Thomas J. McCabe[1], Sabine Lang[1], Keisuke Aoshima[2], Lizhen Wu[3], Jian Cao[4], Qin Yan[1,5,6,7,8]

Title: The Roles of the Histone Demethylase KDM5A in Breast Cancer and Senescence

Introduction: Epigenetic alterations including histone methylation have become an increasingly important topic in cancer pathophysiology, with implications in all stages of cancer development and progression. The histone demethylase KDM5A has been found to be upregulated in several cancers including breast cancer, and its associations with cell cycle regulation and senescence make it a promising therapeutic target, however the underlying mechanisms remain largely unclear. Further examination of KDM5A is warranted to probe its therapeutic potential. Using mouse models of breast cancer and *ex vivo* analyses, our group has demonstrated that KDM5A inhibition leads to senescence phenotypes indicating its potential to prevent mammary tumor growth.

Methods: The mouse and cell culture models utilized in our studies are 1) mouse mammary tumor virus expressing polyoma middle T antigen (MMTV-PyMT) mice with genetic knockout of Kdm5a, 2) a doxycycline-inducible Kdm5a in Kdm5a-/- MMTV-PyMT cells, and 3) an orthotopic model established by injection of the metastatic triple negative breast cancer (TNBC) cell line, 4T1, with LentiCRISPR short guide RNA (sgRNA) against Kdm5a. Tumor cells from all models were used for quantitative real-time polymerase chain reaction (RT-qPCR), and immunohistochemical (IHC) staining was used to investigate the changes in immune cell infiltration of the 4T1 tumors.

Results: Genetic deletion of Kdm5a in the MMTV-PyMT mouse model resulted in mild change in tumor burden, but had no impact on tumor free or overall survival. Remarkably, however, Kdm5a-/- tumor cells from these mice rapidly began to exhibit senescence phenotypes including increased expression of senescence associated secretory phenotype (SASP) markers and hexokinase 2, and an acquired senescent cell morphology with increased media acidification. Consistently, removal of the exogenous KDM5A from the Kdm5a-/- MMTV-PyMT cells resulted in a similar phenotype. These changes suggest that KDM5A inhibition leads to tumor cell senescence, but that changes in the tumor microenvironment (TME) *in vivo* are permissive of cancer progression. In fact, Kdm5a knockout in the orthotopic 4T1 mouse model significantly increased T cell infiltration and decreased tumor growth. Additionally, KDM5A loss or inhibition resulted in increased expression of interferon stimulated genes and cytokines and chemokines.

Conclusions: These studies suggest that KDM5A targeting could be a therapeutic strategy for breast cancer treatment.

Author Affiliations:

1. Department of Pathology, Yale School of Medicine, New Haven, CT 06520
2. Laboratory of Comparative Pathology, Department of Clinical Sciences, Faculty of Veterinary Medicine, Hokkaido University, Kita 18 Nishi 9, Kita-ku, Sapporo, Hokkaido 060-0818 Japan
3. Department of Immunobiology, Yale School of Medicine, New Haven, CT 06520
4. Division of Medical Oncology, Rutgers Cancer Institute of New Jersey, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08903, USA
5. Yale Cancer Center, Yale School of Medicine, New Haven, CT 06520

6. Yale Stem Cell Center, Yale School of Medicine, New Haven, CT 06520
7. Yale Center for Immuno-Oncology, Yale School of Medicine, New Haven, CT 06520
8. Yale Center for Research on Aging, Yale School of Medicine, New Haven, CT 06520