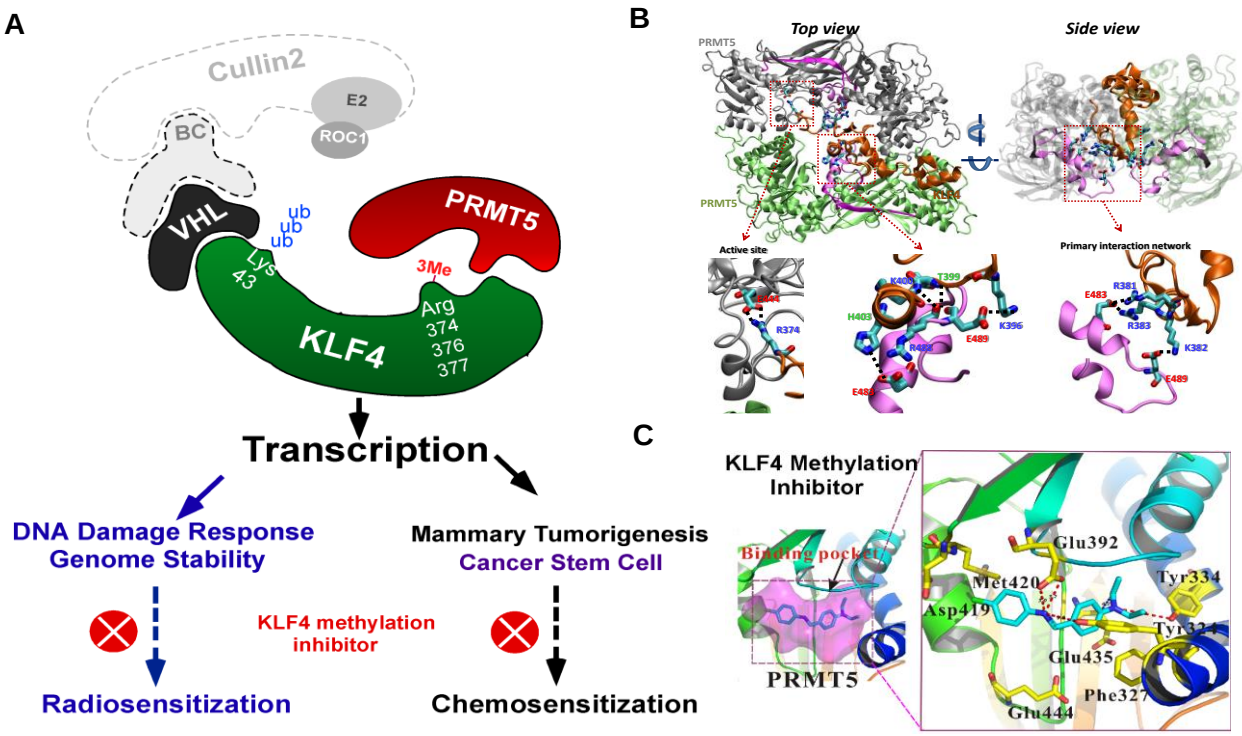
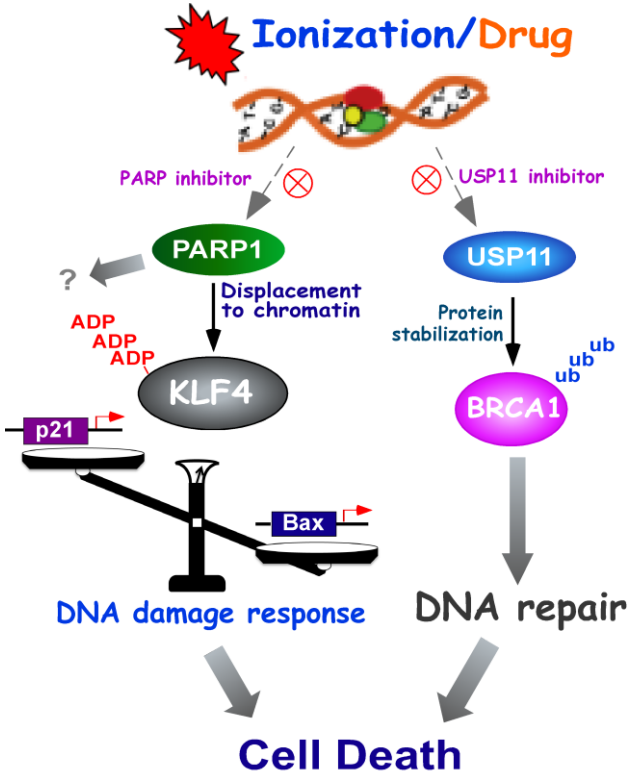
Interplay between ubiquitylation and protein methylation in KLF4-mediated genome stability and breast carcinogenesis



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 ligase), PRMT5 (an arginine protein Methyltransferase) and PARP1 (poly (ADP-ribose) polymerase 1). Results from our initial characterization suggest that precise 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 ubiquitylation and methylation dictates DNA damage response by promotion of p21 and inhibition of Bax. 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, which could be valuable for anti-breast cancer chemotherapy. This project is recently funded by NIH/NCI R01 grant (**CA202963**).

Synergism of the molecular axes PARP1-KLF4 and USP11-BRCA1 in breast cancer treatment

Our recent identification of interaction between KLF4 and PARP1 based on mass spectrometry leads to the characterization of PARP1-mediated KLF4 ADP-ribosylation in the recruitment of KLF4 to chromatin that is a critical step in ensuring KLF4-governed transcriptional function. 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.



Proteolytic regulation of Rad17 in orchestrating checkpoint function 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 egulated 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. One interesting candidate that we identified was Rad17, a checkpoint protein. We are currently investigating the mechanism by which Rad17 is ubiquitylated and degraded in response to DNA damage signal. 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). We are now determining how failure in proteolytic regulation of Rad17 by Cdh1/APC and USP20 would affect genomic integrity and tumorigenesis by various tumor mouse models. In collaboration with clinical group, we are developing a new combinatorial therapy for melanoma/skin cancer in synergistically targeting BRAF and ATR-Cdh1/APC-Rad17-Chk1 checkpoint pathway, using various combinations of BRAF inhibitor, ATR inhibitor, Chk1 inhibitor as well as Cdh1/Cdc20 inhibitor in an animal model.