impact of mismatches on the recombination efficiency. Their reporter was integrated in the genome in such a way that they could use one Crispr/Cas9 guide RNA to cut near the upstream homology region and a second guide RNA to cut varying distances (16 bp to 28.4 kb) from the downstream homology region.

Using their assay [7], the Stark lab showed that the frequency of repeat-mediated deletions (RMDs) increased when the DSB is close to the repetitive elements and declines rapidly with distance, followed by a very gradual decline up to at least 28.4 kb. At the longer distances, the RMD was much more dependent on Rad52 then at shorter distances. The role of Rad52 in SSA is to help anneal homologous or homologous sequences to form heteroduplexes [1], whereas NHEJ and alt-NHEJ pathways are Rad52-independent. They also showed that even a modest level of mismatch between the repetitive sequences (1–3%) significantly inhibited RMD at all distances. This inhibition by mismatches between repetitive elements was largely dependent on Msh2, which would be expected for a process mediated by heteroduplex rejection [9].

This new study tested the dependence of RMD on various proteins involved in DSB repair. For example, loss of KU70 and XRCC4 (proteins involved in c-NHEJ) increased RMD, while loss of CtIP and BRCA1 (which are involved in the resection pathway) was found to decrease RMD. This indicates that there may be competition between the nonresection and resection pathways (Figure 1). However, the authors found that direct regulation by heteroduplex rejection (Msh2-mediated) is a stronger inhibitor of RMD than competition with c-NHEJ. These data indicate that there could be multiple pathways that suppress RMD between the high concentration of repetitive sequences throughout the genome.

The presence of dispersed Alu elements throughout the genome [3], and other repetitive DNA sequences, provides endless opportunities for RMDs that disrupt genome function. The relatively efficient excision of tens of kilobases of DNA at DSBs means that many copies of Alu may be exposed in single-strand regions, leading to many possible rearrangements. The human genome could not survive the resulting instability if it did not have strong regulators inhibiting these RMDs.

There are a number of situations in which some of the DSB repair pathways are either suppressed or defective in humans. Cancer is the most obvious situation in which some of these genes, such as msh2, brca1, and brca2 are commonly defective. Thus, in various cancers one might expect to see increases in the abundance of RMDs and possibly other changes in the nature of repeat-mediated genomic rearrangements that might lead to more genetic instability, which in turn helps the cancer evolve to a more malignant form.

**References**

1. Ceccaldi, R. (2011) Double-strand break repair. Nature 45, 247–254
2. Symington, L.S. and Gautier, J. (2011) Double-strand break repair. Trends Cell Biol. 21, 524–533
3. Lander, E.S. (2004) The fellowship of functional genomics. Genome Res. 14, 1367–1372
4. Canval, J.M. and Lopes, M.R. (2016) Mechanisms underlying structural variant formation in genomic disorders. Genes Dis. 3, 224–238
5. Belancio, V.P. et al. (2008) Mammalian non-LTR retrotransposons: for better or worse, in sickness and in health. Genome Res. 18, 343–358
6. Morales, M.E. et al. (2015) The contribution of alu elements to mutagenic DNA double-strand break repair. PLoS Genet. 11, e1005016
7. Mendez-Dorantes, C. et al. (2016) Repeat-mediated deletions can be induced by a chromosomal break far from a repeat, but multiple pathways suppress such rearrangements. Genes Dev. 32, 524–531
8. Gunn, A. and Stark, J.M. (2012) I-SceI-based assays to examine distinct repair outcomes of mammalian chromosomal double strand breaks. Methods Mol. Biol. 602, 379–391
9. Sugawara, N. et al. (2004) Heteroduplex rejection during single-strand annealing requires Sp1 helicase and mismatch repair proteins Msh2 and Msh6 but not Pms1. Proc. Natl. Acad. Sci. U. S. A. 101, 9215–9220

---

**Forum**

**Rad5, HLTF, and SHPRH: A Fresh View of an Old Story**

Menattallah Elserafy,1,2,3 Arwa A. Abugable,2,3 Reham Atteya,2 and Sherif F. El-Khamisy1,2,†,‡

Not only have helicase-like transcription factor (HLTF) and SNF2 histone-linker PHD-finger RING-finger helicase (SHPRH) proved to be important players in post-replication repair like their yeast counterpart, Rad5, but they are also involved in multiple biological functions and are associated with several human disorders. We provide here an updated view of their functions, associated diseases, and potential therapeutic approaches.

**RAD5, a Gene All Budding Yeast Laboratories Should Know about**

Yeast has served as a prominent model organism in DNA repair research for decades [1]. Rad5 is a RING-finger-containing ubiquitin ligase and helicase that was discovered in budding yeast and was found to play a role in template switching (TS) and translesion synthesis (TLS) post-replication repair (PRR) mechanisms. TS utilizes a clever mechanism using the newly synthesized sister DNA strand as a template to bypass lesions, while TLS switches replicative polymerases to low-fidelity polymerases to overcome lesions [2].
The wild-type W303 yeast strain used by numerous laboratories harbors a RAD5 mutation (rad5-535). Some researchers have started to use a strain in which the mutation has been corrected. Consequently, conflicting results have been reported in the literature by laboratories using the W303 rad5-535 versus W303 RAD5 strains [3]. While it is important to raise the awareness of yeast researchers to the importance of RAD5 genotype in yeast, it is equally important to highlight how the mammalian Rad5 orthologs, such as HLTF and SHPRH, play multiple important roles in mammalian cells.

HLTF and SHPRH; Rad5 Human Orthologs

HLTF is considered to be the closest ortholog to yeast Rad5; it shows higher sequence conservation to Rad5 than to SHPRH. HLTF contains the conserved HIRAN domain that is responsible for 3’-single-stranded (ss)DNA binding [4], and is able to complement UV sensitivity in the Δrad5 yeast strain. It also possesses double-stranded (ds)DNA translocase activity which is utilized to reverse stalled replication forks [5]. Similarly to yeast Rad5, both HLTF and SHPRH are E3 ubiquitin ligases and have been shown to polyubiquitinate PCNA, in cooperation with Ubc13–MMSS2, in response to DNA damage, thereby directing PRR towards the TS repair pathway [6,7]. The homology between Rad5, HLTF, and SHPRH is depicted in Figure 1A [2].

HLTF and SHPRH in Disease

Cancer

A large body of evidence indicates that HLTF is a tumor-suppressor gene [2]. Methylation and silencing of HLTF have been documented in several cancers such as colon, gastric, esophageal, uterine, lung, hepatocellular, and bladder cancer [2,8]. HLTF promoter hypermethylation in the blood of cancer patients has been investigated as a tumor biomarker to predict prognosis and survival. Moreover, in cervical, thyroid, and head and neck cancers, alternative splicing of HLTF mRNA increases the expression of truncated versions lacking functional DNA repair domains [8]. Heterozygosity in the long arm of chromosome 6, where the SHPRH gene resides, has been reported in malignant melanoma, ovarian, and cervical cancers. Furthermore, SHPRH was found to be truncated or to contain missense mutations in tumor cell lines, which is consistent with a tumor-suppressor function [2].

Interestingly, HLTF was found to be one of the genetic determinants conferring sensitivity or resistance to lysosomal autophagy inhibitors such as hydroxychloroquine (HCQ) in several cancer cell lines [8]. HLTF expression was found to be low in HCQ-sensitive cell lines and high in resistant cell lines. HLTF protected against HCQ-induced cell death because it is essential for repairing the resulting reactive oxygen species (ROS)-induced DNA damage [8]. Because lysosome inhibitors are currently in clinical trials for efficacy as single or combined anticancer agents, HLTF expression profiling of patients may be helpful to determine the response [8] (Figure 1B). We suggest designing HLTF inhibitors for anticancer therapy because combining them with autophagy inhibitors could prove to be synergistic. Moreover, one could question whether cancers with downregulated SHPRH expression would also respond to autophagy inhibitors. Similarly, it is tempting to test whether inhibiting SHPRH would also synergize with autophagy inhibitors (Figure 1B).

HLTF and HIV-1

Upon HIV-1 infection of target cells, viral protein R (Vpr) downregulates HLTF [9]. Vpr is known to recruit CRL4 (DCAF1) E3 ligase to mediate HLTF degradation, and thus facilitates viral replication. A highly conserved zinc-binding motif (HHCH) in Vpr is required for the interaction between Vpr and DCAF1 [9]. Mutations in this motif or the addition of a zinc-chelating agent, TPEN N,N,N,N-tetrais-(2-pyridylmethyl) ethylenediamine, was found to disrupt the Vpr–CRL4 (DCAF1) interaction and therefore their function. Nevertheless, TPEN did not affect the assembly of the cellular CRL4 E3 complex. This opens the door to developing novel antiviral therapies against HIV-1 in which the interaction between Vpr and CRL4/DCAF1 is selectively disrupted [9] (Figure 1B).

HLTF and Inflammatory Bowel Disease (IBD)

Gene expression analysis of regenerating gene (REG) family members has shown that the genes encoding REG-Iα (REG1A), REG-IP (REG1B), and REG-IV (REG4) are overexpressed in IBD. Upregulation of REG genes is beneficial on the one hand because it induces the proliferation of intestinal epithelial cells, protecting them from damage caused by the immune system, but on the other hand upregulation increases the risk of developing colon cancer [10].

A recent study investigating the link between REG genes and IBD reported that REG1A, REG1B, and REG4 are overexpressed in the colon of Crohn’s disease patients, and that REG4 is overexpressed in the colon of ulcerative colitis patients [10]. IL-22 was found to induce REG1B overexpression through activating the REG1B promoter region which contains a binding site for HLTF. Knockdown of HLTF was found to decrease the expression of IL-22-induced REG IP [10]. To prevent the development of colon cancer after intestinal epithelium recovery, the study suggested that anti-IL-22 and anti-REG proteins could be administered to patients. We also propose that developing inhibitors that prevent the interaction between HLTF and the REG1B promoter could be useful to reduce the expression of REG-1B after colon regeneration, and thus reduce the risk of colon cancer (Figure 1B).
(A) Homology between Rad5, HLTF, and SHPRH. Similarly to Rad5, HLTF contains the conserved HIRAN domain. All three proteins contain the RING finger domain embedded between the SNF2 helicase motifs. SHPRH also contains a H15 linker histone H1/H5 domain and a PHD-finger domain. Panel adapted, with permission, from [2].

| Protein | Domains | Length (aa) |
|---------|---------|-------------|
| Rad5   | HIRAN - SNF2 - RING | 1169 |
| HLTF   | HIRAN - SNF2 - RING | 1009 |
| SHPRH  | SNF2 - H15 - PHD - RING | 1683 |

(B) Biological functions and associated diseases for HLTF and SHPRH, and potential therapeutic approaches. HLTF plays a role in the regulation of clock-controlled genes in GH4C1 cells, in mouse embryonic and postnatal brain development, and in collagen biogenesis in the heart. In addition, downregulation or truncation of HLTF and SHPRH have been implicated in different cancers. Cancer cells with low HLTF expression were found to be sensitive to lysosomal autophagy inhibitors. HLTF is also involved in REG1B overexpression, increasing the risk of colon cancer in IBD patients. Zinc-chelating agent prevents HLTF degradation by HIV-1. We propose testing the effect of the lysosomal autophagy inhibitors on cancers with low SHPRH levels. We also propose designing HLTF and SHPRH inhibitors to be tested in combination with lysosomal autophagy inhibitors. Abbreviations: aa, amino acids; IBD, inflammatory bowel disease.
HLTF in Biological Functions

HLTF in the Regulation of Clock-Controlled Genes (CCGs)

The majority of CCGs involved in circadian rhythm regulation lack the specific E-box response element required for regulation. A study was conducted on the GH4C1 rat pituitary somatotroph cell line to investigate the mechanism of transcription of prolactin (Prl), a CCG that lacks the specific E-box element. HLTF was found to interact with PIT-1 transcription factor at the P2 region of the Prl promoter, which is different from the specific E-box that is usually bound by CCGs. HLTF and PIT-1 are not rhythmically expressed; however, HLTF interacts with NONO and SFPQ proteins which rhythmically bind to the Prl gene. Therefore, this leads to Prl expression that is necessary for maintaining the normal circadian pattern. These findings have uncovered a novel role for HLTF in the regulation of the oscillatory expression of CCGs that lack the specific E-box response element [11] (Figure 1B).

Concluding Remarks

There is increasing interest among scientists in discovering different roles for the Rad5 domain of helicase-like transcription factor (HLTF) and its orthologs, HLTF and SHPRH in DNA damage tolerance. There is increasing interest among scientists in discovering different roles for the Rad5 domain of helicase-like transcription factor (HLTF) and its orthologs, HLTF and SHPRH in DNA damage tolerance.

HLTF in Mouse Embryonic and Postnatal Development

HLTF has been shown to play crucial roles in embryonic and postnatal development of the murine heart and brain [12,13]. In mouse heart, HLTF is necessary for hypoxia-inducible factor 1α (HIF-1α) regulation to achieve normal collagen biogenesis. In addition, HLTF regulates the expression of the cohesion and condensin complexes, as well as of cell-cycle regulators, during embryonic development of mouse brain. Therefore, a significant percentage of HLTF null mice die, and those that survive suffer from DNA damage, increased apoptosis in brain, brain encephalomalacia, G2/M transition defects, and fibrillar network disorganization [12,13] (Figure 1B).

References

1. Abougabe, A.A. et al. (2017) Personalised medicine: genome maintenance lessons learned from studies in yeast as a model organism. Adv. Exp. Med. Biol. 1007, 157–178
2. Unk, I. et al. (2010) Role of yeast Rad5 and its human ortholog, HLTF and SHPRH in DNA damage tolerance. DNA Repair 9, 257–267
3. Ellersayf, M. and El-Khamisy, S.F. (2018) Choose your yeast strain carefully: the RAD5 gene matters. Nat. Rev. Mol. Cell Biol. Published online April 3, 2018. http://dx.doi.org/10.1038/s41580-018-0005-2
4. Hishiki, A. et al. (2019) Structure of a novel DNA-binding domain of helicase-like transcription factor (HLTF) and its functional implication in DNA damage tolerance. J. Biol. Chem. 296, 13215–13223
5. Blastyak, A. et al. (2010) Role of double-stranded DNA translesion activity of human HLTF in replication of damaged DNA. Mol. Cell. Biol. 30, 684–693
6. Unk, I. et al. (2006) Human SHPRH is a ubiquitin ligase for Mms2-Ubc13-dependent polyubiquitylation of proliferating cell nuclear antigen. Proc. Natl. Acad. Sci. U. S. A. 103, 18107–18112
7. Unk, I. et al. (2008) Human HLTF functions as a ubiquitin ligase for proliferating cell nuclear antigen polyubiquitination. Proc. Natl. Acad. Sci. U. S. A. 105, 3768–3773
8. Piao, S. et al. (2017) ALDH1A1 and HLTF modulate the activity of lysosomal autophagy inhibitors in cancer cells. Autophagy 13, 2056–2071
9. Wang, H. et al. (2017) Inhibition of Vpx-mediated SAMHD1 and Vpr-mediated host helicase transcription factor degradation by selective disruption of viral CRL4 (DCAF1) E3 ubiquitin ligase assembly. J. Virol. 91, e00225-17
10. Tsuchida, C. et al. (2017) Expression of REG family genes in human inflammatory bowel diseases and its regulation. Biochem. Biophys. Rep. 12, 198–205
11. Guillaumond, F. et al. (2011) Chromatin remodeling as a mechanism for circadian-protein transcription rhythmic NONO and SFPQ recruitment to HLTF. FASEB J. 25, 2740–2756
12. Helmer, R.A. et al. (2013) Role of helicase-like transcription factor (Hlf) in the G2/M transition and apoptosis in brain. PLoS One 8, e50225-17
13. Helmer, R.A. et al. (2013) Helicase-like transcription factor Hlf regulates G2/M transition, Wt1/Gab4/Hif-1α cardiac transcription networks, and collagen biogenesis. PLoS One 8, e60461

*Correspondence: s.el-khamisy@sheffield.ac.uk (S.F. El-Khamisy).
https://doi.org/10.1016/j.tig.2018.04.006