

## A Study on *BRCA2-RB1* Co-loss, EMT and Aggressive Therapy Resistant Prostate Cancer

**Goutam Chakraborty**<sup>1</sup>, Joshua Armenia<sup>2,3</sup>, Ying Z. Mazzu<sup>1</sup>, Subhiksha Nandakumar<sup>2</sup>, Konrad H. Stopsack<sup>1</sup>, Mohammad Atiq<sup>1</sup>, Kazumasa Komura<sup>1,4</sup>, Kalyani Chadalavada<sup>5</sup>, Yuki Yoshikawa<sup>1</sup>, Nabeela Khan<sup>1</sup>, Yu Chen<sup>1,6</sup>, Wassim Abida<sup>1</sup>, Lorelei A. Mucci<sup>7</sup>, Gwo-Shu Mary Lees<sup>8</sup>, Gouri Nanjangud<sup>5</sup>, Philip W. Kantoff<sup>1</sup>

<sup>1</sup>Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY

<sup>2</sup>Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center, New York, NY

<sup>3</sup>Current address: IMED Biotech Unit, AstraZeneca, Cambridge, UK

<sup>4</sup>Department of Urology, Osaka Medical College, Osaka, Japan

<sup>5</sup>Molecular Cytogenetics Core Facility, Memorial Sloan Kettering Cancer Center, New York, NY

<sup>6</sup>Human Oncology Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY

<sup>7</sup>Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA

<sup>8</sup>Department of Medicine, Dana-Farber Cancer Institute, Boston, MA

**Background:** The normal structure of DNA is essential for healthy cell growth, but DNA is sometimes damaged or mutated. DNA damage repair (DDR) genes help maintain DNA integrity. Pathologic variants of DNA damage repair (DDR) pathway genes are prevalent in a substantial subset of men who develop metastatic castration resistance prostate cancer (mCRPC). Therefore, study on the aberrations of this DDR pathway components, present in men with localized prostate cancer (PC), could be used to identify those with the highest risk of developing lethal PC, and therapy could be based on specific somatic or germline findings. *BRCA2* plays a crucial role in DDR through its role in homologous recombination repair (HRR). In two recent studies of men with advanced or metastatic PC, *BRCA2* alterations were seen in 13.3% of patients; 5.3% of patients had inherited (germline) aberrations. *BRCA2* is located on chromosome 13q in close proximity to the *RB1* gene. Recent sequencing studies have revealed that co-deletion (homozygous and heterozygous) of *RB1* and *BRCA2* is present in a significant fraction of primary PCs. Recently we observed that patients with primary PC who have *BRCA2-RB1* co-deletion have significantly shorter recurrence-free survival compared to patients with deletion of neither or with *RB1* or *BRCA2* deletion alone.

**Experimental design:** Building on this clinical data, we used CRISPR and shRNA to develop an *BRCA2-RB1* knockout experimental PC cell line systems and subjected them to *in-vitro* studies and transcriptomic analyses. We have also developed a 3-color FISH assay to detect genomic deletions in human PC cells and patients derived mCRPC organoids.

**Results:** In human prostate cancer LNCaP cell loss of *BRCA2* leads to androgen-independent growth. Most importantly we observed that co-loss of *BRCA2* and *RB1* in LNCaP cells induces an EMT-like invasive and for the first time we demonstrate that human prostate cancer cells exhibit a distinct phenotype upon co-loss of *BRCA2-RB1* which may lead to aggressive disease. We also showed that co-elimination of *BRCA2-RB1* induced EMT transcription factor SLUG (*SNAI2*) expression. Knockdown of SLUG reduces Matrigel invasion in *BRCA2-RB1*-null prostate cancer cells. Therefore, we suggest SLUG might play an important role in *BRCA2-RB1* loss-mediated EMT transformation of prostate cancer cells and may be a potential driver of aggressive disease. We have also demonstrated that treatment with the PARPi caused significant cell growth inhibition on *BRCA2-RB1* null PC cells and *BRCA2-RB1* deleted mCRPC organoids.

**Conclusion** Our finding suggests that concurrent deletion of *BRCA2-RB1* is most likely a driver of therapy resistant aggressive PC rather than the consequence of exposure to therapy. We propose that screening for *BRCA2-RB1* deletion early could be implemented to identify those at highest risk of aggressive PC and provide an opportunity for early intervention and alternative treatments.

**Conflict of Interest:** None