BIOGRAPHICAL SKETCH

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NAME: Kashiwagi, Satoshi

eRA COMMONS USER NAME (credential, e.g., agency login): skashiwagi1

POSITION TITLE: Assistant Professor of Medicine, Harvard Medical School; Assistant Immunologist, Massachusetts General Hospital

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
Keio University School of Medicine, Tokyo, Japan	M.D.	03/1997	Medicine
Keio University Hospital, Tokyo, Japan	Residency	05/1998	Obstetrics and Gynecology
Saiseikai Kanagawa-Ken Hospital, Yokohama, Japan	Residency	05/1999	Obstetrics and Gynecology
Otawara Red-Cross Hospital, Tochigi-Prefecture, Japan	Residency	05/2000	Obstetrics and Gynecology
Tokyo Dental Collage Ichikawa General Hospital, Chiba-Prefecture, Japan	Clinical Fellowship	05/2001	Obstetrics and Gynecology
Keio University Graduate School of Medicine, Tokyo, Japan	Ph.D.	03/2004	Biochemistry /Obstetrics and Gynecology
Massachusetts General Hospital, Boston	Postdoctoral	12/2006	Radiation Oncology
Massachusetts General Hospital, Boston	Postdoctoral	12/2008	Cardiovascular Medicine
Massachusetts General Hospital, Boston	Postdoctoral	05/2009	Immunology

A. Personal Statement

The goal of the proposed research is to establishing a small laser prototype device as vaccine adjuvant for clinical use, which was supported by STTR Phase I from NIAID/NIH. A central focus of my laboratory at Massachusetts General Hospital (MGH) has been to develop a new class of energy-based, physical immunological adjuvants. I have the expertise and research experience to accomplish the work at MGH described in this proposal. I have a broad background in the field of reactive oxygen species (ROS)/ nitric oxide (NO) pathophysiology and have more recently developed original ideas around laser-tissue interactions, which results in ROS and/or NO mediated signaling. My PhD thesis in Japan and postdoctoral fellowships engaged in basic science elucidating the role of ROS/NO in immune cell trafficking and tumor angiogenesis where I gained a wide array of expertise in laser technology and bioimaging, including fluorescence recovery after photobleaching technology (FRAP) using visible lasers (Kashiwagi S, *Am J Physiol*, 1997), intravital confocal laser microscopy (Kashiwagi S, *Circ Res*, 2002), and intravital multiphoton laser microscopy (Kashiwagi S, *J Clin Invest*, 2005; Kashiwagi S, *Nat Med*, 2008). Having had a strong interest in molecular and cellular mechanisms which induce immune activity through the activation of specific molecular pathways including NO and/or ROS mediated signaling, I took a faculty position in the Vaccine and Immunotherapy Center within the Division of Infectious Disease Medicine at MGH, and embarked on novel research projects of the development of a laser-based vaccine adjuvant. I have been supported by Bill & Melinda Gates Foundation and MGH internal grants towards the development of a new immunological adjuvant. I am now funded through NIH R01 and R41 grants to further explicate the function and mechanisms of near-infrared lasers in stimulating vaccine immune responses and develop a small and economical laser device to adjuvant vaccines that could be used in the clinic. We have made significant progress in this research fields to date, pioneering a new

concept that light exposure of the skin can induce specific immune stimulatory responses (Kashiwagi S, *PLoS One*, 2013). Based on our findings that a new kind of adjuvant that consists of laser light rather than chemicals can increase vaccine efficacy, we have developed a portable laser device that is able to deliver such laser light to augment immune response. In summary, with my broad background in the fields of reactive oxygen species, immunology, bioimaging, and laser medicine, I have demonstrated an ability to create a synergy of interdisciplinary collaboration between laser technology and vaccinology, which is essential for success in the proposed research.

B. Positions and Honors

Positions and Employment

2009-2015 Instructor in Medicine and Assistant in Immunology, Vaccine and Immunotherapy Center, Infectious Disease Division, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA

2015-pres Assistant Professor of Medicine and Assistant Immunologist, Vaccine and Immunotherapy Center, Infectious Disease Division, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA

Other Experience and Professional Memberships

2009-pres Regular Member, American Association for Immunologists
2009-pres Active Member, American Association for Cancer Research
2012-pres Associate Editor, American Journal of Cancer Biology

Ad hoc Reviewer (in the past 3 years): Molecular Cancer Research, Tumor Biology, Marine Drugs, Developmental Biology, American Journal of Cancer Biology, International Journal of Molecular Science, Journal of Molecular Medicine, Acta Biochimica et Biophysica Sinica.

Honors

1995 Noda Award, Keio University School of Medicine Students' Research Program
2001 Investigator Award, Japanese Foundation of Cardiovascular Research
2006 Excellence Award, 21st Century COE program of Japan Society for the Promotion of Science

Patents

1. International Application Number: PCT/US2008/002004 (8/21/2008), entitled: Modulation of Nitric Oxide Signaling to Normalize Tumor Vasculature.
2. International Application Number: PCT/US2012/053282 (9/1/2012), entitled: Laser Adjuvants for Enhancing Immune Response.

C. Contribution to Science

1. My early work determined the underlying molecular mechanisms of how reactive oxygen species (ROS), carbon monoxide (CO) and nitric oxide (NO) regulate vascular tone and immune cell trafficking. We developed a novel platform for NO bioimaging and micro-angiography using rodent models. We also provided critical pieces of information on the regulation of blood pressure and the interaction between blood vessels and immune cells by showing the distinct mechanisms of the regulation of blood pressure by ROS, CO and NO. We pioneered a new concept that physiological roles of these gaseous molecules are governed by spatial and temporal distribution of their sources in tissue. I served as the primary investigator or co-investigator in all of these studies.
    a. Suematsu M, **Kashiwagi S**, Sano T, Goda N, Shinoda Y, Ishimura Y. Carbon monoxide as an endogenous modulator of hepatic vascular perfusion. *Biochem Biophys Res Commun* 1994; 205(2): 1333-1337. PMID: 7802666.
    b. **Kashiwagi S**, Kajimura M, Yoshimura Y, Suematsu M. (2002). Nonendothelial source of nitric oxide in arterioles but not in venules: Alternative source revealed in vivo by diaminofluorescein microfluorography. *Circ Res*, 91: e55-e64. PMID: 12480826.

c. Kudo A, **Kashiwagi S**, Kajimura M, Yoshimura Y, Uchida K, Arii S, Suematsu M. (2004). Kupffer cells alter organic anion transport through multidrug resistance protein 2 in the post-cold ischemic rat liver. *Hepatology*, 34(4): 1099-1109. PMID: 15057914.

2. Although it was understood that NO is ubiquitously expressed in malignant tumors and that it promoted tumor progression, the role of NO in intratumoral blood vessel formation was poorly understood. We found that NO facilitates the remodeling of blood vessels in solid tumors using genetic mouse models and with the use of a novel NO imaging technique, multiphoton microscopy, we demonstrated spatial and temporal distribution of NO. In addition, we demonstrated that selective modulation of NO around tumor vessels restores oxygen delivery by blood vessels and enhances sensitivity of tumor to radiation therapy because the efficacy of radiotherapy is highly dependent on local oxygen tension in solid tumor. This discovery aided the development of new strategies to remodel tumor vessels by modulating NO for anti-cancer therapy. I served as the primary investigator in these studies. We also published a thorough review on related studies in this field in 2006, which has been cited more than 500 times by peers.
    a. **Kashiwagi S**, Izumi Y, Gohongi T, Demou ZN, Xu L, Huang PL, Buerk DG, Munn LL, Jain RK, Fukumura D. (2005). NO mediates mural cell recruitment and vessel morphogenesis in murine melanomas and tissue-engineered blood vessels. *J Clin Invest***,** 115(7): 1816–1827. PMID: 15951843; PMCID: PMC1143589.
    b. **Kashiwagi S**, Tsukada K, Xu L, Miyazaki J, Kozin SV, Tyrrell JA, Sessa WC, Gerweck LE, Jain RK, Fukumura D. (2008). Perivascular nitric oxide gradients normalize tumor vasculature. *Nat Med***,** 14(3): 255-257. PMID: 18278052.
    c. Fukumura D, **Kashiwagi S**, Jain RK. (2006). Role of nitric oxide in tumour progression. *Nat Rev Cancer*, 6(7): 521-534. PMID: 16794635.

3. Metabolic syndrome and insulin resistance is the single leading cause of heart disease. We found that NO production through phosphorylation of endothelial nitric oxide synthese (eNOS) was central for the regulation of insulin sensitivity in muscle and adipose tissue using unique genetic tools, and that modulation of eNOS activity could correct insulin resistance in metabolic diseases. This is the first demonstration that NO can be used for treatment of metabolic syndrome in the clinic. In view of the large number of patients affected, developing a better method in the prevention of metabolic syndrome and ischemic heart disease will have a material impact on the lives of many patients. I served as the primary investigator or co-investigator in these studies.
    a. **Kashiwagi S**, Atochin DN, Li Q, Schleicher M, Pong T, Sessa WC, Huang PL. (2013). eNOS phosphorylation on serine 1176 affects insulin sensitivity and adiposity. *Biochem Biophys Res Commun*, 431(2): 284-290. PMID: 23291238; PMCID: PMC3576142.
    b. Li Q, Atochin D, **Kashiwagi S**, Earle J, Wang A, Mandeville E, Hayakawa K, d'Uscio L, Lo E, Katusic Z, Sessa W, Huang P. (2013). Deficient eNOS phosphorylation is a mechanism for diabetic vascular dysfunction contributing to increased stroke size. *Stroke*, 44(11): 3183-3188. PMID: 23988642; PMCID: PMC3864831.

4. Solid tumor is known to effectively evade host immune attack. We demonstrated that a chemokine, CXCL12, plays an important role in evasion mechanisms and AMD3100, a specific inhibitor for CXCR4 (a receptor of CXCL12), significantly reduced tumor progression in a murine model of ovarian cancer. These preclinical data now form the basis of a new clinical trial in which the application of AMD3100 to the treatment of ovarian cancer will be studied in collaboration at MGH. I served as a co-first and corresponding author in this work.
    a. Righi E, **Kashiwagi S****, Yuan J, Santosuosso M, Leblanc P, Ingraham R, Forbes B, Edelblute B, Collette B, Xing D, Kowalski M, Mingari MC, Vianello F, Birrer M, Orsulic S, Dranoff G, and Poznansky MC*****. (2011). CXCL12/CXCR4 blockade induces multimodal anti-tumor effects that prolong survival in an immunocompetent mouse model of ovarian cancer. *Cancer Res*, 71(16): 5522-5534. *******equally contributed*. PMID: 21742774; PMCID: PMC3959864.

5. Although vaccines are the most effective and economical medical interventions for personal and public health, modern vaccines are generally inefficient and often require an addition of a vaccine adjuvant to achieve significance. Unfortunately, many current or candidate adjuvants have been known to induce significant side effects, resulting in very few vaccine adjuvants being approved by regulatory agencies to

date. We demonstrated that short treatment of the small vaccine skin site with near-infrared laser selectively activates specific signaling pathway in skin and skin-resident dendritic cells (antigen presenting cells), significantly enhancing protective immune responses to skin vaccination without tissue damage or inflammation in a clinically relevant mouse model of influenza. In addition, we showed that equivalent laser doses used in mice is safe and tolerable in humans in a clinical study. This basic research gathered many attentions because of its potential immediate and profound impact on the current vaccine practice. The laser adjuvant could entirely replace conventional chemical vaccine adjuvant without inducing side effects. Lasers have been already employed for various medical applications for decades and their safety is well established. **In this proposal, we will accelerate translation of these basic studies to clinical application.** We also published the first-of-its-kind thorough review on this technology in 2014. I served as the primary investigator and corresponding author in these work.

a. **Kashiwagi S**, Yuan J, Forbes B, Hibert ML, Lee ELQ, Whicher L, Goudie C, Yang Y, Chen T, Edelblute B, Collette B, Edington L, Trussler J, Nezivar J, Leblanc P, Bronson R, Tsukada K, Suematsu M, Dover J, Brauns T, Gelfand J, Poznansky MC. (2013) Near-infrared laser adjuvant for influenza vaccine. *PLoS One*, 8(12):e82899. PMID: 24349390; PMCID: PMC3859633.
b. **Kashiwagi S**, Brauns T, Gelfand J, Poznansky MC. (2014). Laser vaccine adjuvants: History, progress, and potential. *Hum Vaccin Immunother*, 10(7):1892-907. PMID: 25424797; PMCID: PMC4186024.

Complete List of Published Work in My Bibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/satoshi.kashiwagi.1/bibliography/44103563/public/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

5 R01 AI105131-02 Kashiwagi (PI) 09/01/13 – 08/31/17
NIH/NIAID
Near-infrared laser to replace chemical vaccine adjuvant
This proposal explores the cellular and molecular mechanisms of a non-tissue damaging continuous wave near-infrared laser to enhance adaptive immune responses, and develops a safe and effective immunologic adjuvant for skin and mucosal vaccination.
Role: PI

Completed Research Support

1 R41 AI114012-01 Callahan (PI) and subcontracted Kashiwagi (PI) 07/01/14 - 06/30/15
NIH/NIAID
Development of small near-infrared laser system capable of improving immune responses to vaccines (laser-based adjuvant).
This proposal involves testing the adjuvant function of a prototype handheld NIR laser. Our goal is to develop a prototype of a handheld device that could be commercially produced for less than $1,000 per unit.
Role: subcontract PI at MGH

Kashiwagi (PI) 11/01/11-04/31/13
Grand Challenges Explorations Round7/Bill & Melinda Gates Foundation
Dermal laser for dose sparing of pediatric polio vaccine
The major goal of this study is to test whether pre-treating the skin at the site of vaccination with laser light to stimulate antigen-presenting cells will result in a stronger immune response to the polio vaccination. The laser-based technology could reduce the number of vaccinations required to protect children from polio.
Role: PI

Kashiwagi (PI) 04/01/13-08/31/13
MGH ECOR Formulaic Bridge Funding (internal)
Near-infrared laser to replace chemical vaccine adjuvant

This proposal explores the cellular and molecular mechanisms of a non-tissue damaging continuous wave near-infrared laser to enhance adaptive immune responses, and develops a safe and effective immunologic adjuvant for skin and mucosal vaccination.
Role: PI

Poznansky, Kashiwagi (PI) 01/01/12-12/31/13
Disposition of the Federal Share of Proton Program Income (internal)
CXCR4/CXCL12 as biomarkers for ovarian cancer explored through CXCR4 blockade
The major goal of this study is to explore whether CXCL12 and/or CXCR4 can serve as biomarkers for tumor progression and response to treatment in this disease using an established mouse model of ovarian cancer and an extensive human tissue bank from ovarian cancer patients.
Role: Co-PI