MUSC
HOLLINGS CANCER CENTER
A National Cancer Institute Designated Cancer Center

T Cell Signaling and Immuno-Metabolism Lab

Principle Investigator: Shikhar Mehrotra, Ph.D.

Departmental Affiliation: Surgery (primary), Microbiology & Immunology (secondary)

The goal of our lab is to develop strategies for improving cancer immunotherapy and autoimmune vitiligo.
Our primary focus is to ***determine the immunometabolic factors that modulate TCR receptor signaling and alter T cell function or survival in tumor bearing host and autoimmune prone model***.

Models: Several knock-out, overexpressing and TCR transgenic mouse models, along with engineered human T cells expressing tumor epitope specific T Cell Receptor (TCR) are used for *in vitro* and *in vivo* studies. Models for spontaneous autoimmune vitiligo and adoptive T cell immunotherapy studies are available and used routinely in the lab.

Lab Focus 1: Understanding T cell Death Pathways to Increase Persistence of Anti-Tumor T cells

Caspase-independent death pathway in effector T cells. Our studies showed that, upon repetitive TCR stimulation by cognate antigen effector, T cells underwent cell death that was not dependent upon capases. Rather, it involved activation of the JNK pathway and reactive oxygen species (ROS). Using anti-oxidants resulted in less accumulation of ROS, reduced p-JNK and rescued T cells from cell death. We believe that this strategy has translational implications in adoptive T cell immunotherapy (ACT), where activated T cell is chronically activated by the tumor antigen. Strategies to rescue T cell death and increase persistence will be key for successfully using ACT.

Selected publications:
1.Kesarwani P, Murali AK, Al-Khami AA, **Mehrotra S**. Redox regulation of T-cell function: from molecular mechanisms to significance in human health and disease. *Antioxid Redox Signal.* 2013; 18(12):1497-534. PMCID: PMC3603502.
2.Norell H, Martins da Palma T, Lesher A, Kaur N, Mehrotra M, Naga OS, Spivey N, Olafimihan S, Chakraborty NG, Voelkel-Johnson C, Nishimura MI, Mukherji B, **Mehrotra S.** Inhibition of superoxide generation upon T-cell receptor engagement rescues Mart-1(27-35)-reactive T cells from activation-induced cell death. *Cancer Res.* 2009; 69(15):6282-9. PMCID: PMC2719828.
3.Mehrotra S, Chhabra A, Chattopadhyay S, Dorsky DI, Chakraborty NG, Mukherji B. Rescuing melanoma epitope-specific cytolytic T lymphocytes from activation-induced cell death, by SP600125, an inhibitor of JNK: implications in cancer immunotherapy. *J Immunol*. 2004; 173(10):6017-24.
4.US Patent: Methods for improving immunotherapy by enhancing survival of antigen-specific cytotoxic T lymphocytes. US patent publication # US 20070003531 A1 (Jan 4th 2007).

Trainees from Mehrotra Lab

S. No.	Name	Year(s) at MUSC	Current information
	Post-doctoral fellow's		
1.	Navtej Kaur. Ph.D.	2008-2011	Current info unavailable
2.	Amir Al-khami, Ph.D	2010-2011	Principal Scientist Cancer Immunology and Immunotherapy *Pfizer* San Francisco, CA
3.	Pravin Kesarwani, Ph.D.	2010-2015	Scientist Immunotherapy Research, Beaumont Health Department of Radiation Oncology, Michigan
4.	Anuradha Murali, Ph.D.	2011-2013	Manager of Clinical Trials Gastroenterology Associates Orangeburg, South Carolina
5.	Quan Fang, M.D. Ph.D.	2011-2012	General Surgery Practice in Charleston
6.	Shilpak Chatterjee, Ph.D.	2011-2018	Senior Scientist Cancer Biology & Inflammatory Disorder *CSIR-Indian Institute of Chemical Biology* Kolkata - 700 032 (India)
7.	Krishnamurthy Thyagarajan, Ph.D.	2011-2015	Senior Development Scientist *Beckman Coulter* Bengaluru, Karnataka, India
8.	Bharathi Viswanathan, Ph.D.	2012-2013	Patent Attorney *De Penning and De Penning* Bengaluru, Karnataka, India
9.	Anirban Banerjee, Ph.D.	2013-2014	Research Scientist Stony Brook University, NY
10.	Paramita Chakraborty, Ph.D.	2015-present	
	Under-graduate student		
1.	Mazen Al-Hommrani	2014-2016	Awarded the best Undergraduate Award in 2016 Went back to Saudi Arabia
	Research Specialist's		
1.	Natali D. Spivey	2007-2010	Current info unavailable
2.	Osama S. Naga	2008-2010	Moved to Indiana University Dental School Currently Practices as a General Dentist with the Willamette Dental Group, Corvallis, OR
3.	Ya Ying Zheng	2011-2013	Ph.D. Student, University of Albany, NY
4.	Myra Soloshchenko	2012-2015	Ph.D. Student, MUSC
5.	Christine Marking	2013-2013	Federal ϕb
6.	Kyle Toth	2015-2016	MD student at MUSC
7.	Mahvash Husain	2016-2017	MD student at MUSC
8.	George Washington	2017-2018	Retired after 30 years of service at MUSC
9.	Zachariah Hedley	2018-present	

Lab Focus 2: Deciphering role on anti-oxidants in shaping anti-tumor T cell phenotype and function

Role of cellular anti-oxidant system in anti-tumor effector and memory T cell phenotype. We recently established a direct correlation between long-lived central memory (Tcm) cells and anti-oxidant capacity. Tumor reactive T cells with CD62Lhi Tcm-like phenotype exhibit higher levels of anti-oxidant cell surface thiols (c-SH), intracellular glutathione (iGSH), anti-oxidant enzymes catalase, superoxide dismutase, thioredoxin and Nrf2. CD62Lhi Tcm-like T cells with c-SH-hi phenotype show distinct metabolic commitment with less glucose uptake and express lower level of glycolytic enzymes. Further, we confirmed that c-SH expression can be used as a biomarker for long-lived anti-tumor T cells, because tumor reactive T cells sorted on the basis of c-SH expression and adoptively transferred to treat murine melanoma B16-F10 established subcutaneously in immunocompetent C57BL/6 mice showed that c-SH-hi T cells persisted longer and controlled tumor long-term as compared to c-SH-lo T cells. We believe that understanding the role of thiol/thioredoxin in TCR signaling is important to dissect the unique molecular imprint that results in persistence of T cells in oxidative tumor microenvironment.

Selected publications:
1.Kesarwani P, Thyagarajan K, Chatterjee S, Palanisamy V, **Mehrotra S**. Anti-oxidant capacity and anti-tumor T cell function: A direct correlation. *Oncoimmunology.* 2015; 4(1):e985942. PMCID: PMC4368125.
2.Kesarwani P, Al-Khami AA, Scurti G, Thyagarajan K, Kaur N, Husain S, Fang Q, Naga OS, Simms P, Beeson G, Voelkel-Johnson C, Garrett-Mayer E, Beeson CC, Nishimura MI, **Mehrotra S**. Promoting thiol expression increases the durability of antitumor T-cell functions. *Cancer Res.* 2014; 74(21):6036-47. PMCID: PMC4216764.
3.Kaur N, Naga OS, Norell H, Al-Khami AA, Scheffel MJ, Chakraborty NG, Voelkel-Johnson C, Mukherji B, **Mehrotra S**. T cells expanded in presence of IL-15 exhibit increased antioxidant capacity and innate effector molecules. *Cytokine*. 2011; 55(2):307-17. PMCID: PMC3595556.
4.US patent: Process to Generate Superior Anti-Tumor Memory Cells. Provisional application filed 05/15/2015 for MUSC-FRD Technology ID: P1569. PCT filed 04/28/2017.

Lab focus 3: Targeting metabolites and metabolic pathways to modulate T cell survival and function

Targeting immune-metabolic axis to overcome tumor induced immunosuppression and improve immunotherapeutic potential of anti-tumor T cells. We also have shown that *ex vivo* programming of Th17 cells in the presence of conventionally used TGFbeta could result in upregulation of ectonucelotidase CD39 and CD73 that results in accumulation of adenosine and immunosuppression. However, using IL1b to program Th17 cells *ex vivo* could overcome immunosuppression and could prove beneficial for long-term tumor control. Thus, we are further developing strategies to identify the best *ex vivo* programming conditions that bring together the best effector phenotype with reduced susceptibility to immunosuppression by targeting ectonucleotidase expression in T cell and tumor microenvironment. Since ectonuclotidase expression controls the availability of ATP and thereby modulate mitochondrial metabolism, we have initiated studies that link energy metabolism, suppression and T cell metabolism in cancer or autoimmunity.

Selected publications:
1.Chatterjee S, Thyagarajan K, Kesarwani P, Song JH, Soloshchenko M, Fu J, Bailey SR, Vasu C, Kraft AS, Paulos CM, Yu XZ, **Mehrotra S**. Reducing CD73 expression by IL1β-Programmed Th17 cells improves immunotherapeutic control of tumors. *Cancer Res.* 2014; 74(21):6048-59. PMCID: PMC4216762.
2.Banerjee A, Thyagarajan K, Chatterjee S, Chakraborty P, Kesarwani P, Soloshchenko M, Al-Hommrani M, Andrijauskaite K, Moxley K, Janakiraman H, Scheffel MJ, Helke K, Armenson K, Palanisamy V, Rubinstein MP, Mayer EG, Cole DJ, Paulos CM, Voelkel-Johnson C, Nishimura MI, ***Mehrotra S***. Lack of p53 augments anti-tumor functions in cytolytic T cells. *Cancer Res.* 2016;76:5229-40. PMID: 27466285
3.Chatterjee S, Thyagarajan K, Kesarwani P, Song JH, Soloshchenko M, Fu J, Bailey SR, Vasu C, Kraft AS, Chatterjee S, Daenthanasanmak A, Chakraborty P, Meek M, Dhar P, Paneerselvam S, Nygen H, Toth K, Al-Homrani M, Zhang J, Mehrotra M, Ball L, Beeson G, Husain S, Garrett-Mayer E, Hardiman G, Nishimura MI, Beeson CC, Gubbels-Bupp M, Wu J, Ogretmen B, Paulos CM, Rathmell J, Yu XZ, ***Mehrotra S***. CD38-NAD$^+$ Axis Regulates Potent Immunotherapeutic Anti-Tumor T cell Response. *Cell Metabolism,* 2018;27:85-100. PMID: 29129787
4.US patent: CD38-mediated Metabolic Axis in Anti-Tumor Immunotherapy. Provsional application filed 11/09/2016 for MUSC-FRD Technology ID: P1716. PCT filed 11/09/2017.

Lab focus 4: Immunometabolic modulation of T cell subsets in autoimmune Vitiligo

Novel transgenic mice with human tyrosinase TCR. In order to readily obtain the T cells with human TCR for *in vivo* tumor studies, we generated a novel transgenic mice using the human tyrosinase reactive TCR isolated from an HLA-A2+ patient with metastatic melanoma. We named this mouse model "h3T" and extensively used CD8+ or CD4+ T cells from these mice (both bear class I restricted tyrosinase TCR) for *in vitro* and *in vivo* tumor studies. The h3T model was further developed on an HLA-A2 background and named h3T-A2. The h3T-A2 mice develop spontaneous vitiligo within 6 weeks. We have shown that quantitatively increasing the regulatory T cells (either by adoptive transfer or rapamycin treatment) halts vitiligo progression. These two novel TCR transgenic strains (h3T and h3T-A2) provide a unique resource for addressing questions relevant to tumor immunity and auto-immunity.

Selected publications
1.Eby JM, Kang HK, Klarquist J, Chatterjee S, Mosenson JA, Nishimura MI, Garrett-Mayer E, Longley BJ, Engelhard VH, **Mehrotra S**, Le Poole IC. Immune responses in a mouse model of vitiligo with spontaneous epidermal de- and repigmentation. *Pigment Cell Melanoma Res.* 2014; 27(6):1075-85. PMCID: PMC4470702.
2.Chatterjee S, Eby JM, Al-Khami AA, Soloshchenko M, Kang HK, Kaur N, Naga OS, Murali A, Nishimura MI, Le Poole IC, **Mehrotra S**. A quantitative increase in regulatory T cells controls development of vitiligo. *J Invest Dermatol.* 2014; 134(5):1285-94. PMCID: PMC3989443.
3.Husain S, Abdul Y, Webster C, Chatterjee S, Kesarwani P, **Mehrotra S**. Interferon-gamma (IFN-γ)-mediated retinal ganglion cell death in human tyrosinase T cell receptor transgenic mouse. *PLoS One*. 2014;9(2):e89392. PMCID: PMC3938457.
4.Mehrotra S, Al-Khami AA, Klarquist J, Husain S, Naga O, Eby JM, Murali AK, Lyons GE, Li M, Spivey ND, Norell H, Martins da Palma T, Onicescu G, Diaz-Montero CM, Garrett-Mayer E, Cole DJ, Le Poole IC, Nishimura MI. A coreceptor-independent transgenic human TCR mediates anti-tumor and anti-self immunity in mice. *J Immunol.* 2012; 189(4):1627-38. PMCID: PMC3674773.

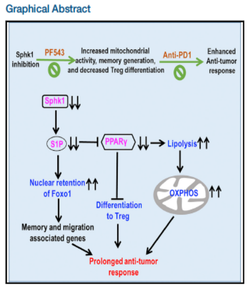
Recent Publications

Article

Cell Reports

Pro-Survival Lipid Sphingosine-1-Phosphate Metabolically Programs T Cells to Limit Anti-tumor Activity

Graphical Abstract



Authors
Paramita Chakraborty, Silvia G. Vaena, Krishnamurthy Thyagarajan, ..., Xue-Zhong Yu, Besim Ogretmen, Shikhar Mehrotra

Correspondence
ogretmen@musc.edu (B.O.), mehrotr@musc.edu (S.M.)

In Brief
Chakraborty et al. define the role for SphK1/S1P signaling via engaging lipid transcription factor PPARγ to attenuate lipolysis and spare respiratory capacity in T cells. Genetic ablation or pharmacological inhibition of Sp[illegible] expression limits intrinsic S1P le improves T cell-mediated anti-tu immunotherapeutic control.

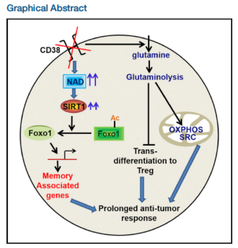
Highlights
- S1PR-independent intrinsic S1P signaling activates PPARγ to skew the Treg/Th17 balance
- S1P-PPARγ activation inversely correlates with lipolysis and regulates Tcm phenotype
- Inhibiting SphK1/S1P/PPARγ signaling improves T cell-mediated tumor control

Article

Cell Metabolism

CD38-NAD$^+$Axis Regulates Immunotherapeutic Anti-Tumor T Cell Response

Graphical Abstract



Authors
Shilpak Chatterjee, Anusara Daenthanasanmak, Paramita Chakraborty, ..., Jeffery Rathmell, Xue-Zhong Yu, Shikhar Mehrotra

Correspondence
mehrotr@musc.edu

In Brief
Chatterjee et al. show that intracellular NAD$^+$ levels control the anti-tumor potential of hybrid Th1/17 cells through the NAD$^+$-Sirt1-Foxo1 axis. Expression of the NADase CD38 inversely correlates with NAD+ levels and regulates anti-tumor T cell response. Genetic ablation or antibody-mediated targeting of CD38 on T cells improves tumor control.

Translational Cancer Mechanisms and Therapy

Clinical Cancer Research

Targeting PIM Kinase with PD1 Inhibition Improves Immunotherapeutic Antitumor T-cell Response

Shilpak Chatterjee[1], Paramita Chakraborty[1], Anusara Daenthanasanmak[2], Supinya Iamsawat[2], Gabriela Andrejeva[3], Libia A. Luevano[4], Melissa Wolf[3], Uday Baliga[5], Carsten Krieg[2], Craig C. Beeson[6], Meenal Mehrotra[6], Elizabeth G. Hill[7], Jeffery C. Rathmell[3], Xue-Zhong Yu[2], Andrew S. Kraft[4], and Shikhar Mehrotra[1]

JBC ARTICLE

Thioredoxin-1 improves the immunometabolic phenotype of antitumor T cells

Received for publication, November 16, 2018, and in revised form, March 25, 2019 Published, Papers in Press, April 10, 2019, DOI 10.1074/jbc.RA118.006753

Paramita Chakraborty[‡1], Shilpak Chatterjee[‡1], Pravin Kesarwani[‡1], Krishnamurthy Thyagarajan[§], Supinya Iamsawat[§], Annika Dalheim[¶], Hung Nguyen[§], Shanmugam P. Selvam[‖], Patrick Nasarre[‖], Gina Scurti[¶], Gary Hardiman[**], Nilanjana Maulik[‡‡], Lauren Ball[§§], Vamsi Gangaraju[‖], Mark P. Rubinstein[¶], Nancy Klauber-DeMore[§], Elizabeth G. Hill[¶¶], Besim Ogretmen[‖], Xue-Zhong Yu[§], Michael I. Nishimura[¶], and Shikhar Mehrotra[‡2]

From the Departments of [‡]Surgery, [§]Microbiology and Immunology, [‖]Biochemistry and Molecular Biology, [**]Nephrology, [§§]Pharmaceutical and Biomedical Sciences, and [¶¶]Public Health, Hollings Cancer Center, Medical University of South Carolina, Charleston, South Carolina 29425, the [¶]Department of Surgery, Loyola University, Maywood, Illinois 60153, and the [‡‡]Department of Surgery, University of Connecticut Health Center, Farmington, Connecticut 06030

Edited by Luke O'Neill

Contact:
E-mail: mehrotr@musc.edu; Phone: 843-792-9195; Lab location: HO503