NPG

DNA DAMAGE

Running in parallel

The repair of DNA interstrand crosslinks (ICLs) is a coordinated mechanism that requires the intra-S-phase checkpoint machinery and the Fanconi anaemia repair pathway. By studying these pathways, Simon Boulton and colleagues have found that the 3'-5' superfamily 2 helicase HELQ is important to prevent germ cell attrition and tumour development.

A role for HELQ in ICL repair had been suggested by the results of previous studies in *Drosophila melanogaster* and *Caenorhabditis elegans*. To investigate a repair function in vertebrate cells, Boulton and colleagues generated *Helq*^{ΔC/ΔC} mice in which the carboxyl terminus of HELQ is truncated. No detectable HELQ is evident in *Helq*^{ΔC/ΔC} mice and, although they have no obvious developmental defects, breeding experiments using *Helq*^{ΔC/ΔC} pairs showed that there was a fertility defect — male and female mice had regions of atrophy in their testis and ovaries, respectively. As the mice aged it became obvious that they were also more prone to tumour development than control mice; for example, 40% of female mice developed ovarian tumours and 30% developed pituitary tumours. Examination of *Helq*^{ΔC/+} females indicated a similar, although less aggressive, phenotype. This shows that the loss of a single allele of *Helq* causes haploinsufficiency in mice.

The phenotype of *Helq*^{ΔC/ΔC} mice is reminiscent of defects that are evident in mouse models of Fanconi anaemia, which include hypersensitivity to the presence of ICLs and a predisposition to developing tumours. Cells isolated from *Helq*^{ΔC/+} mice were also hypersensitive to the ICL-generating agent mitomycin C (MMC) and

to the replication-blocking agent camptothecin (CPT). A similar hypersensitivity to ICLs was also evident in human cells in which HELQ was knocked down using small interfering RNA.

To examine the potential relationship between HELQ and the Fanconi anaemia pathway more closely, the authors generated *Helq*^{ΔC/ΔC} mice in which Fanconi anaemia complementation group D2 (*Fancd2*) was also knocked out (*Helq*^{ΔC/ΔC}*Fancd2*^{-/-} mice). Although these mice again seemed to be developmentally normal, male *Helq*^{ΔC/ΔC}*Fancd2*^{-/-} mice, for example, had much greater testicular atrophy than either *Helq*^{ΔC/ΔC} mice or *Fancd2*^{-/-} mice, and the *Helq*^{ΔC/ΔC}*Fancd2*^{-/-} mice showed increased sensitivity to MMC and CPT compared with either the *Helq*^{ΔC/ΔC} or the *Fancd2*^{-/-} mice. These findings indicate that HELQ and FANCD2 function in parallel ICL repair pathways. To understand this relationship further, the authors carried out co-immunoprecipitation using Flag-tagged HELQ and mass spectrometry. The proteins that bound to HELQ included many proteins associated with DNA repair, such as the replication checkpoint serine/threonine protein kinase ATR, the single-stranded DNA-binding protein RPA70 (also known as RPA1), the RAD51 paralogue complex, BCDX2 (which is required for homologous recombination), and the FANCD2–FANCI heterodimer. All of these proteins respond to the presence of stalled replication forks, and the use of green fluorescent protein-tagged HELQ showed that HELQ localization to stalled replication forks is lost in the absence of ATR. This indicates that there is a role for HELQ in an ATR-mediated repair pathway.

Further examination of replication dynamics, checkpoint activation, double-strand break formation and the integrity of homologous recombination in mouse and human cells that were deficient for HELQ indicated that, although the checkpoint response seems to be intact in these cells, the ability to efficiently repair stalled replication forks is compromised. Indeed, although RAD51, RPA70 and γH2AX all accumulate at sites of damaged replication forks, they persist for much longer in HELQ-deficient cells than they do in normal cells. A DNA repair defect in the absence of HELQ was further suggested by the persistence of DNA double-strand breaks induced by MMC and by a twofold to threefold reduction in the frequency of homologous recombination compared with control cells. Interestingly, HELQ deficiency also compromised the survival of human and mouse cells in the presence of inhibitors of poly(ADP-ribose) polymerase (PARP), which is a hallmark of homologous recombination-deficient cells. Moreover, RAD51D, which is a component of the BCDX2 complex, is involved in resistance to PARP inhibitors.

On the basis of their findings, the authors conclude that HELQ is important for both germ cell maintenance and suppression of tumorigenesis in mammals. These findings might also help to explain the association of non-synonymous variants of HELQ with upper aerodigestive tract cancer, mostly in smokers, and with early menopause. Moreover, the findings indicate a need for screening for HELQ mutations in human cancers, particularly breast and ovarian cancers, because of their association with defects in homologous recombination.

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