広島大学

Yosuke Hashimoto
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Molecular Systems Pharmaceutics (Uchida lab),
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My research interests are *CLDN5* gene and claudin-5 protein. Claudin-5 is the most enriched component of the tight junctions associated with the blood-brain barrier (BBB). The expression/function of CLDN-5 is critical in determining the onset/frequency/severeness of a range of CNS diseases, such as epilepsy, psychiatric disorders and cognitive decline.

I have developed anti-claudin-5 monoclonal antibodies that can inhibit barrier forming function of claudin-5. Added to this, I have discovered a novel pathogenic *de novo CLDN5* missense mutation that causes alternating hemiplegia of childhood with epilepsy and brain calcification. I also discovered RNA-binding protein pumulio-1 controls translational level of claudin-5 mRNA and its binding ability to pumilio-1 is attenuated in claudin-5 mRNA with rs10314 mutation which is located in 3'-UTR of claudin-5 mRNA.

CAREER PROFILE (Education and Employment)

Apr 2008—Mar 2012	B.S, Pharmacology, Tokushima University, Japan
Apr 2012—Mar 2014	M.S, Pharmacology, Tokushima University, Japan
Apr 2014—Mar 2017	PhD, Pharmacology, Osaka University, Japan
Apr 2015—Mar 2017	Research fellow (doctoral course) of the Japan Society for the Promotion of Science (JSPS), Osaka University, Japan
March 2017	PhD (pharmacology)
Apr 2017—Jun 2019	Post-doctoral research fellow of the JSPS, Nagoya University, Japan
Jun 2019—Sep 2022	Visiting researcher, Trinity College Dublin (TCD), Ireland
Jun 2019—Mar 2020	Post-doctoral research fellow of the JSPS, TCD, Ireland
Apr 2020—Sep 2022	Overseas research fellow of the JSPS, TCD, Ireland
Oct 2022—Jun 2024	Post-doc research fellow, TCD, Ireland
Jul 2024—Present	Assistant Professor, Hiroshima University, Japan

Summary of Research Achievements

Original article	(first author)	**12**
	(co-author)	**9**
	Total publication	**21**
	Corresponding publication	**1**
Review articles	(first author)	**9**
	Total publication	**9**
	Corresponding publication	**3**
Other articles	**(Japanese)**	**4**
	(English)	**2**
Research Grants	**From JSPS**	**3**
	From others	**3**
Intellectual properties		**2**
Invited presentations	**(English)**	**2**

Original Articles

1. **Y. Hashimoto (co-corresponding author)**, C. Greene, N. Hanley, N. Hudson, D. Henshall, K.J. Sweeney, D.F. O'Brien, M. Campbell. Pumilio-1 mediated translational control of claudin-5 at the blood-brain barrier. ***Fluids Barriers CNS*** 21, 52 (2024). doi: 10.1186/s12987-024-00553-5.
2. **Y. Hashimoto**, C. Besmond, N. Boddaert, A. Munnich, M. Campbell. A loss of function mutation in *CLDN25* causing Pelizaeus-Merzbacher-like leukodystrophy. ***Hum. Mol. Genet.*** 33(12), 1055-1063 (2024). doi: 10.1093/hmg/ddae038.
3. **Y. Hashimoto**, K. Poirier, N. Boddaert, L. Hubert, M. Aubart, A. Kaminska, M. Alison, I. Desguerre, A. Munnich, M. Campbell. Recurrent *de novo* mutations in *CLDN5* induce an anion-selective blood-brain barrier and alternating hemiplegia. ***Brain*** 145(10), 3374-3382 (2022). doi: 10.1093/brain/awac215.
4. K. Tachibana, **Y. Hashimoto**, K. Shirakura, Y. Okada, R. Hirayama, Y. Iwashita, I. Nishino, Y. Ago, H. Takeda, H. Kuniyasu, M. Kondoh. Safety and efficacy of an anti-claudin-5 monoclonal antibody to increase blood-brain barrier permeability for drug delivery to the brain in a non-human primate. ***J. Control. Release*** 336, 105-111 (2021). doi: 10.1016/j.jconrel.2021.06.009.
5. T. Shimizu, A.S. Abu Lila, Y. Kawaguchi, Y. Shimazaki, Y. Watanabe, Y. Mima, **Y. Hashimoto**, K. Okuhira, G. Storm, Y. Ishima, T. Ishida. A novel platform for cancer vaccines: antigen-selective delivery to splenic marginal zone B cells via repeated injections of PEGylated liposomes. ***J. Immunol.*** 201(10), 2969-2976 (2018). doi: 10.4049/jimmunol.1701351.
6. T. Shimizu, A.S. Abu Lila, M. Awata, Y. Kubo, Y. Mima, **Y. Hashimoto**, H. Ando, K. Okuhira, Y. Ishima, T. Ishida. A cell assay for detecting anti-PEG immune response against PEG-modified therapeutics. ***Pharm. Res.*** 35(11), 223 (2018). doi: 10.1007/s11095-018-2505-3.
7. T. Shimizu, A.S. Abu Lila, R. Fujita, M. Awata, M. Kawanishi, **Y. Hashimoto**, K. Okuhira, Y. Ishima, T. Ishida. A hydroxyl PEG version of PEGylated liposomes and its impact on anti-PEG IgM induction and on the accelerated clearance of PEGylated liposomes. ***Eur. J. Pharm. Biopharm.*** 127, 142-149 (2018). doi: 10.1016/j.ejpb.2018.02.019.
8. **Y. Hashimoto**, W. Zhou, K. Hamauchi, K. Shirakura, T. Doi, K. Yagi, T. Sawasaki, Y. Okada, M. Kondoh, H. Takeda. Engineered membrane protein antigens successfully induce antibodies against extracellular regions of claudin-5. ***Sci. Rep.*** 8, 8383 (2018). doi: 10.1038/s41598-018-26560-9.
9. **Y. Hashimoto**, T. Hata, M. Tada, M. Iida, A. Watari, Y. Okada, T. Doi, H. Kuniyasu, K. Yagi, M. Kondoh. Safety evaluation of a human chimeric monoclonal antibody that recognizes the extracellular loop domain of claudin-2. ***Eur. J. Pharm. Sci.*** 117, 161-167 (2018). doi: 10.1016/j.ejps.2018.02.016.
10. **Y. Hashimoto**, K. Shirakura, Y. Okada, H. Takeda, K. Endo, M. Tamura, A. Watari, Y. Sadamura, T. Sawasaki, T. Doi, K. Yagi, M. Kondoh. Claudin-5-binders enhance permeation of solutes across the blood-brain barrier in a mammalian model. ***J. Pharmacol. Exp. Ther.*** 363(2), 275-283 (2017). doi: 10.1124/jpet.117.243014.
11. **Y. Hashimoto**, Y. Kawahigashi, T. Hata, X. Li, A. Watari, M. Tada, A. Ishii-Watabe, Y. Okada, T. Doi, M. Fukasawa, H. Kuniyasu, K. Yagi, M. Kondoh. Efficacy and safety evaluation of claudin-4-targeted antitumor therapy using a human and mouse cross-reactive monoclonal antibody. ***Pharmacol. Res. Perspect.*** 4(5), e00266 (2016). doi: 10.1002/prp2.266.
12. **Y. Hashimoto**, M. Tada, M. Iida, S. Nagase, T. Hata, A. Watari, Y. Okada, T. Doi, M. Fukasawa, K. Yagi, M. Kondoh. Generation and characterization of a human-mouse chimeric antibody against the extracellular domain of claudin-1 for cancer therapy using a mouse model. ***Biochem. Biophys. Res. Commun.*** 477(1), 91-95 (2016). doi: 10.1016/j.bbrc.2016.06.025.
13. N. Tarashima, H. Ando, T. Kojima, N. Kinjo, **Y. Hashimoto**, K. Furukawa, T. Ishida, N. Minakawa. Gene silencing using 4′-thioDNA as an artificial template to synthesize short hairpin RNA without inducing a detectable innate immune response. ***Mol. Ther. Nucleic Acids*** 5, e274 (2016). doi: 10.1038/mtna.2015.48.
14. T. Shimizu, Y. Mima, **Y. Hashimoto**, M. Ukawa, H. Ando, H. Kiwada, T. Ishida. Anti-PEG IgM and

complement system are required for the association of second doses of PEGylated liposomes with splenic marginal zone B cells. ***Immunobiology*** 220(10), 1151-1160 (2015). doi: 10.1016/j.imbio.2015.06.005.

15. Y. Mima, **Y. Hashimoto**, T. Shimizu, H. Kiwada, T. Ishida. Anti-PEG IgM is a major contributor to the accelerated blood clearance of polyethylene glycol-bonjugated Protein. ***Mol. Pharm.*** 12(7), 2429-2435 (2015). doi: 10.1021/acs.molpharmaceut.5b00144.
16. M. Kawanishi, **Y. Hashimoto**, T. Shimizu, I. Sagawa, T. Ishida, H. Kiwada. Comprehensive analysis of PEGylated liposome-associated proteins relating to the accelerated blood clearance phenomenon by combination with shotgun analysis and conventional methods. ***Biotechnol. Appl. Biochem.*** 62(4), 547-555 (2015). doi: 10.1002/bab.1291.
17. **Y. Hashimoto**, T. Shimizu, A.S. Abu Lila, T. Ishida, H. Kiwada. Relationship between the concentration of anti-polyethylene glycol (PEG) immunoglobulin M (IgM) and the intensity of the accelerated blood clearance (ABC) phenomenon against PEGylated liposomes in mice. ***Biol. Pharm. Bull.*** 38(3), 417-424 (2015). doi: 10.1248/bpb.b14-00653.
18. Y. Saito, **Y. Hashimoto**, M. Arai, N. Tarashima, T. Miyazawa, K. Miki, M. Takahashi, K. Furukawa, N. Yamazaki, A. Matsuda, T. Ishida, N. Minakawa. Chemistry, properties, and *in vitro* and *in vivo* aMolecular Therapy-Nucleic Acidspplications of 2′-*O*-methoxyethyl-4′-thioRNA, a novel hybrid type of chemically modified RNA. ***Chembiochem*** 15(17), 2535-2540 (2014). doi: 10.1002/cbic.201402398.
19. **Y. Hashimoto**, A.S. Abu Lila, T. Shimizu, T. Ishida, H. Kiwada. B cell-intrinsic toll-like receptor 7 is responsible for the enhanced anti-PEG IgM production following injection of siRNA-containing PEGylated lipoplex in mice. ***J. Control. Release*** 184, 1-8 (2014). doi: 10.1016/j.jconrel.2014.04.003.
20. **Y. Hashimoto**, Y. Uehara, A.S. Abu Lila, T. Ishida, H. Kiwada. Activation of TLR9 by incorporated pDNA within PEG-coated lipoplex enhances anti-PEG IgM production. ***Gene Ther.*** 21(6), 593-598 (2014). doi: 10.1038/gt.2014.32.
21. **Y. Hashimoto**, T. Shimizu, Y. Mima, A.S. Abu Lila, T. Ishida, H. Kiwada. Generation, characterization and *in vivo* biological activity of two distinct monoclonal anti-PEG IgMs. ***Toxicol. Appl. Pharmacol.*** 277(1), 30-38 (2014). doi: 10.1016/j.taap.2014.03.002.

Review Articles

1. **Y. Hashimoto (co-corresponding author)**, C. Greene, A. Munnich, M. Campbell. The *CLDN5* gene at the blood-brain barrier in health and disease. ***Fluids Barriers CNS*** 20, 22 (2023). doi: 10.1186/s12987-023-00424-5.
2. **Y. Hashimoto (co-corresponding author)**, M. Campbell, K. Tachibana, Y. Okada, M. Kondoh. Claudin-5: a pharmacological target to modify the permeability of the blood-brain barrier. ***Biol. Pharm. Bull.*** 44(10), 1380-1390 (2021). doi: 10.1248/bpb.b21-00408.
3. **Y. Hashimoto**, K. Tachibana, M. Kondoh. Tight junction modulators for drug delivery to the central nervous system. ***Drug Discov. Today*** 25(8), 1477-1486 (2020). doi: 10.1016/j.drudis.2020.05.007.
4. **Y. Hashimoto (co-corresponding author)**, M. Campbell. Tight junction modulation at the blood-brain barrier: current and future perspectives. ***Biochim. Biophys. Acta Biomembr.*** 1862(9), 183298 (2020). doi: 10.1016/j.bbamem.2020.183298.
5. **Y. Hashimoto**, K. Tachibana, S.M. Krug, J. Kunisawa, M. Fromm, M. Kondoh. Potential for tight junction protein-directed drug development using claudin binders and angubindin-1. ***Int. J. Mol. Sci.*** 20(16), 4016 (2019). doi: 10.3390/ijms20164016.
6. **Y. Hashimoto**, Y. Okada, K. Shirakura, K. Tachibana, M. Sawada, K. Yagi, T. Doi, M. Kondoh. Anti-claudin antibodies as a concept for development of claudin-directed drugs. ***J. Pharmacol. Exp. Ther.*** 368(2), 179-186 (2019). doi: 10.1124/jpet.118.252361.
7. **Y. Hashimoto**, K. Yagi, M. Kondoh. Roles of the first-generation claudin binder, *Clostridium perfringens* enterotoxin, in the diagnosis and claudin-targeted treatment of epithelium-derived cancers. ***Pflugers Arch.*** 469(1), 45-53 (2017). doi: 10.1007/s00424-016-1878-6.

8. **Y. Hashimoto**, M. Fukasawa, H. Kuniyasu, K. Yagi, M. Kondoh. Claudin-targeted drug development using anti-claudin monoclonal antibodies to treat hepatitis and cancer. ***Ann. N. Y. Acad. Sci.*** 1397(1), 5-16 (2017). doi: 10.1111/nyas.13337.

9. **Y. Hashimoto**, K. Yagi, M. Kondoh. Current progress in a second-generation claudin binder, anti-claudin antibody, for clinical applications. ***Drug Discov. Today*** 21(10), 1711-1718 (2016). doi: 10.1016/j.drudis.2016.07.004.

MISC

1. **Y. Hashimoto**, M. Campbell. Eyes on glutamate in angiogenesis and barrier formation. ***Neuron*** 112(12), 1895-1897 (2024). doi: 10.1016/j.neuron.2024.05.020.

2. 橋本洋佑. Claudin-5 を創薬標的とする研究. ***Drug Delivery System (DDS 学会機関誌)*** 38(3), 254-255 (2023). doi: 10.2745/dds.38.254.

3. I. Desguerre, M. Aubart, **Y. Hashimoto**, K. Poirier, A. Kaminska, M. Alison, N. Boddaert, A. Munnich, M. Campbell. Reply: *de novo* mutations in *CLDN5*: alternating hemiplegia of childhood or not? ***Brain*** 146(8), E59-E60 (2023). doi: 10.1093/brain/awad054.

4. 橋本洋佑. アイルランドでの研究生活. ***製剤機械技術学会誌*** 30(1), 57-59 (2021).

5. 橋本洋佑，橘敬祐，近藤昌夫. 血液脳関門制御抗体の開発. ***BIO INDUSTRY*** 37(2), 34-44 (2020).

6. 橋本洋佑，近藤昌夫，竹田浩之. タイトジャンクションシール制御技術を利用した中枢神経疾患治療薬のための DDS 開発. ***Drug Delivery System (DDS 学会機関誌)*** 34(5), 374-384 (2019). doi: 10.2745/dds.34.374.

7. 橋本洋佑，八木清仁，近藤昌夫. Claudin の創薬ターゲットとしてのインパクト. ***和光純薬時報*** 83(3), 5-7 (2015).

Awards

2023/10 **A Travel Award** to the RD2023 meeting from The XXth International Symposium on Retinal Degenerations (RD2023) and the BrightFocus Macular Fast Track.

2014/01 **Kohraku award** (good student award) from The University of Tokushima.

2013/11 **Best Presentation Award** from 35th symposium of biomembrane-drug interactions.

2012/05 **A scholarship** from Nagai foundation from Nagai memorial foundation.

Research Grants

1. **Hiroshima University,** an internal competitive research grant for researches focused on infection diseases (2024/09–2025/08: 1,000,000 JPY). A preliminary study to ameliorate long COVID syndrome-associated brain fog by improving the blood-brain barrier integrity.

2. **Irish Research Council,** New Foundations, strand 2 (2020/07–2021/03: 5,000 EUR). Genome-wide screening to discover novel claudin-5 regulating genes for establishing the strategy to normalize the permeability of blood-brain barrier.

3. **JSPS**, KAKENHI for Young Researcher (2019/04–2020/03: 3,900,000 JPY). Visualization of gene expression around the blood-brain barrier by keeping spatial information.

4. **Chubei-Itoh foundation**, Research Grant (2019/04–2020/03: 500,000 JPY). The development of a novel strategy to modulate the blood-brain barrier by anti-claudin-5 antibody.

5. **JSPS**, Research Grant for JSPS Post-doctoral Research Fellow (2017/04–2020/03: 4,420,000 JPY). The development of a novel strategy to modulate the blood-brain barrier permeability by tight-junction binders. 2017/04–2020/03.

6. **JSPS**, Research Grant for JSPS Research Fellow (2015/04–2017/03: 2,300,000 JPY). The development of claudin-5 binders for drug delivery into the brain. 2015/04–2017/03.

Intellectual Property

1. Patent, Application number: JP7108956B2. Anti-CLDN-5 antibody, and drug containing said antibody
2. Patent, Application number: JP6900051B2. Claudin 5 antibody and medicament containing the antibody

Invited Presentations in international conferences

1. *CLDN5* Missense Mutations in Neurological Diseases. Gordon Research Conference on Barriers of the CNS. 2024/06/10.
2. A novel *de novo* missense mutation in *CLDN5* found in patients with alternating hemiplegia of childhood. 5th Mini-symposium on the blood-brain barrier: From basic to clinical research. 2023/03/03.

Presentations in International Conferences

1. **Y. Hashimoto**, N. Hudson, G. Porkoláb, J. O'Callaghan, N. Hanley, C. Greene, M. Kondoh, M. Deli, M. Campbell. Identification of novel negative regulators of blood retina barrier integrity by genome-wide screening; Relevance to novel treatments for age-related macular degeneration (poster presentation). XXth International Symposium on Retinal Degeneration RD2023. 2023/10-23-27.
2. **Y. Hashimoto**. Discovery of a novel pathogenic de novo CLDN5 mutation associated with alternating hemiplegia of childhood (oral presentation). 12th UK and Ireland Early Career BBB Symposium. 2022/10/27.
3. **Y. Hashimoto**, K. Poirier, N. Boddaert, L. Hubert, M. Aubart, I. Desguerre, A. Munnich, M. Campbell. Functional analysis of a novel pathogenic *de novo CLDN5* mutation associated with alternating hemiplegia (poster presentation). 24th International symposium on signal transduction at the blood–brain barriers. 2022/09/21-23.
4. **Y. Hashimoto**, C. Greene, C. Delaney, N. Hudson, M. Campbell. Pumilios-mediated translational control of claudin-5 (poster presentation). Gordon Research Seminar/Conference, Barriers of the CNS. 2022/06/11-17.
5. **Y. Hashimoto**, Y. Kawahigashi, T. Hata, X. Li, K. Yagi, M. Kondoh. Safety profile and anti-tumor activity of a new generated human/mouse cross-reactive anti-claudin-4 monoclonal antibody in mice (poster presentation). European Cancer Congress (ECCO). 2015/09/25-29.