OVERVIEW OF MY RESEARCH INTERESTS

Posttranslational modifications such as ubiquitylation, methylation, ADP-ribosylation as well as phosphorylation orchestrate genome stability (DNA damage response/DNA repair), cell growth, signal transduction, apoptosis and tumorigenesis. Posttranslational modifications act as critical molecular switches or fine-tune operators that determine the activation, deactivation or subcellular localization of functional proteins. Emerging evidence has drawn attention to the modulation of regulatory proteins in response to extrinsic/intrinsic signaling being executed simultaneously by multiple posttranslational modifications.

Research interests in my laboratory seek to address how defects in the ubiquitin-proteasome system (E3 ligase/deubiquitinase), protein methyltransferase and poly (ADP-ribose) polymerase 1 (PARP1) would result in genomic instability, abnormal cell division or apoptosis, and aberrant signal transductions (e.g., ER, TGF-β and EGFR) that predispose otherwise normal cells to become cancerous tumor cells. The ultimate objective is to integrate our basic research with clinical translational studies that would allow the development of new anti-cancer therapy thereby fully exploiting our knowledge of posttranslational modifications. To achieve our goals, we have developed a multidisciplinary approach that includes biochemical, cell biological and genetic analyses as well as the use of animal models and analyses of clinical samples. Below is a brief description of our research projects for the next five years:

RESEARCH PROJECTS

Posttranslational Modifications in Genome Stability, Carcinogenesis and Cancer Treatment

(1) Rad17 proteolysis in checkpoint termination and tumorigenesis

Genotoxic stress, such as environmental radiation and chemical mutagens, results in genomic instability leading to cancer. Both DNA-damage response and DNA repair are tightly regulated by posttranslational modifications. The pivotal role of posttranslational modification has been demonstrated in the recognition of DNA damage lesion sites, activation of DNA damage checkpoint response, recruitment of DNA repair elements and termination of DNA damage checkpoint following metabolic recovery from genotoxic stress. To systematically search for proteins that are ubiquitylated and degraded in response to genotoxic stress and to further examine their impact on genomic integrity and carcinogenesis, we have performed a high-throughput screening as well as large-scale immunoblotting analyses (Fig 1A). One interesting candidate that we identified was Rad17, a checkpoint protein. We are currently investigating the mechanism by which Rad17 is ubiquitylated and degraded after cellular exposure to UV radiation or DNA replication stress. By purifying protein complex followed by mass spectrometry, we recently identified functional interaction between Rad17 and Cdh1/APC (E3 ligase) as well as between Rad17 and USP20 (deubiquitinase) (Fig. 1B). We are now determining how failure in proteolytic regulation of Rad17 by Cdh1/APC and USP20 would affect genomic integrity and tumorigenesis by utilizing a melanomagenesis mouse model and 3D human skin reconstitution model (Fig. 1C). In addition, we are developing a new combinatorial...
therapy for melanoma by synergistically targeting BRAF and ATR-Cdh1/APC-Rad17-Chk1 checkpoint pathways using various combinations of BRAF inhibitor (GSK1120212), Temozolomide, ATR inhibitor and Chk1 inhibitor in an animal model.

(2) Identification of crosstalk between ubiquitylation and protein methylation in determining KLF4 protein stability in DNA damage response and cancer treatment

Kruppel-like factor 4 (KLF4) is an important regulator of cell-fate decision, including DNA damage response, inflammation, apoptosis, and stem cell renewal. Its critical impact in breast cancer formation was recently uncovered by the TCGA (The Cancer Genome Atlas) study as well as human breast cancer specimen tissue array. Surprisingly, recent studies have sketched an ambivalent nature for KLF4 in tumorigenesis as either a tissue specific tumor suppressor (in colorectal cancer) or oncogene (in breast cancer), although the underlying mechanism as to how it switches functions remains unclear. In addition, how KLF4 is regulated in response to various environmental factors such as DNA damage signal remains largely unknown. To explore the mystery, we have purified KLF4 protein complexes followed by identification of its physiological binding partners using mass spectrometry. This effort has led to the identification of several KLF4 physiological interacts including VHL (a ubiquitin protein ligase), PRMT5 (an arginine protein methyltransferase) and PARP1 (poly (ADP-ribose) polymerase I) (Fig. 2A). Results from our initial characterization suggest that precised KLF4 protein levels were determined by VHL/VBC and PRMT5. While KLF4 is targeted by VHL/VBC for ubiquitylation and degradation, PRMT5-mediated methylation antagonizes KLF4 ubiquitylation thereby stabilizing KLF4. Given that KLF4 is a pivotal cellular-fate factor after exposure to DNA damage, we are now addressing how the crosstalk between

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Figure 1. (A) Identification of Rad17 as a DNA damage induced degradable protein by high-throughput screening. (B) Time-dependent Rad17 proteolysis governs the termination of DNA damage checkpoint signal ensuring metabolic recovery. (C) Validation of impact of Rad17 proteolysis on tumorigenesis using melanoma mouse model.

Figure 2. (A) Identification and characterization of VHL, PRMT5 and PARP1 as KLF4 physiological binding partners by using mass spectrometry. (B) Crosstalk between PRMT5 and VHL/VBC determines KLF4 levels ensuring DNA damage response. (C) Structure and computational modeling of catalysis of KLF4 methylation by PRMT5. (D) PARylation of KLF4 regulates its chromatin displacement. Synergistic blockade of PARP1-KLF4 and USP11- BRCA1 axis could be an efficient strategy to kill breast cancer cells.
ubiquitylation and methylation dictates DNA damage response by promotion of p21 and inhibition of Bax (Fig. 2B). In addition, using a protein structural and computational modeling analysis, we are elucidating the mechanisms of how PRMT5 catalyzes KLF4 methylation and how KLF4 methylation counteracts KLF4 ubiquitylation for stabilization. The ultimate goal is to develop small molecule inhibitors that could intercept PRMT5-mediated KLF4 methylation based on information obtained through structural modeling (Fig. 2C), which could be valuable for chemotheraphy.

(3) Synergism of the molecular axes PARP1-KLF4 and USP11-BRCA1 in breast cancer treatment
Identification of interaction between KLF4 and PARP1 leads to the characterization of PARP1-mediated KLF4 ADP-ribosylation in the displacement of KLF4 to chromatin that is a critical step in ensuring KLF4-governed transcriptional function (Fig. 2D). Given the vital role of KLF4 in determining the cell fate in response to DNA damage through regulating p21 and Bax, demonstration of KLF4 PARylation by PARP1 has revealed a new paradigm as to how cancer cells are sensitized to radiation or chemotherapeutic agents. We are now elucidating the mechanism by which KLF4 is modified by PARylation and how ADP-ribosylation of KLF4 leads to recruitment of KLF4 onto chromatin. In addition, we recently observe that inhibition of BRCA1 deubiquitination, a mechanism that stabilizes BRCA1, results in enhanced efficacy for PARP inhibitor in killing breast cancer cells. We thus propose a synthetic lethality based strategy to treat breast cancer cells with either positive or negative BRCA1 background using combination of PARP1 and USP11 inhibitors in patient-driven breast cancer xenograft mouse model (Fig. 2D).

Posttranslational Modifications in Signal Transduction and Breast Tumor Initiation and Invasion

(1) Crosstalk between estrogen receptor and TGF-β signaling in the development of mammary gland and breast cancer
Defective regulation of estrogen receptor (ER) and transforming growth factor beta (TGF-β) signaling pathways can predispose breast cells towards carcinogenesis. Our recent findings have revealed a previously unknown mechanism that centers on the interplay between KLF4, an oncogenic transcriptional factor, and VHL/VBC (E3 ligase) as well as between KLF4 and Cdh1/APC (E3 ligase) in cell cycle control and proliferation. Their functional interaction is critical in orchestrating the crosstalk between ER and TGF-β signaling pathways, which in turn determines whether breast cells retain their homeostasis or are transformed to initiate oncogenic growth. Our observations have provided insight into the pathological mystery previously observed by us and others that over 70% of human mammary cancers exhibited cellular accumulation of KLF4 and decreased levels of VHL protein and that disruption in Cdh1 function in mice often results in the genesis of breast tumor or its enhanced progression. We are currently studying the molecular basis of the interplay between KLF4 and VHL/VBC as well as KLF4 and Cdh1/APC in their regulation of ER and TGF-β signal transduction and determining how impaired KLF4 proteolytic regulation due to dysregulated VHL/VBC or Cdh1/APC would impact breast tumorigenesis using a breast cancer animal model (Fig. 3). Outcomes from this project could provide a more effective strategy to modulate the ER signaling pathway by exploiting components of the VHL/VBC-Cdh1/APC-KLF4 cascade. Controlling the ER signaling pathway has long been a key focus in the field to develop new methods for chemoprevention and endocrine therapy for breast cancer treatment.

(2) Killing breast tumor-initiating cell by synergizing molecular axes of PARP-KLF4 and EGFR-KLF4
One of the most recent striking findings in stem cell and tumor-initiating cell field is that PARP1 could elevate iPS induction-rate by 100 folds, while ionizing irradiation significantly enhances subpopulation of breast tumor-initiating cell resulting in increases in cancer heterogeneity. We and others have observed that KLF4 is an important factor that facilitates the effect of PARP1 and ionizing irradiation. In addition, we also observed EGFR2 is critical to alter subpopulation of breast tumor-initiating cell. Accordingly, we are now addressing the mechanism of how PARP1, ionizing irradiation and EGFR synergistically orchestrate KLF4 resulting in increased breast
tumor-initiating cell population, and we are therefore proposing a synthetic lethality based strategy to treat breast tumor-initiating cell using combination of PARP1 and EGF2 inhibitors in human breast cancer xenograft mouse model (Fig. 4). We are now preparing to submit an R21 application pertaining to our project to develop a targeted therapy for breast cancer treatment.

Posttranslational Modifications in Mitotic Regulation and Tumorigenesis

Posttranslational modifications in mitotic regulation and cancer treatment

Control of G2/M transition and mitotic progression during the cell cycle is crucial for DNA damage checkpoint response and maintenance of chromosomal stability. Defects in either G2/M checkpoint or maintenance of chromosomal integrity often results in malignant cellular transformation. Development of cancer therapies through sensitizing G2/M transition or inducing mitotic catastrophe has became an attractive strategy for anti-cancer therapies. We recently identified two new players (ARID1A and USPx) that govern G2/M transition and chromosomal stability. While current deep sequencing analyses have revealed a clinical connection between genetic mutations on ARID1A with ovarian and breast cancers, our pathological studies have demonstrated a tight association between aberrant expression of USPx with breast cancer. We have recently found that both ARID1A and USPx are regulated by phosphorylation, ubiquitylation and sumoylation during cell cycle and identified such regulators upstream of ARID1A or USPx. We are currently studying the molecular mechanism by which ARID1A or USPx is regulated during transition from G2 to mitosis as well as during chromatid segregation and cytokinesis and how impaired regulation of ARID1A or USPx would impact breast carcinogenesis by using a breast cancer animal model (Fig. 6). In addition, we are now developing chemical modulators of ARID1A or USPx that could sensitize cells to Taxol and other anti-mitotic drugs as part of a breast cancer treatment.
Awards and Honors:

2012-present  Hillman Foundation Award for Cancer Research
2006-2010  American Cancer Society Scholar Award
2004-2007  V Scholar (The V Foundation for Cancer Research)
2004-2006  CMRF Award (Competitive Medical Research Fund)
1999-2002  Helen Hay Whitney Post-doctoral Fellowship Award
1999  Leukemia Society of America Post-doctorial Fellowship Award
       (Declined due to other award)
1996  Vincent du Vigneaud Award, Cornell University Medical College

Professional Association:

2003-present  American Association of Cancer Research
1998-present  American Association of Cell Biology
1996-present  American Association for The Advancement of Science

Editorial Board:

Journal of Biological Chemistry, Editorial Broad Member (2011-2016)

Service on National Grant Review Panels:

Molecular Oncogenesis Study Section (MONC), NIH, Permanent member (2013-2019)
Special panel study section ZRG1 BCMB-A, NIH, Ad Hoc Reviewer (2014)
Molecular Oncogenesis Study Section (MONC), NIH, Ad Hoc Reviewer (2011, 2012 and 2013)
Radiation Tumor Biology Study Section (RTB), NIH, Ad Hoc Reviewer (2008)
NIH F30, F31 and F32 study section, Ad Hoc Reviewer (2012 and 2013)

Service on International Grant Review Panels:

Cancer Research United Kingdom, Ad Hoc Reviewer (2010, 2011 and 2012)
Chinese NSF, Ad Hoc Reviewer (2005-present)

Journal Reviews (selected):

Molecular Cell
EMBO Journal
PNAS, Nature Communication
Cancer Research
MCB, MBC, Oncogene
Clinical Cancer Research
FASEB Journal

Service on University of Pittsburgh Committee:

University of Pittsburgh Patent Review Committee, Review Member (2012)
Cell Biology Admission Committee, Chair (2006-2009)
International Graduate Admission Committee, Member (2003-2006)
Cell Biology Faculty Search Committee, Member (2005-present)
Molecular and Cellular Cancer Biology Faculty Search Committee, Member(2004-present)
Molecular Virology Faculty Search Committee, Member (2005, 2006)
Conference Organizer:
Co-organizer of Annual Pittsburgh Ubiquitin-Proteasome Conference

Seminars and Invited Lectureships:

2014  The role of proteolysis in cell cycle, genomic integrity and carcinogenesis. Harvard Medical School
2014  Coordination between ubiquitination and deubiquitination in genome stability and cancer formation. Mayo Clinic
2014  Impact of posttranslational modification in DNA damage response and tumorigenesis. University of Southern California
2013  Crosstalk between ubiquitylation and methylation in tumorigenesis. Science 2013 in Pittsburgh
2013  Impact of UPS: from Kruppling development to tumorigenesis. International Symposium of Cancer, Cancer Stem Cell and Therapy. China
2013  Ubiquitin-proteasome system: from biological process to diseases. School of Pharmacy University of Pittsburgh
2013  Crosstalk between protein di-methylation and ubiquitylation in carcinogenesis. SCBA in China.
2012  The role of UPS in DNA damage response and cancer formation. New York University
2012  Cross-talk between estrogen receptor and TGF-b signaling in carcinogenesis. University of Pittsburgh, Children’s Hospital
2012  Post-translational modification of KLF4 in genomic integrity and cell cycle control. Emory University School of Medicine
2011  Ubiquitin-proteasome in DNA damage response and cancer. The University of Hong Kong
2011  Regulation of KLF4 Turnover Reveals an Unexpected Tissue Specific Role of pVHL in Tumorigenesis. Bioscience for 21st Century: Emerging Frontiers and Evolving Concepts
2011  APC pathway in cell cycle, genomic integrity and carcinogenesis. University of Pennsylvania
2010  Regulation of genomic integrity by Cdh1/APC-Rad17 cascade in DNA damage checkpoint and melanomagenesis. Wistar Institute
2010  Interplay between APC/Cdh1 and Rad17 in regulating DNA damage checkpoint and carcinogenesis. Science 2010 in Pittsburgh
2010  Regulation of stem cell division and differentiation by UPS. International Forum on Stem Cell. Tianjin, China
2010  The role of proteolysis in cell cycle, genomic integrity and carcinogenesis. duPont Hospital for Children, Nemours Biomedical Research
2010  The role of UPS in cell cycle control and carcinogenesis. Department of Urology, University of Pittsburgh School of Medicine
2009  The role of Cdh1/APC in cell cycle, differentiation and cancer formation. The National Taiwan University
2009  The role of UPS in genomic integrity and carcinogenesis. Institute of Molecular Biology, Academia Sinica, Taiwan
2008  Targeting the APC-separase cascade ensuring the mitotic progression. Peking Union Medical University, China
2007  APC pathway in tumorigenesis. Robert Wood Johnson Medical School
2007  The role of Anaphase-Promoting Complex in the control of cell cycle and beyond. Cornell University
2007  Mitotic Progression is modulated by TGF-β signaling. International Conference of Protein Modification (Beijing)
2006  Anaphase-Promoting Complex and its biological function. Clemson University
2006  The role of Cdh1 in genomic integrity and cellular differentiation. 2006 Pittsburgh Ubiquitin Conference
2005  The role of ubiquitin-dependent proteolysis in the control of the cell cycle and tumor formation. University of Pittsburgh Senior Vice Chancellor's Research Seminar

2005  Biological regulation by ubiquitin-proteasome pathway. Fudan University School of Medicine, China

2005  Ubiquitylation and tumorigensis/Beijing Union Medical University, China

2004  Ubiquitylation in Cancer. Department of Medicine, School of Medicine University of Pittsburgh

2004  The role of proteolysis in modulating signal transduction. Conference of Science 2004 in Pittsburgh

2004  The role of ubiquitylation in regulating genomic integrity/Children's Hospital. UPMC


2004  Degradation of the SCF component Skp2 in cell-cycle phase G1 by the anaphase-promoting complex. Pittsburgh Cancer Institute

2003  Ubiquitin-dependent proteolysis in normal and cancer cells/Science 2003 in Pittsburgh

2003  Role of proteolysis in the control of cell cycle/International Conference of Cancer Biology in Pittsburgh

2002  Role of ubiquitin-dependent proteolysis in the control of cell cycle and differentiation. NIH/NCI/Bethesda

2002  The role of APC in TGF-b signaling pathway and carcinogenesis. Department of Cell Biology, University of Pittsburgh School of Medicine

2002  The anaphase-promoting complex mediates TGF-b signaling by targeting SnoN for destruction. UT-San Antonio/San Antonio

2001  Identification of multiple CDH1 homologues in vertebrates conferring different substrate specificities. Helen Hay Whitney postdoctoral fellow symposium/New York

1997  The role of Src, Syk and Btk in mediating G protein signaling. Department of Cell Biology, Harvard Medical School

1997  Tyrosine kinases in G protein signaling pathways. Dana Farber Cancer Institute

1997  Role of tyrosine kinase in G protein signaling. Institute of Harvard Medicine/Boston

1997  Tyrosine kinases in G protein signaling pathways. Columbia University School of Medicine

1997  Tyrosine kinases in activation of the MAP kinase cascade by G-protein-couple receptors. Rockefeller University/New York

1996  The role of tyrosine kinase in mediating G protein signaling. Tri-Institute Annual Scientific Conference (Cornell University Medical College, Memorial Sloan- Kettering Cancer Center and Rockefeller University)

Research Support:

On-Going Support

1 R01 CA154695  Wan, Yong (PI)  NCI/NIH  7/1/11-6/31/16
insight in the Regulation of Rad17 in Genomic Integrity and Carcinogenesis

Melanoma/Skin Cancer SPORE (pilot)

Wan, Yong (PI)  NCI/NIH  7/1/13-6/31/14
A Novel Strategy for Melanoma Treatment by Targeting Both BRAF and Genome Stability

Hillman Foundation Award for Cancer Research 7/1/12-6/31/15
The role of KLF4 in breast cancer

Completed Research Support

1 R01 CA115943  Wan, Yong (PI)  NCI/NIH  7/1/05-6/31/11
Activation of APC by TGF-b Suppresses Breast Cancer

RSG-05-027-01-CCG Wan (PI)  ACS  1/1/05-12/31/10
A Novel Strategy to Dissect Ubiquitylation in Genomic Integrity and Cancer

CMRF  Wan (PI) University of Pittsburgh  1/1/04-12/31/05
Ubiquitin-Regulatory Circuitry in Cancer Formation
Ubiquitin-Mediated Circuitry in Genome Stability

V-703509 Wan (PI) V Foundation of Cancer Research 1/1/05-12/31/08
Functional Proteomic Study of Protein degradation in Tumorigenesis

Pittsburgh Women Cancer Fund Wan, Yong (PI) 1/1/13-12/31/13
Proteolytic regulation in breast carcinogenesis

Pending

1R01CA179215-01 Wan, Yong (PI) NCI/NIH
Regulation of Krüppel-like factor 4 by ubiquitin-proteasome in tumorigenesis

1R01CA179222-01 Wan, Yong (PI) NCI/NIH
Arginine methylation of KLF4 by PRMT5 in genomic integrity and tumorigenesis

Selected Publications:


**Manuscripts Submitted and Prepared:**


**Published Abstracts:**


2010. Proteolysis of Rad17 by Cdh1/APC involves in checkpoint termination and recovery from genotoxic stress. Abstract (Cold Spring Harbor Conference)


Wan Y., Kurosaki T., and Huang X-Y. 1996. Tyrosine kinases in activation of the MAP kinase cascade by G protein-couple receptors. Abstract (Gordon Conference)

Teaching Responsibility:

5. Foundation Course (2005)
Graduate Committee:

George Wu (Ph. D. thesis mentor)
Joe X. Qia (Ph. D. thesis mentor)
Mingjing He (Ph. D. thesis mentor)
Michael Preston (Thesis committee member)
Shashikanth Sriram (Thesis committee member)
Anne Lipton (Thesis committee member)
Min Jae Lee (Thesis committee member)
Jee Young An (Thesis committee member)
Christopher Guerriero (Comprehensive exam committee member)
Michelle A. Wood (Comprehensive exam committee member)
Mark R. Silvis (Comprehensive exam committee member)
James Thieman (Comprehensive exam committee member)

Trainee:

(Current lab member)
Zhuan Zhou  Post-doctoral fellow
Nianhong Chen  Post-doctoral fellow
Xu Lin  Post-doctoral fellow
Dong Hu  Post-doctoral fellow
Mingjing He  Graduate student
Amit Shetty  Undergraduate student

(Previous lab member)
Armin Gamper  Post-doctoral fellow
Liyong Zhang  Post-doctoral fellow
Weijun Liu,  Post-doctoral fellow
Shouhui Yang  Post-doctoral fellow
Wenqi Li,  Post-doctoral fellow
Takeo Fujita,  Post-doctoral fellow
Jing Wu,  Post-doctoral fellow
Chi-Hoon Park  Post-doctoral fellow
Guang Jin  Post-doctoral fellow
Wenjun Zong  Post-doctoral fellow
George Wu,  Graduate student
Joe X. Qiao  Graduate student
Hyun Kim  MD student (fellowship)
Katherine Yang  Undergraduate student, Carnegie Mellon University
Andrew Lu  Undergraduate student, Carnegie Mellon University
Jennifer Kim  Undergraduate student, Carnegie Mellon University
Kathy Chiapaikeo  Undergraduate student, Carnegie Mellon University
Xiao Xiao  Undergraduate student, University of Pittsburgh
Kelvin Cheng  Undergraduate student, Carnegie Mellon University
Part Parekh  Undergraduate student, Carnegie Mellon University
Qi Yang,  Graduate student University of Pittsburgh School of Public Health
Judy Wu  MD student, Drexel University School of Medicine