Editorial

A novel mechanism of regulation of SHPRH by circular RNA, circ-SHPRH in glioblastoma

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Maintenance of genomic integrity is critical for the control of cell proliferation and survival. The genome is susceptible to DNA damage, which generates DNA lesions blocking the progression of DNA replication. Cells have multiple mechanisms to respond to DNA damage during replication; stabilization of the stalled replication fork, bypass the damage using specific DNA polymerases or recombination, or converting the damage into a second lesion. Responses by cells to DNA damage by these mechanisms can either activate DNA-repair pathway or programmed cell death (1). Failure or abnormal responses to DNA damage results in accumulation of mutations and promotes transformation of normal cells into cancer cells.

A recent paper by Zhang *et al.* described a potential role of short circular form of the SHPRH (SNF2 histone linker PHD RING helicase) as a suppressor of glioma tumorigenesis (2). In this paper, authors characterized circular RNAs (circRNAs) from glioma and adjacent normal brain tissues by using deep RNA-seq with computational approaches. They found that a circular form of *SHPRH* gene encodes a novel short form of SHPRH protein (SHPRH-146aa), which was less expressed in glioblastoma patient samples compared to surrounding normal brain tissues. They showed that SHPRH-146aa affected the stability of a full-length SHPRH protein and suggested that it possibly acted as a tumor suppressor in brain.

circRNAs are covalently closed RNAs generated by a back-splice event of pre-mRNA. A downstream 5' splice donor site is ligated to an upstream 3' acceptor site (3). Due to its absence of free 5' and 3' termini, circRNAs are resistant to degradation by RNA exonuclease and have long half-lives (4). Notably circRNAs are enriched in neural tissues especially in brain (5). The low proliferation rate of neuronal cells may account for the high level of circRNAs in brain as more stable circRNAs can accumulate without degradation. In contrast, the expression of circRNAs are shown to be less abundant in cancerous tissues with high proliferation rate underscoring the relation between circRNAs and proliferation (6). Recent studies have suggested functions of circRNAs in regulating gene expression; some circRNAs regulate gene expression by competing with host mRNAs for miRNA binding as miRNA sponges and other circRNAs has been reported to associate with RNA polymerase II and promote the transcription of their parental genes (7,8). Thus circRNAs can be functionally involved in carcinogenesis either by generating novel cancer promoting circRNAs or by regulating expression of oncogenic proteins (3). These features also make circRNAs as potential cancer biomarkers.

Zhang *et al.* provided evidence that the circSHPRH contained 440 nucleotides and the expression level was decreased in glioblastoma cells compared to adjacent normal brain tissues. For the detection of protein coding circRNAs, Zhang *et al.* used the approach with deep RNA sequencing with ribosomal RNA depletion. The authors further validated the circSHPRH was translated to a functional 144 amino acids of SHPRH (circSHPRH) by targeted mass spectrometry and LC-MS. The circSHPRH seems to protect the degradation or enhance the stability of the full length SHPRH since the expression of

full-length SHPRH protein was increased following overexpression of circSHPRH (2). These results imply that the circSHPRH contributes to genome stability from replication stress through regulating the full length SHPRH.

Human *SHPRH* gene is located at the chromosome 6q 24 and has been suggested as a putative tumor suppressor (9). It is a functional ortholog of *S. cerevisiae* Rad5, a DNA repair protein having Swi2/Snf2 chromatin remodeling and DNA dependent ATPase activity. Rad5 also contains a RING finger motif that mediates ubiquitin ligase activity and this activity is important for polyubiquitination of proliferating cell nuclear antigen (PCNA) in post replication repair (PRR) pathway (10-12).

PCNA, the replicating sliding clamp is an evolutionary well conserved protein found in all eukaryotes and plays an essential role in DNA replication and repair. The proper control of replication process is crucial for genomic integrity and when replication forks encounter DNA damage, they can stall to repair DNA lesions by DNA damage response mechanisms. However, persistently stalled replication forks may fail to restart and collapse to cause severe genome instability (13). To suppress these detrimental effects, cells have evolved the PRR pathways; either translesion synthesis (TLS) bypassing DNA lesions by TLS polymerases or template switching (TS) to the nascent strand of sister chromatids (14). The posttranslational modifications of PCNA regulate the choice of the PRR pathways. Upon various DNA damage and replication stress, PCNA is either mono- or polyubiquitinated on the lysine 164 (K164) residue (15). Monoubiquitination of PCNA by Rad18 and Rad6 promotes error-prone DNA lesion bypasses by recruiting TLS polymerases to the replication forks whereas Rad5 dependent K63-linked polyubiquitination of PCNA facilitates error-free TS pathway (15). The K63-linked polyubiquitination of PCNA promotes the TS in a proteasome independent manner and this is different from canonical K48-linked polyubiquitination that leads protein degradation (16).

Human SHPRH physically interacts with the human PCNA, RAD18, and ubiquitin-conjugating enzyme UBC13 and also promotes DNA damage induced PCNA polyubiquitination (17,18). Human Rad5 family includes SHPRH and helicase like transcription factor (HLTF). Both SHPRH and HLTF are involved in polyubiquitination of PCNA and loss of either SHPRH or HLTF increases genome instability following DNA damage suggesting they are functional homologs of Rad5 (17,19). The predicted functional domains in SHPRH include SWI2/SNF2, RING helicase and PHD domain (9,20). Zhang *et al.* showed a translated form of human circSHPRH is generated from exons 26–29 harboring helicase domain of SHPRH. They found that the overexpression of SHPRH-146aa increased the level of full length SHPRH protein without affecting the level of mRNA. The SHPRH-146aa also includes putative ubiquitination site that can be a target of ubiquitin ligase (2). The SHPRH-146aa may protect SHPRH from ubiquitination *in vivo* and enhance its stability, which could promote tumor suppressive functions of SHPRH through regulation of posttranslational modification of PCNA upon DNA damage.

In addition to functions in DNA repair and PCNA polyubiquitination, SHPRH has been shown to regulate ribosomal RNA (rRNA) transcription through its plant homeodomain (PHD) (21). The PHD of SHPRH interacts with histone H3 in an mTOR-dependent manner and the presence of SHPRH is important to recruit RNA polymerase I to the rDNA promoter (21). Transcription of rRNA accounts for more than 50% of RNA synthesis in a cell and rRNA transcription is involved in many cellular processes including cell cycle regulation, differentiation, and metabolic processes (22-24). Due to its multiple physiological functions, abnormal rRNA transcription has been reported in many cancers (25). Several point mutations of SHPRH gene were found in multiple human cancer cells (17). Although it is still unclear which function of SHPRH has link to carcinogenesis, it is possible that the SHPRH-146aa secures the regulation of rRNA transcription by SHPRH to suppress tumorigenesis.

Zhang *et al.* explored the potential clinical implications of SHPRH-146aa and observed down-regulation in 81% glioblastoma samples and patients with higher level of SHPRH146aa in their tumor survive longer. Moreover, overexpression of SHPRH-146aa resulted in lower tumorigenicity suggesting SHPRH-146aa as a potential prognostic biomarker of glioblastoma.

The study provides a novel insight of potential role of circRNAs in carcinogenesis and the clinical implication of translated peptides. In additions, this study uncovers a new mechanism of regulation of SHPRH protein stability and its importance in glioblastoma. Further studies for molecular mechanism of SHPRH-146aa as a tumor suppressor will deepen our understandings of coding circRNAs and improve the prognosis of cancer.

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Footnote

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