BIOGRAPHICAL SKETCH

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NAME: Hu, Chih-Chi

eRA COMMONS USER NAME (credential, e.g., agency login): chihchihu

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
China Medical University, Taiwan	B.S.	06/97	Public Health
National Sun Yat-Sen University, Taiwan	M.S.	06/99	Cancer Biology
New York University School of Medicine, USA	M.S.	06/04	Pharmacology
New York University School of Medicine, USA	Ph.D.	06/06	Pharmacology
Whitehead Institute for Biomedical Research, USA	Post-doc.	10/10	Immunology

A. Personal Statement

My laboratory investigates the biology of the endoplasmic reticulum (ER) in normal and malignant B cells. In addition to studying the assembly of secretory and integral membrane proteins in the ER, we also investigate the ER stress response elicited by the loss of ER functionally important proteins like IRE-1, XBP-1s, BiP and Derlin-2 in normal and malignant B cells. The work from my laboratory has established that the IRE-1/XBP-1s pathway is an attractive drug target for the therapy of the most common lymphoproliferative disorder, chronic lymphocytic leukemia (CLL). Because we still don't know what exactly activates this pathway in CLL, my laboratory continues to identify and characterize proteins that associate with ER stress sensors. By investigating IRE-1-interacting proteins, we discovered that STING is an interacting protein of IRE-1, and that activating STING using its agonists induces strong and rapid apoptosis of CLL, B cell lymphoma and multiple myeloma in vitro and in vivo. To translate our work into clinical use, we have developed potent IRE-1/XBP-1 pathway inhibitors, such as B-I09, D-F07 and their derivatives, and used them to treat leukemia in mice and human CLL cells. We also demonstrated that targeting XBP-1s to turn on the regulated IRE-1-dependent decay of μS mRNA can lead to the suppression of secretory IgM (sIgM) and the inhibition of immunosuppressive functions of myeloid-derived suppressor cells to allow for reactivation of anti-CLL immunity in mice. In addition to research, I am dedicated to cancer research training and education. Previously, I served as the chair of Admissions Committee to recruit cancer biology Ph.D. students to University of Pennsylvania and sat on a T32 training committee at the Wistar Institute to mentor predoctoral and postdoctoral fellows. I was appointed in 2023 as the Assistant Director for Training and Education at the Houston Methodist Neal Cancer Center (HMNCC) and became the Associate Director in 2024. I implement new cancer-relevant training and coalesce a structured training and mentoring program for trainees working in the field of cancer within the HMNCC. Since 2023, I launched various training and educational programs for pre- and post-doctoral fellows across the research programs to provide access to proctored-seminars, responsible conduct of research (RCR) course, rigor and reproducibility course, outside speakers, and career advancement. I also aim to cultivate the next generation of cancer-focused researchers and clinicians while actively engaging underrepresented individuals in biomedical and cancer research. My strong enthusiasm in cancer research training and education in addition to my ability to advise predoctoral and postdoctoral fellows in experimental design, data analyzes, manuscript writing and oral presentations makes me a qualified trainer in this T32 training grant application.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2024-present Associate Director for Cancer Research Training Education Coordination, Houston Methodist Neal Cancer Center, Houston, TX, USA
2023-2024 Assistant Director for Cancer Research Training Education Coordination, Houston Methodist Neal Cancer Center, Houston, TX, USA
2022-present Professor of Cancer Biology in Medicine, Weill Cornell Medical College, Cornell University, New York, NY, USA
2022-2023 Ad Interim Innovative Therapeutics Program Leader, Houston Methodist Neal Cancer Center, Houston, TX, USA
2021-present Adjunct Professor, Texas A&M University College of Medicine, Houston, TX, USA
2020-present Professor, Houston Methodist Academic Institute, Houston, TX, USA
2020-2021 Professor, Department of Pathology and Laboratory Medicine, The Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA
2020-2021 Adjunct Professor, Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA, USA
2020-2020 Professor, The Wistar Institute, Philadelphia, PA, USA
2015-2019 Associate Professor, Department of Pathology and Laboratory Medicine, The Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA
2014-2019 Associate Professor, The Wistar Institute, Philadelphia, PA, USA
2010-2014 Assistant Member, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA
2006-2010 Postdoctoral Fellow, Whitehead Institute for Biomedical Research, Cambridge, MA, USA
2001-2006 Graduate Student, New York University School of Medicine, New York, NY, USA
1999-2001 Second-Lieutenant Teaching & Research Officer, National Defense Medical Center, Taiwan
1997-1999 Graduate Assistant, Institute of Biological Sciences, National Sun Yat-Sen University, Taiwan
1995-1997 Undergraduate Assistant, Department of Physiology, China Medical University, Taiwan

Honors and Awards

2012 Hollis Brownstein Research Grant, Leukemia Research Foundation
2012 American Cancer Society Institutional Research Grant
2011 Miles for Moffitt Award, Florida Bank
2010 Whitehead Appreciation Award, Whitehead Institute for Biomedical Research
2009 WIPDA Education Award, Whitehead Institute for Biomedical Research
2004 Young Investigator Travel Award, The Society for Basic Urologic Research
1999 Honorary Member of the Phi Tau Phi Scholastic Honor Society
1999 Presidential Travel Award, National Sun Yat-Sen University

Recent Grant Review Service:

1. NIH/NCI Study Section: R21 and R03 Review Meeting, Video-assisted review meeting, March 22, 2023.
2. NIH Study Section: Oncological Sciences Fellowship Study Section (F09B), Video-Based Zoom Meeting, March 18-19, 2021.
3. NIH Study Section: Basic Mechanisms in Immunology Study Section Peer Review Meeting; Special Emphasis Panel/Scientific Review Group 2020/10 ZRG1 IMM-S (99) S, Video-Assisted Meeting, August 6, 2020.
4. NIH Study Section: Special Emphasis Panel/Scientific Review Group 2020/10 ZRG1 IMM-S (02) M, Video-Assisted Meeting, July 23, 2020.
5. NIH/NCI Study Section: BMCT/MCT1 Mechanisms of Cancer Therapeutics - 1, Pentagon City, VA, June 6-7, 2019.
6. NIH/NCI Study Section: 2019/01 BMCT/MCT1 Mechanisms of Cancer Therapeutics - 1, San Diego, CA, October 11-12, 2018.
7. NIH/NCI Study Section: 2018/10 ZCA1 SRB-A (O1) R; SEP-3: NCI Clinical and Translational Exploratory/Developmental Studies, Washington, D.C., June 5-6, 2018.
8. NIH/NCI Study Section: R03 & Clinical and Translational R21, ZCA1 TCRB-V(O1), Washington, D.C., June 28-29, 2017.

C. Contribution to Science

1. In 1999, I was drafted into the Taiwanese army as a second lieutenant. After six months in boot camps, I was reassigned to teach and to do research. I isolated RC-RNase, a ribonuclease from ovaries of the bullfrogs, *Rana catesbeiana*, purchased from local markets. RC-RNase shares ~40% protein homology with Onconase, a ribonuclease from ovaries of *Rana pipiens*. Because Onconase was in clinical trials for cancer therapy, I investigated the cytotoxicity of RC-RNase in cancer cell lines. I found that RC-RNase synergizes with interferon-γ, and it kills cancer in a differentiation-dependent manner. Onconase was not commercially available. To conduct a direct comparison, I obtained Onconase from the Alfacell company in USA, and showed that RC-RNase kills only cancer cells, while Onconase kills both cancer and normal cells.

a. Hu CCA, Lee YH, Tang CHA, Cheng JT and Wang JJ. 2001. Synergistic cytotoxicity of *Rana catesbeiana* ribonuclease and IFN-γ on hepatoma cells. **Biochem. Biophys. Res. Commun.** Feb. 9; 280(5): 1229-36. PMID: 11162659.
b. Hu CCA, Tang CHA, and Wang JJ. 2001. Caspase activation in response to cytotoxic *Rana catesbeiana* ribonuclease in MCF-7 cells. **FEBS Lett.** Aug. 10; 503(1): 65-68. PMID: 11513856.
c. Wei CW, Hu CCA, Tang CHA, Lee MC, and Wang JJ. 2002. Induction of differentiation rescues HL-60 cells from *Rana catesbeiana* ribonuclease-induced cell death. **FEBS Lett.** Nov. 20; 531(3): 421-426. PMID: 12435586.
d. Tang CHA, Hu CCA, Wei CW, and Wang JJ. 2005. Synergism of *Rana catesbeiana* ribonuclease and IFN-γ triggers distinct death machineries in different human cancer cells. **FEBS Lett.** Jan. 3; 579(1): 265-270. (Co-first author) PMID: 15620724.

2. In 2001, I started as a graduate student at NYU to study the assembly of four integral membrane proteins (uroplakin (UP)Ia, UPIb, UPII, and UPIII). UPIa and UPIb are tetraspanin proteins, while UPII and UPIII are single-pass transmembrane proteins. The four uroplakins form two-dimensional (2-D) crystals covering almost the entire bladder apical surface, functioning as one of the best biological permeability barriers in nature to block invasion of toxic substances from the urine. I solved a puzzle that existed in the urothelial biology field for more than a decade. In 1990, the discovery of uroplakin started with the use of a monoclonal antibody called AE31 which stains the urothelium in a differentiation-dependent manner. The AE31 epitope was named 'UPI'; however, its identity remained mysterious as the AE31 antibody did not recognize any cloned uroplakins. I discovered that the AE31 antibody recognizes a composite epitope consisting of two far separated domains in pro-UPII (the precursor of UPII), suggesting that pro-UPII assumes a hairpin-like structure. The prosequence of UPII contains three N-linked glycosylation sites. I showed that all three sites are modified by complex-type glycans in vivo while only two contains complex glycans in cultured cells. Using 2-D blue-native/SDS-PAGE, I showed that uroplakin assembly in cultured urothelial cells is blocked at the heterodimeric stage possibly due to decreased glycosylation of the UPII prosequence, providing an explanation for the lack of 2-D crystals in cultured cells. By mutagenesis experiments, I discovered that the UPII prosequence contains a disulfide bond and showed that disulfide formation and glycosylation of the UPII prosequence are indispensable for uroplakin assembly. Most prosequences of proteins are assumed to be degraded after cleavage; however, the UPII prosequence associates tightly with the mature UPII sequence after furin cleavage, and they traffic together to the apical surface of the bladder. My findings led to the construction of a model of urothelial plaque assembly, in which I illustrated how the four uroplakins interact with one another mechanistically. This model also established tetraspanin proteins as "maturation-facilitators" for their partners. The impact of this model is ongoing. For instance, we recently demonstrated that the failure of CD19 to bind to its tetraspanin partner, CD81, is an acquired resistance mechanism of B-cell acute lymphoblastic leukemia to CD19-directed chimeric antigen receptor T-cell (CART) immunotherapy.

a. Hu CCA, Liang FX, Zhou G, Tu L, Tang CHA, Zhou J, Kreibich G, and Sun TT. 2005. Assembly of urothelial plaques: tetraspanin function in membrane protein trafficking. **Mol. Biol. Cell.** Sep. 1; 16(9): 3937-3950. PMCID: PMC1196309.
b. Hu CCA, Bachmann T, Zhou G, Liang FX, Ghiso J, Kreibich G, and Sun TT. 2008. Assembly of a membrane receptor complex: roles of the uroplakin II prosequence in regulating uroplakin bacterial receptor oligomerization. **Biochem. J.** Sep. 1; 414(2): 195-203. PMCID: PMC4048928.
c. Bagashev A, Sotillo E, Tang CHA, Black KL, Perazzelli J, Seeholzer SH, Argon Y, Barrett DM, Grupp SA, Hu CCA, and Thomas-Tikhonenko A. 2018. CD19 alterations emerging after CD19-directed immunotherapy cause retention of the misfolded protein in the endoplasmic reticulum. **Mol. Cell Biol.** Oct. 15; 38(21): e00383-18. PMCID: PMC6189457.

d. Liao Y, Tham DKL, Liang FX, Chang J, Wei Y, Reddy SP, Sall J, Ren SJ, Chicote JU, Arnold LL, Hu CCA, Romih R, Andrade LR, Rindler MJ, Cohen SM, DeSalle R, Garcia-España A, Ding M, Wu XR, and Sun TT. 2019. Mitochondrial lipid droplet formation as a detoxification mechanism to sequester and degrade excessive urothelial membranes. **Mol. Biol. Cell.** Nov. 15; 30(24): 2969-2984. PMCID: PMC6857570.

3. When I was a post-doctoral fellow, I became fascinated by the functions of the endoplasmic reticulum (ER). I studied the ER functions in B cells because differentiated B cells (or plasma cells) produce a dramatically expanded ER for the production and secretion of antibodies to fight infections. Misfolded antibodies are unavoidable byproducts in the ER upon massive production of antibodies by plasma cells. If not properly destructed by ER-associated degradation (ERAD), they were believed to activate the XBP-1 protein, which was arguably one of the most important players in correcting protein misfolding problems and expanding the size of the ER. Thus, I chose XBP-1 to peek into the biology of the ER. Surprisingly, my initial pursuit of the function of XBP-1 in folding proteins led to production of massive amounts of negative (yet clear) results, suggesting that XBP-1 is not activated by misfolded antibodies and XBP-1 plays minimal role in correcting misfolded proteins in B cells. These conclusions were reached by disabling B cells of their capability in making antibodies and by deleting the XBP-1 gene from the B cells. My investigation established that XBP-1 is activated by differentiation cues, and XBP-1-deficient B cells do not accumulate misfolded proteins based on examinations of over 20 receptors and factors that are folded in the ER. My work further revealed new roles for XBP-1 in regulating B cell receptor (BCR) signaling; in regulating the expression of important transcription factors in B cells; in maintaining proper lipid synthesis and protein glycosylation in B cells; and in colonization of stimulated B cells into the bone marrow for sustention of antibody production. These functions of XBP-1 in B cells are documented in Janeway's Immunobiology textbook, and my work was cited. On a relevant note, we recently discovered that the ER-resident STING protein can engage ERAD to regulate the BCR and BCR signaling.

a. Hu CCA, Dougan SK, McGehee AM, Love JC, and Ploegh HL. 2009. XBP-1 regulates signal transduction, transcription factors and bone marrow colonization in B cells. **EMBO J.** Jun. 3; 28(11): 1624-1636. PMCID: PMC2684024.
b. McGehee AM, Dougan SK, Klemm EJ, Shui G, Park B, Kim YM, Watson N, Wenk MR, Ploegh HL, and Hu CCA. 2009. XBP-1-deficient plasmablasts show normal protein folding but altered glycosylation and lipid synthesis. **J. Immunol.** Sep. 15; 183(6): 3690-3699. PMCID: PMC4053221.
c. Dougan SK, Hu CCA, Paquet ME, Greenblatt MB, Kim J, Lilley BN, Watson N, and Ploegh HL. 2011. Derlin-2 deficient mouse reveals an essential role for protein dislocation in chondrocytes. **Mol. Cell Biol.** March; 31(6): 1145-1159. PMCID: PMC3067910.
d. Tang CHA, Lee AC, Chang S, Xu Q, Shao A, Lo Y, Spalek WT, Pinilla-Ibarz JA, Del Valle JR, and Hu CCA. 2021. STING regulates BCR signaling in normal and malignant B cells. **Cell. Mol. Immunol.** Apr.; 18(4): 1016-1031. PMCID: PMC8115116.

4. Subtilase cytotoxin produced by a deadly bacterium can cause fatal 'hamburger' disease among meat lovers. I became interested in this toxin because it specifically cleaves and inactivates an ER protein called BiP. My study showed that this toxin specifically cleaves newly synthesized BiP in the ER while leaving 'aged' BiP uncleaved. These data suggested that the 'supposedly homogeneous' ER may in fact contain subcompartments since the toxin can only access newly synthesized BiP at the biosynthetic sites. This study also revealed a novel strategy by which this deadly bacterium evades attack from our immune system by hijacking antibody light chains in the ER to block antibody secretion from B cells. My laboratory recently discovered that this toxin can trigger phosphorylation at serine 729 (S729) of IRE-1 to activate regulated IRE-1-dependent decay (RIDD) of the mRNA of secretory μ (μS) heavy chains, highlighting yet another mechanism used by this bacterium to dismantle the capability of B cells to produce antibodies. We also showed that XBP-1s deficiency can similarly induce phosphorylation at S729 of IRE-1 and turn on RIDD in B cells. Interestingly, targeting XBP-1s to trigger RIDD of μS mRNA in B cells can lead to the suppression of secretory IgM (sIgM) and the inhibition of immunosuppressive functions of myeloid-derived suppressor cells to allow for reactivation of anti-CLL immunity in mice. Furthermore, our results also showed that RIDD is an important mediator for reducing chronic graft-versus-host disease (cGVHD) pathogenesis through targeting XBP-1s in B cells.

a. Hu CCA, Dougan SK, Virreira-Winter S, Paton AW, Paton, JC, and Ploegh HL. 2009. Subtilase cytotoxin cleaves newly synthesized BiP and blocks antibody secretion in B lymphocytes. **J. Exp. Med.** Oct. 26; 206(11): 2429-2440. PMCID: PMC2768844.

b. Tang CHA, Chang S, Paton AW, Paton JC, Gabrilovich DI, Ploegh HL, Del Valle JR, and Hu CCA. 2018. Phosphorylation of IRE1 at S729 regulates RIDD in B cells and antibody production after immunization. **J. Cell Biol.** May. 7; 217(5): 1739-1755. PMCID: PMC5940306.
c. Tang CHA, Chang S, Hashimoto A, Chen YJ, Kang CW, Mato AR, Del Valle JR, Gabrilovich DI, and Hu CCA. 2018. Secretory IgM exacerbates tumor progression by inducing accumulations of MDSCs in mice. **Cancer Immunol. Res.** Jun.; 6(6): 696-710. PMCID: PMC5984713.
d. Choi HJ, Tang CHA, Tian L, Wu YX, M. Sofi MH, Ticer T, Schutt SD, Hu CCA, and Yu XZ. 2021. XBP-1s promotes B cell pathogenicity in chronic GVHD by restraining the activity of regulated IRE-1α-dependent decay. **Front. Immunol.** Oct. 1; 12: 705484. PMCID: PMC8517405. (**Co-corresponding author**)

5. Inspired by the role of XBP-1 in regulating BCR signaling, my laboratory started to examine the role of XBP-1 in CLL, the survival of which requires BCR signaling. We chose to use the Eμ-TCL1 mouse model to study CLL because ~90% of human CLL patient samples express the TCL1 protein, and TCL1 overexpression in B cells causes CLL in mice without fail. We showed that the TCL1 oncoprotein associates with XBP-1 and turns on vital ER proteins to support CLL growth. For the first time, an oncoprotein was demonstrated to directly regulate the ER function. We also sought collaborations to develop inhibitors to target the expression of XBP-1 by inhibiting its activator, IRE-1. Our work established that genetic deletion or pharmacological inhibition of XBP-1 can stall malignant progression of CLL in mice. Our inhibitors also induced apoptosis in primary human leukemic cells. Our inhibitors, when combined with ibrutinib, exert a strong synergistic cytotoxicity against leukemia, lymphoma and multiple myeloma. Our finding in synergism is important because our inhibitors can be used to help ibrutinib to achieve higher cytotoxicity in CLL cells at a lower dose, addressing ibrutinib's toxicity issue. We recently developed a novel potent IRE-1 inhibitor, in which we masked the functional aldehyde group to allow for strong emission of blue fluorescence, useful for tracking the inhibitor inside the cell and in vivo. Importantly, we discovered that the modification of the hydroxy group adjacent to the aldehyde can stabilize the 'cap' used to mask the aldehyde, allowing precise control of the inhibitory activity. We installed a photocage onto the hydroxy group, and showed that the inactive photocaged inhibitor can be converted by UV irradiation to become an active inhibitor. We had also taken advantage of this novel chemical feature to develop tumor-specific inhibitors. Importantly, we share with other investigators all mouse models, cell lines, chemical tools and immunological reagents that we have developed to facilitate their important and exciting research.
a. Kriss CL, Pinilla-Ibarz JA, Mailloux AW, Powers JJ, Tang CHA, Kang CW, Zanesi N, Epling-Burnette PK, Sotomayor EM, Croce CM, Del Valle JR, and Hu CCA. 2012. Overexpression of TCL1 activates the endoplasmic reticulum stress response: a novel mechanism of leukemic progression in mice. **Blood.** Aug. 2; 120(5): 1027-1038. PMCID: PMC3680046.
b. Tang CHA, Ranatunga S, Kriss CL, Cubitt CL, Tao J, Pinilla-Ibarz JA, Del Valle JR, and Hu CCA. 2014. Inhibition of ER stress-associated IRE-1/XBP-1 pathway reduces leukemic cell survival. **J. Clin. Invest.** Jun. 2; 124(6): 2585-2598. PMCID: PMC4038575.
c. Shao A, Kang CW, Tang CHA, Cain C, Xu Q, Phoumyvong C, Del Valle JR, and Hu CCA. 2019. Structural tailoring of a novel fluorescent IRE-1 RNase inhibitor to precisely control its activity. **J. Med. Chem.** Jun. 13; 62(11): 5404-5413. PMCID: PMC6628725.
d. Shao A, Xu Q, Spalek WT, Cain CF, Kang CW, Tang CHA, Del Valle JR, and Hu CCA. 2020. Development of tumor-specific IRE-1 inhibitors for B cell cancer therapy. **Mol. Cancer Ther.** Dec.; 19(12): 2432-2444. PMCID: PMC7773214.

Complete List of Published Work in MyBibliography: http://www.ncbi.nlm.nih.gov/sites/myncbi/chih-chi.hu.1/bibliography/45908280/public/?sort=date&direction=ascending