KIDNEY CANCER

SETD2 affects DNA replication

The consequences of SETD2 (histone-lysine N-methyltransferase SETD2, also known as Su(var), enhancer of zeste, trithorax-domain containing 2) loss of function on nucleosome structure, replisome occupancy, replication fork progression and DNA repair by homologous replication in clear cell renal cell carcinoma (ccRCC) have been reported by Kanu and colleagues in a recent article published in *Oncogene*.

SETD2 is the main methyltransferase responsible for trimethylating histone-3 at lysine-36 (H3K36me3) and it is recruited to sites of active transcription by RNA polymerase II. The *SETD2* gene is estimated to be mutated in 4–8% of ccRCCs and distinct mutations have been observed in spatially separated regions of the same tumour. Kanu *et al.* collated a data set of 450 ccRCC tumours with copy number information and 293 ccRCC tumours with somatic mutation data from The Cancer Genome Atlas. MutSig analysis on this sample set identified 14 significantly-mutated genes, including *SETD2*. Loss of heterozygosity (LOH) at the *SETD2* locus was observed in 391 of 396 samples that had LOH at the *VHL* locus. Nonsynonymous mutations or homozygous deletions of *SETD2* were found in 34 samples and paired data were available for 30 of these samples. Of the paired samples, 28 showed LOH at the *SETD2* locus, and one had homozygous deletion of *SETD2*, giving a total of 29 samples that were mutated at both alleles—collectively termed SETD2_{mut}. Samples with LOH at the SETD2 locus but no mutation of the gene were called SETD2_{LOH}.

Analysis of genome stability (which could be influenced by transcriptional aberrations) showed that SETD2_{mut} tumours did not have more chromosomal breakpoints with respect to H3K36me3 sites, or more chromosomal complexity than samples from normal adult kidneys or SETD2_{LOH} tumours. Breakpoint regions from SETD2_{LOH} tumours showed significant depletion of H3K36me3 marks ($P < 0.05$), whereas SETD2_{mut} samples had marginal H3K36me3 enrichment at breakpoint sites ($P < 0.05$). However, no depletion of H3K27me3 was observed at breakpoint regions, suggesting that H3K36me3 depletion at breakpoints in tumours is specific. Immunohistochemical analysis of tumour samples demonstrated a negative correlation between both SETD2 and H3K36me3 staining and DNA double strand breaks. The authors conclude that these data indicate that SETD2 is protective against chromosomal breaks, and this protection is specific to H3K36me3 sites. This observation is suggestive of a role for SETD2 in maintaining genome stability through a mechanism involving H3K36me3.

In vitro studies showed that DNA repair by homologous recombination was reduced in RCC cell lines in which SETD2 had been silenced by small-interfering RNA, and SETD2-silenced cells also had lower H3K36me3 expression than control cells. These results support the hypothesis that the absence of SETD2 could exacerbate DNA damage *in vivo*. Investigation of chromatin organization revealed reduced nucleosome compaction in normally trimethylated regions of SETD2-silenced cells. The stability of the replication complex is dependent on the reassembly of the nucleosomes behind the advancing replication fork. DNA fibre analysis showed SETD2 depletion resulted in significantly slower replication fork progression, an accumulation of cells in S phase and longer S phase duration. These observations are suggestive of defective nucleosome assembly during S phase in the absence of SETD2, contributing to replication fork instability and leading to genomic instability in SETD2-depleted cells.

Speaking to *Nature Reviews Urology*, Nnennaya Kanu, lead author on the paper, said: “This is the first time a histone modification has been shown to impact upon replication stress.” Jiri Bartek, one of the principle investigators, added: “DNA repair inhibitors are emerging as a new and promising strategy to treat certain cancers, our results could have implications for choice of treatment in the near future.”

Louise Stone

Original article Kanu, N. *et al.* SETD2 loss-of-function promotes renal cancer branched evolution through replication stress and impaired DNA repair. *Oncogene* doi:10.1038/onc.2015.24