

Presenting Author Name

Manpreet Singh

Phonetic spelling of your (full) name

muhn-PREET SING

Presenting Author Category

Masters Student

Supervisor Name

Dr. Sabine Hombach-Klonisch

Research Category

Basic Science

Abstract Title

Patient-derived HMGA2K56E missense mutant renders MEFs sensitive to replication stress

Authors

Name	Role	Profession
Manpreet Singh	Presenting Author	Graduate
Thatchawan Thanasupawat	Co Author	Research Associate
Dr. Thomas Klonisch	Co Author	Full Professor
Dr. Sabine Hombach-Klonisch	Co Author	Full Professor

Background

High-Mobility Group A2 (HMGA2) protein is expressed during embryonic development and in adult stem cells. Our lab created a knockin mouse model for a patient-derived HMGA2 missense mutation (HMGA2K56E) that recapitulated the growth retardation phenotype in the patient with Silver-Russell Syndrome (SRS). We previously showed that HMGA2 protects stalled replication forks (RF) and enhances activity of the DNA damage sensor PARP1, thereby promoting DNA damage repair.

Objective

We hypothesize that HMGA2K56E negatively impacts protection of RFs resulting in impaired recovery of damaged replication forks.

Methods

Mouse embryonic fibroblasts (Hmga2WT and Hmga2K56E MEFs) were exposed to 2mM of DNA replication-blocking hydroxyurea (HU) for 24h followed by recovery. Western blots (WBs) were performed to assess the duration of double strand DNA damage (γH2AX) and stalled forks (p-RPA). Poly(ADP-ribose) [PAR] immunofluorescence (IF) on fixed MEF cells was performed to quantify PARP activity as indicator of

DNA damage. IF detection for replication protein A (RPA) foci marked stalled forks in single nuclei. 53BP1 IF foci served as marker for non-homologous end-joining (c-NHEJ) of double-strand DNA breaks in Hmga2WT and Hmga2K56E MEFs.

Results

WBs revealed that Hmga2K56E, but not Hmga2WT, MEFs retain increased γ H2AX expression after removal of HU. Hmga2K56E MEF cells showed an increased number of PAR+ nuclei upon 4 h HU exposure. Higher levels of p-RPA32 upon recovery indicated persistent, stalled forks upon release from HU in Hmga2K56E MEFs. Hmga2 co-localization with RPA- foci was observed in WT and K56E MEFs. Upon HU removal, higher numbers of 53BP1 foci were observed in Hmga2K56E compared to Hmga2WT MEFs.

Conclusion

HMGA2K56E MEFs are slower to recover from HU-induced stalled RF when compared to Hmga2WT MEFs. This may impact cell proliferation and differentiation during developmental exposure to endogenous DNA and RF damage, contributing to the developmental growth reduction observed in Hmga2K56E transgenic mice.