient serum. We incubated J774 cells labeled with [d 7] cholesterol (d 7-C) with patient serum, and d 7-C extracted from the cell culture medium was quantified by liquid chromatography-quadrupole time-of-flight mass spectrometry. The assay coefficient of variation of five consecutive measurements of three sets of samples ranged from 7.3% to 9.5%, and the interassay coefficient of variation determined by measuring 3 samples four times ranged from 4.1% to 8.5%, both indicating good precision. The CEC levels were measured for 41 outpatients with serum high-density lipoprotein cholesterol levels of 36 to 94 mg/dl (mean, 61.7 ± 18.0 mg/dl) under cyclic adenosine monophosphate. Results were suggested that positive correlation between CEC levels using the stable isotope and radioisotope methods. It was stronger than the correlation between measurements using the fluorescence and radioisotope methods ($r = 0.73$, $P < 0.0001$ vs. $r = 0.55$, $P < 0.001$). Therefore, our newly developed using stable isotope method can be considered useful as a non-radioisotope method and thus deserves evaluation in future clinical studies.

Effect of smoking inflammatory response to smoking cessation on human gingival fibroblast and periodontal ligaments cells

The purpose of this study was to investigate the inflammatory response of human gingival fibroblasts and periodontal ligament cells during smoking (during nicotine stimulation) and the effect of repair period during smoking cessation (interruption of nicotine stimulation).

Both cells were obtained from healthy periodontal tissue. The cells were cultured until they reached confluence, replaced with a medium containing 1 μg/ml nicotine, and cultured for 24 hours. After that, the supernatant was replaced with a nicotine-free medium and the culture was carried out for 48 hours. Culture supernatants at each time point after nicotine stimulation and after nicotine discontinuation were collected, and interleukin 6 production was measured with enzyme-linked immunosorbent assay. Interleukin 6 production increased significantly ($p < 0.001$) in both cells after nicotine stimulation, but decreased significantly after nicotine discontinuation ($p < 0.001$). Scanning electron microscopy revealed many depressions on the surface of the cell membrane due to nicotine stimulation.

From these facts, it was demonstrated that smoking probably had an adverse effect on cells, and the possibility of a cell repair effect by smoking cessation was shown.

Development of the adenovirus vector systems

We have developed a protocol for curing HBV infection with an adenovirus vector (AdV). We established the efficient detection system of HBV genome replication applying AdVs (HBV103-AdV system) and identified several promising compounds. Furthermore, we succeeded in efficient cleavage of the HBV genome using a hepatocyte-specific genome editing system by AdV and we identified several promising genomic RNA candidates.

Rapid identification and quantification of Lactobacillus rhamnosus*-targeting real-time polymerase chain reaction using a TaqMan probe*

Lactobacillus rhamnosus is a gram-positive, rod-shaped bacterium and is commonly used as a probiotic to maintain intestinal health. Recently, surveillance of Lactobacillus bacteremia was conducted using biochemical or conventional polymerase chain reaction (PCR) assay; however, these assays are unable to quantify the target, and might detect a small number of DNA fragments or yield a false-positive result. In this study, we developed an *L. rhamnosus*-targeting quantitative PCR assay, which produces accurate and reproducible results based on the specificity of a TaqMan probe targeting the unique 16S ribosomal DNA sequence of *L. rhamnosus*. The assay specifically detected the targeted bacterium, *L. rhamnosus*, and no non-specific signals were generated under the study conditions. Using genomic DNA from the bacterial cells of *L. rhamnosus* (101 to 106 cells), the cycle threshold value showed a linear trend (R2 = 0.9993). This *L. rhamnosus*-targeting quantitative PCR assay can contribute to advance research into the effects of the organism on microflora, microbial infections, and the host.

Protective actions of urocortin family peptide on pancreatic β-cells

It has been reported that urocortin family peptides exert cellular protective actions. We are now investigating actions of urocortin family peptides, especially, urocortin III, against toxic actions to pancreatic β-cells, such as hyperglycemic condition and nicotine exposure which resulted in reduced insulin release. As the first step of these approach, we tried to investigate the action of urocortin III on such conditions by insulin release. Urocortin III facilitate insulin release at the hyperglycemic condition and recovered the suppressive effect on insulin release by nicotine.

Publications

Funamizu N, Lacy CR, Kamada M, Yanaga K, Manome Y. MicroRNA-200b and -301 are associated with gemcitabine response as biomarkers in pancreatic carcinoma cells. *Int J Oncol.* 2019 Mar; **54**(3): 991-1000. doi: 10.3892/ijo.2019.4676. Epub 2019 Jan 7. PMID: 30628651.

Nozaki A, Atsukawa M, Kondo C, Toyoda H, Chuma M, Nakamuta M, Uojima H, Takaguchi K, Ikeda H, Watanabe T, Ogawa S, Itokawa N, Arai T, Hiraoka A, Asano T, Fujioka S, Ikegami T, Shima T, Ogawa C, Akahane T, Shimada N, Fukunishi S, Abe H, Tsubota A, Genda T, Okubo H, Mikami S, Morishita A, Moriya A, Tani J, Tachi Y, Hotta N, Ishikawa T, Okanoue T, Tanaka Y, Kumada T, Iwakiri K, Maeda S; KTK49 Liver Study Group. The effectiveness and safety of glecaprevir/pibrentasvir in chronic hepatitis C patients with refractory factors in the real world: a comprehensive analysis of a prospective multicenter study. *Hepatol Int.* 2020 Mar; **14**(2): 225-238. doi: 10.1007/s12072-020-10019-z. Epub 2020 Mar 3. PMID: 32128704.

Toyoda H, Atsukawa M, Watanabe T, Nakamuta M, Uojima H, Nozaki A, Takaguchi K, Fujioka S, Iio E, Shima T, Akahane T, Fukunishi S, Asano T, Michitaka K, Tsuji K, Abe H, Mikami S, Okubo H, Okubo T, Shimada N, Ishikawa T, Moriya A, Tani J, Morishita A, Ogawa C, Tachi Y, Ikeda H, Yamashita N, Yasuda S, Chuma M, Tsutsui A, Hiraoka A, Ikegami T, Genda T, Tsubota A, Masaki T, Iwakiri K, Kumada T, Tanaka Y, Okanoue T. Marked heterogeneity in the diagnosis of compensated cirrhosis of patients with chronic hepatitis C virus infection in a real-world setting: A large, multicenter study from Japan. *J Gastroenterol Hepatol.* 2020 Aug; **35**(8): 1420-1425. doi: 10.1111/jgh.14982. Epub 2020 Jan 31. PMID: 31950525.

Okubo T, Atsukawa M, Tsubota A, Yoshida Y, Arai T, Iwashita AN, Itokawa N, Kondo C, Iwakiri K. Relationship between serum vitamin D level and sarcopenia in chronic liver disease. *Hepatol Res.* 2020 May; **50**(5): 588-597. doi: 10.1111/hepr.13485. Epub 2020 Jan 22. PMID: 31914479.

Atsukawa M, Tsubota A, Takaguchi K, Toyoda H, Iwasa M, Ikegami T, Chuma M, Nozaki A, Uojima H, Hiraoka A, Fukunishi S, Yokohama K, Tada T, Kato K, Abe H, Tani J, Okubo H, Watanabe T, Hattori N, Tsutsui A, Senoh T, Yoshida Y, Okubo T, Itokawa N, Nakagawa-Iwashita A, Kondo C, Arai T, Michitaka K, Iio E, Kumada T, Tanaka Y, Takei Y, Iwakiri K. Analysis of factors associated with the prognosis of cirrhotic patients who were treated with tolvaptan for hepatic edema. *J Gastroenterol Hepatol.* 2020

Jul; **35**(7): 1229-1237. doi: 10.1111/jgh.14965. Epub 2020 Jan 14. PMID: 31881554.
Saeki C, Takano K, Oikawa T, Aoki Y, Kanai T, Takakura K, Nakano M, Torisu Y, Sasaki N, Abo M, Matsuura T, Tsubota A, Saruta M. Comparative assessment of sarcopenia using the JSH, AWGS, and EWGSOP2 criteria and the relationship between sarcopenia, osteoporosis, and osteosarcopenia in patients with liver cirrhosis. *BMC Musculoskelet Disord.* 2019 Dec 26; **20**(1): 615. doi: 10.1186/s12891-019-2983-4. PMID: 31878909; PMCID: PMC6933666.
Arai T, Atsukawa M, Tsubota A, Kawano T, Koeda M, Yoshida Y, Tanabe T, Okubo T, Hayama K, Iwashita A, Itokawa N, Kondo C, Kaneko K, Kawamoto C, Hatori T, Emoto N, Iio E, Tanaka Y, Iwakiri K. Factors influencing subclinical atherosclerosis in patients with biopsy-proven nonalcoholic fatty liver disease. *PLoS One.* 2019 Nov 13; **14**(11): e0224184. doi: 10.1371/journal.pone.0224184. PMID: 31721770; PMCID: PMC6853607.
Takano K, Saeki C, Oikawa T, Hidaka A, Mizuno Y, Ishida J, Takakura K, Nakano M, Torisu Y, Amano K, Ishikawa T, Zeniya M, Tsubota A, Saruta M. IgM response is a prognostic biomarker of primary biliary cholangitis treated with ursodeoxycholic acid and bezafibrate. *J Gastroenterol Hepatol.* 2020 Apr; **35**(4): 663-672. doi: 10.1111/jgh.14900. Epub 2019 Dec 11. PMID: 31677185.
Toyoda H, Atsukawa M, Watanabe T, Nakamuta M, Uojima H, Nozaki A, Takaguchi K, Fujioka S, Iio E, Shima T, Akahane T, Fukunishi S, Asano T, Michitaka K, Tsuji K, Abe H, Mikami S, Okubo H, Okubo T, Shimada N, Ishikawa T, Moriya A, Tani J, Morishita A, Ogawa C, Tachi Y, Ikeda H, Yamashita N, Yasuda S, Chuma M, Tsutsui A, Hiraoka A, Ikegami T, Genda T, Tsubota A, Masaki T, Tanaka Y, Iwakiri K, Kumada T. Real-world experience of 12-week direct-acting antiviral regimen of glecaprevir and pibrentasvir in patients with chronic hepatitis C virus infection. *J Gastroenterol Hepatol.* 2020 May; **35**(5): 855-861. doi: 10.1111/jgh.14874. Epub 2019 Nov 19. PMID: 31609495.
Kato K, Shimada N, Atsukawa M, Abe H, Itokawa N, Matsumoto Y, Agata R, Tsubota A. Single nucleotide polymorphisms associated with elevated alanine aminotransferase in patients receiving asunaprevir plus daclatasvir combination therapy for chronic hepatitis C. *PLoS One.* 2019 Jul 10; **14**(7): e0219022. doi: 10.1371/journal.pone.0219022. PMID: 31291311; PMCID: PMC6619746.
Ikeda H, Watanabe T, Atsukawa M, Toyoda H, Takaguchi K, Nakamuta M, Matsumoto N, Okuse C, Tada T, Tsutsui A, Yamashita N, Kondo C, Hayama K, Kato K, Itokawa N, Arai T, Shimada N, Asano T, Uojima H, Ogawa C, Mikami S, Ikegami T, Fukunishi S, Asai A, Iio E, Tsubota A, Hiraoka A, Nozaki A, Okubo H, Tachi Y, Moriya A, Oikawa T, Matsumoto Y, Tsuruoka S, Tani J, Kikuchi K, Iwakiri K, Tanaka Y, Kumada T. Evaluation of 8-week glecaprevir/pibrentasvir treatment in direct-acting antiviral-naïve noncirrhotic HCV genotype 1 and 2infected patients in a real-world setting in Japan. *J Viral Hepat.* 2019 Nov; **26**(11): 1266-1275. doi: 10.1111/jvh.13170. Epub 2019 Aug 9. PMID: 31278795.
Toyoda H, Atsukawa M, Uojima H, Nozaki A, Tamai H, Takaguchi K, Fujioka S, Nakamuta M, Tada T, Yasuda S, Chuma M, Senoh T, Tsutsui A, Yamashita N, Hiraoka A, Michitaka K, Shima T, Akahane T, Itobayashi E, Watanabe T, Ikeda H, Iio E, Fukunishi S, Asano T, Tachi Y, Ikegami T, Tsuji K, Abe H, Kato K, Mikami S, Okubo H, Shimada N, Ishikawa T, Matsumoto Y, Itokawa N, Arai T, Tsubota A, Iwakiri K, Tanaka Y, Kumada T. Trends and Efficacy of Interferon-Free Anti-hepatitis C Virus Therapy in the Region of High Prevalence of Elderly Patients, Cirrhosis, and Hepatocellular Carcinoma: A Real-World, Nationwide, Multicenter Study of 10 688 Patients in Japan. *Open Forum Infect Dis.* 2019 Apr 15; **6**(5): ofz 185. doi: 10.1093/ofid/ofz185. PMID: 31123693; PMCID: PMC6524830.
Atsukawa M, Tsubota A, Toyoda H, Takaguchi K, Nakamuta M, Watanabe T, Michitaka K, Ikegami T, Nozaki A, Uojima H, Fukunishi S, Genda T, Abe H, Hotta N, Tsuji K, Ogawa C, Tachi Y, Shima T, Shimada N, Kondo C, Akahane T, Aizawa Y, Tanaka Y, Kumada T, Iwakiri K. The efficacy and safety of glecaprevir plus pibrentasvir in 141 patients with severe renal impairment: a prospective, multicenter study. *Aliment Pharmacol Ther.* 2019 May; **49**(9): 1230-1241. doi: 10.1111/apt.15218. Epub 2019 Mar 14. PMID: 30873651.
Arai T, Atsukawa M, Tsubota A, Koeda M, Yoshida Y, Okubo T, Nakagawa A, Itokawa N, Kondo C, Nakatsuka K, Masu T, Kato K, Shimada N, Hatori T, Emoto N, Kage M, Iwakiri K. Association of vitamin D levels and vitamin D-related gene polymorphisms with liver fibrosis in patients with biopsy-proven nonalcoholic fatty liver disease. *Dig Liver Dis.* 2019 Jul; **51**(7): 1036-1042. doi: 10.1016/j.dld.2018.12.022. Epub 2019 Jan 9. PMID: 30683615.
Atsukawa M, Tsubota A, Toyoda H, Takaguchi K, Nakamuta M, Watanabe T, Tada T, Tsutsui A, Ikeda H, Abe H, Kato K, Uojima H, Ikegami T, Asano T, Kondo C, Koeda M, Okubo T, Arai T, Iwashita-Nakagawa A, Itokawa N, Kumada T, Iwakiri K. Efficacy and safety of ombitasvir/paritaprevir/ritonavir and ribavirin for chronic hepatitis patients infected with genotype 2a in Japan. *Hepatol Res.* 2019 Apr; **49**(4): 369-376. doi: 10.1111/hepr.13292. Epub 2019 Jan 2. PMID: 30485638.
Shimizu T, Miyazaki O, Iwamoto T, Usui T, Sato R, Hiraishi C, Yoshida H. A new method for measuring cholesterol efflux capacity uses stable isotope-labeled, not radioactive-labeled, cholesterol. *J Lipid Res.* 2019 Nov; **60**(11): 1959-1967. doi: 10.1194/jlr.D086884. Epub 2019 Aug 27. PMID: 31455616; PMCID: PMC 6824490.
Oguro A, Shigeta T, Machida K, Suzuki T, Iwamoto T, Matsufuji S, Imataka H. Translation efficiency affects the sequence-independent +1 ribosomal frameshifting by polyamines. *J Biochem.* 2020 Aug 1;

168(2): 139-149. doi: 10.1093/jb/mvaa032. PMID: 32181810.

Mostafa D, Takahashi A, Yanagiya A, Yamaguchi T, Abe T, Kureha T, Kuba K, Kanegae Y, Furuta Y, Yamamoto T, Suzuki T. Essential functions of the CNOT7/8 catalytic subunits of the CCR4-NOT complex in mRNA regulation and cell viability. *RNA Biol.* 2020 Mar; **17**(3): 403-416. doi: 10.1080/15476286.2019.1709747. Epub 2020 Jan 10. PMID: 31924127; PMCID: PMC6999631.

Saito T, Kuma A, Sugiura Y, Ichimura Y, Obata M, Kitamura H, Okuda S, Lee HC, Ikeda K, Kanegae Y, Saito I, Auwerx J, Motohashi H, Suematsu M, Soga T, Yokomizo T, Waguri S, Mizushima N, Komatsu M. Autophagy regulates lipid metabolism through selective turnover of NCoR1. *Nat Commun.* 2019 Apr 5; **10**(1): 1567. doi: 10.1038/s41467-019-08829-3. PMID: 30952864; PMCID: PMC6450892.

Yokoyama-Mashima S, Yogosawa S, Kanegae Y, Hirooka S, Yoshida S, Horiuchi T, Ohashi T, Yanaga K, Saruta M, Oikawa T, Yoshida K. Forced expression of DYRK2 exerts anti-tumor effects via apoptotic induction in liver cancer. *Cancer Lett.* 2019 Jun 1; **451**: 100-109. doi: 10.1016/j.canlet.2019.02.046. Epub 2019 Mar 6. PMID: 30851422.

Okai C, Itani Y, Furuta A, Mizunoe Y, Iwase T. Rapid Identification and Quantification of Lactobacillus rhamnosus by Real-Time PCR Using a TaqMan Probe. *Jpn J Infect Dis.* 2019 Sep 19; **72**(5): 323-325. doi: 10.7883/yoken.JJID.2019.102. Epub 2019 Apr 26. PMID: 31061362.