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Shugoshin: From a perspective of clinical disorders

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ABSTRACT

Proper and timely segregation of cellular genome is an important and a prime requirement of all cell division programmes. Mis-segregation of chromosomes and resulting aneuploidy leads to several clinical consequences. Over the years, shugoshin emerges as a key protein factor involved in the segregation of genetic material in dividing cells. Deletion or altered level of shugoshin is reported in several human malignancies, as a result, shugoshin now emerges as an important tumour associated gene and a possible target for cancer therapy. Apart from the role in cancer, recent studies also showed the involvement of shugoshin in several other clinical disorders. Through this review, we tried to highlight the clinical relevance of shugoshin.

INTRODUCTION

Proper cell division is a foremost requirement for reproduction as well as for the survival and continuity of every species. Mis-segregation of the genome during cell division leads to aneuploidy, which is closely associated with numerous medical consequences ranging from tumorigenesis to sterility, mental retardation, spontaneous abortion, and other birth-related defects [1-6]. To make sure that, the genetic blueprint is duplicated and distributed precisely during cell division, cell employs several mechanisms operating either independently or in coordination with one another. Proper and timely removal of cohesin is an example of one such mechanism. Cohesin, a multiprotein complex holds sister chromatids together since DNA duplication in S-phase till the onset of anaphase. The premature or untimely loss of cohesion as a result of abrupt separase activity leads to chromosome mis-segregation. Hence cohesin cleavage by separase is kept under tight cellular control [7,8]. Apart from its prime role of holding sister chromatids together, cohesin is also known for its involvement in diverse cellular processes discussed elsewhere [9]. The detailed account of cohesin and separase falls outside the scope of the present review and same are summarized elsewhere [8,10]. Apart from the timely cleavage of cohesin, several other mechanisms including DNA damage checkpoint (DDC), spindle assembly checkpoint (SAC), separase activation, centriole duplication (and maybe more which remains unidentified) make sure that the genetic endowment of the cells or organisms i.e. its genome is duplicated and separated properly. The detailed discussion of all such mechanisms is difficult in the present review, may require separate volume and can be found elsewhere [8,11-16].

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In this review, we will mainly focus on the shugoshin, a protein factor required for the protection of centromeric cohesin. The intended purpose of this review is to familiarize readers with the medical conditions in which shugoshin was implicated. Before discussing clinical association of shugoshin, we will gave bird eye view of shugoshin identification, cellular localization, conserved nature, distribution in eukaryotes and finally shugoshin as an emerging tumour associated gene and as a potential target for cancer therapy. The present review does not include description of shugoshin in a cell cycle (mitosis and meiosis) as those topics are already discussed in the recent reviews [17-20].

SHUGOSHIN BACKGROUND

Shugoshin (for guardian spirit from Japan) is a homo-dimeric phospho-protein belonging to a shugoshin protein family [21,22]. Shugoshin is conserved from single-celled yeast to multicellular mammals including humans. Shugoshin shares several structural features with other members of the shugoshin family including a basic region at the C-terminus which is essential for centromere binding, chromosome localization and an N-terminal coiled-coil domain that may regulate its dimerization and interaction with other proteins [23,-26]. Initially, shugoshin was discovered in the fruit fly, *D. melanogaster* as a peri-centromeric protein (at the time referred as MEI-S332) required for the protection of Rec8 (meiotic-specific cohesin subunit) from separase action and its persistence during meiosis-I [27-29]. Later, a protein factor with a function equivalent to MEI-S332 was discovered in other eukaryotic species including yeast, insects, vertebrates and plants [30-35].

Based on the sequence (at gene and protein level) and structural analysis it is observed that eukaryotic species studied till date possess either one or two genes coding for shugoshin (referred as *SGO1* and *SGO2*), although several splicing isoforms of shugoshin have been reported in higher eukaryotes [36]. Table1, shows the number of genes coding for shugoshin in different species. The reason why some species (example *Saccharomyces cerevisiae*) possess only one gene for shugoshin and others two (example fission yeast, humans) remains elusive. In human cells, a combined total of 10 splicing isoforms (for *SGO1* and *SGO2*) have been identified (http://www.uniprot.org/uniprot/?query=hugoshin%2C+homo+sapiens&sort=score). Information related to different isoforms of *SGO1* and *SGO2* including number of amino acid residues, molecular mass is given in table 2. Size or number of amino acid residues in shugoshin and molecular mass vary significantly across different eukaryotic species [35] as well as among

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different isoforms within a same species (example *H. sapiens*, table 2). It is important to mention that different shugoshin paralogue is known to exhibit different properties depending on the species under consideration. The expression pattern or profile of shugoshin paralogs may be cell cycle-dependent (i.e., mitosis or meiosis). For example, in fission yeast *SGO2* is expressed in both mitosis and meiosis while *SGO1* is meiosis-specific [30]. Just like fission yeast, mice *SGO2* is required for the completion of meiosis but not for mitosis suggesting cell cycle specific expression [37].

Although shugoshin is present in all the eukaryotic species studied till date and shugoshin-based protection of centromeric cohesin is conserved across different eukaryotic species, cells of *C. elegans* use different strategy which is independent of shugoshin. Unlike other species, chromosomes segregation in *C. elegans* relies on an alternative mechanism which involves *LAB-1* (Long Arm of the Bivalent) [38]. Study from *C. elegans* raised the possibility of shugoshin independent cohesin protection in other species. Why cells of *C. elegans* use this alternative mechanism despite the presence of shugoshin remains open question. Whether shugoshin independent protection of centromeric cohesin is exclusive to worms species also remain matter of future investigation.

CELLULAR LOCALIZATION

So far shugoshin has been detected or observed in the nucleus in close association of chromosomes at kinetochore or centromere, at spindle pole body (or SPB, the functional equivalent of centrioles of higher eukaryotes) and more recently at the sub-telomeric region of the chromosome [30,39-43]. Cellular localization of shugoshin in yeast and the mammalian cell is shown in Figure 1A and 1B respectively. By looking at Figure 1, it can be inferred that the overall cellular localization of shugoshin is similar in yeast and mammalian cells despite a huge evolutionary distance. Similar cellular localization of shugoshin in yeast and mammalian cells shows the importance or suitability of yeast as a model system. It is important to mention that in budding yeast, the nuclear membrane always remains intact (closed mitosis) and SPB always remain embedded in the nuclear envelope. In the case of mammalian cells, the nuclear envelope is lost completely (open mitosis), and centrioles always remain in the cytoplasm. Therefore, the possibility of novel shugoshin interactors and role in yeast as well as in mammalian cells cannot be ruled out. Therefore, identification of novel interactors or function represents both opportunities and challenges in front of contemporary

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biologist working in this direction. We gave comparative localization of human and yeast cells as great deal of information related to shugoshin is gathered using yeast.

SHUGOSHIN AS TUMOUR ASSOCIATED GENE

Being an important player in cell cycle, cells make sure that shugoshin is present only when and where needed [44]. Cells also maintain optimum level of shugoshin by regulating its expression and degradation through APC/C (anaphase promoting complex/cyclosome) [45]. Complete absence or altered level (both more and less than the optimum level) of shugoshin may lead to tumorigenesis. Plenty of studies are now available supporting both the oncogenic as well as tumour suppressor nature of shugoshin. Therefore, it will be fair enough to consider shugoshin as a tumour associated gene (Figure 2). Cellular abundance of shugoshin is so critical that even one-fold change in its cellular abundance is enough for tumour induction [46].The role of shugoshin in cancer is confirmed not only from studies in non-human subjects like rats or mice but also from tissue samples collected from actual cancer patients. Through this section of this review, we highlighted some of the recent studies where complete absence or altered level of shugoshin was associated with cancer.

SHUGOSHIN AS TUMOUR SUPPRESSOR GENE

Decreased level or complete absence of shugoshin was observed in head and neck cancer [47], nasopharyngeal carcinoma [48], neuroblastoma [49], and prostate cancer [50,51]. Homozygous deletion of *SGOL2* was observed in different types of human tumours including head and neck cancer [52], small-cell lung carcinoma [53], cervical carcinoma [54], and neuroblastoma [55]. It important to mention that absence or deletion of either shugoshin (i.e., *SGOL1* or *SGOL2* for shugoshin like in humans) can lead to cancer. Among the 46 colorectal cancer cases, hSgo1 mRNA expression was decreased in the tumour tissue in comparison with the corresponding normal tissue [56]. Heterozygous deletion of sgo1+/- leads to systemic chromosome instability in mice [57] and the formation of ACF (aberrant crypt foci) in mice heterozygous for shugoshin-1 [58]. Treatment with the carcinogen azoxymethane (oxide of azomethane, carcinogenic and neurotoxic chemical compound used in biological research) caused sgo1+/- ME-CIN model mice to develop HCCs (Hepatocellular carcinoma) within 6 months, whereas control mice developed no HCC ($P < 0.003$) [59]. These studies showed the tumour suppressor nature of shugoshin.

Although *SGO1*+/- mice are viable and fertile but showed enhanced colonic tumorigenesis. Enhanced CIN (Cervical intraepithelial neoplasia) observed in sgo1-deficient mice leads to an

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increase in the formation of an ACF and an accelerated development of tumours on exposure to azoxymethane [60]. The *SGOL1-P1* (one of the splicing form of shugoshin in humans) transcript containing an exon-skip of exon 3 that results in a stop codon occurring within exon 4 whose over-expression in human HCT116 cell line resulted in an increased number of cells with aberrant chromosome alignment, precociously separated chromatids and delayed mitotic progression, occasionally followed by inaccurate distribution of the chromosomes [61]. Down-regulation of shugoshin-1 leads to CIN in colorectal cancer cells [56]. Mice heterozygous for shugoshin-1 showed mild proneness to spontaneous lung and liver cancers. In a recent study using adoptive (T/B-cell based) immunity-deficient RAG1(-/-) (Recombination activating gene) sgo1(-/+) double-mutant mice developed lung adenocarcinomas more aggressively compared to sgo1(-/+) or RAG1(-/-) mice, suggesting immune system involvement in CIN-mediated lung carcinogenesis [62]. Up-regulated expression of shugoshin was observed in 82% of hepatocellular carcinoma (HCC) and correlated with elevated alpha-fetoprotein and early disease onset of HCC while depletion of shugoshin-1 reduced cell viability of hepatoma cell lines including HuH7, HepG2, Hep3B, and HepaRG due to persistent activation of the spindle assembly checkpoint [63].

SHUGOSHIN AS AN ONCOGENE

Increased expression and level of shugoshin was reported in human leukemia [64], and breast cancers [65,66]. Similarly, overexpression of *SGOL1-B1* in an NSCLC (Non-small-cell lung carcinoma) cell line induced aberrant chromosome mis-segregation, precociously separated chromatids, and delayed mitotic progression. A Higher level of SGO1-B mRNA was related to taxane [Diterpenes, compounds originally identified in plant genus Taxus (yews), used in cancer chemotherapy e.g., Paclitaxel and docetaxel] resistance, while the forced down-regulation of SGO1-B increased the sensitivity to the taxane [67]. Expression of *SGO1C* (a non-functional isoform of shugoshin) alone induced aberrant mitosis similar to depletion of *SGO1A*, promoting premature sister chromatid separation, activation of the spindle assembly checkpoint, and mitotic arrest suggesting that the expression of *SGO1C* is tightly regulated to prevent dominant-negative effects of *SGO1A* and genome instability [68]. In another clinical study, expression of SGO1 in human prostate tumors were higher than that of adjacent normal tissues and were positively correlated with the poor prognosis of prostate cancer patients [69]. Some of the studies mentioned above clearly showed the oncogenic nature of shugoshin. mentioned above clearly showed the oncogenic nature of shugoshin.

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Based on the studies mentioned in this section, it can be said that shugoshin can acts as an important target for medical intervention in cancer therapy. Not only complete loss of shugoshin but, the altered level of shugoshin can also lead to cancer. Whether shugoshin association with cancer is due to chromosome mis-segregation or due to derailment of other cellular pathways as a result of complete absence or altered level of shugoshin remains a topic of future research. Since the altered level of shugoshin is associated with diverse cancer, and chemicals [example BPA or Bisphenol A, used as plasticizer in plastic industries] can potentially alter its expression, it might be possible that increased incidences of tumour and associated altered shugoshin level may be linked and need further research [70]. The identification of chemicals that can modulate transcription of shugoshin as well as other tumour associated gene would be an important field of future research.

Both complete loss or absence as well as increased expression or increased cellular abundance of shugoshin leads to cancer. Whether oncogenic and tumour suppressor nature of shugoshin modulate same cellular pathways remains unknown.

SHUGOSHIN IN OTHER CLINICAL DISORDERS

In last section we mentioned the tumour associated nature of shugoshin. But cancer or tumour is not only clinical conditions where shugoshin is involved. Research over last several years also implicated shugoshin in other serious medical conditions. Through this section we would like to draw attention towards some of those clinical disorders. Example, recently it was shown that mutations of SGO2 (frameshift, p.Glu485Lysfs*5) and CLDN14 collectively cause coincidental Perrault syndrome which is a rare autosomal genetic disorder characterized by sensorineural hearing loss (SNHL) in males and females and ovarian dysfunction in females [71,72]. Some of the studies in last few years also showed the involvement of shugoshin in neurological disorders like late-onset Alzheimer's disease (LOAD) [73,74]. It is important to mention that not all the diseased conditions are due to chromosome instability, which is induced by impaired shugoshin function of centromeric cohesion. For example, Chronic Atrial and Intestinal Dysrhythmia (CAID) is not due to chromosome instability due to impaired shugoshin at the centromere [75,76].

FINAL CONCLUSIONS AND FUTURE DIRECTIONS

Based on the results from different scientific groups working on a various model system and tissue samples from cancer patients, it can be clearly said that shugoshin is an important tumour associated gene. Apart from role in cancer, shugoshin is also involved in several other medical

conditions. It important to mention that shugoshin associated pathies may also happen even when there is no observed chromosomal instability and shugoshin may still be involved. The way shugoshin is associated with different clinical conditions, it may be possible the list of shugoshin associated pathies may increase in the future. In such a scenario, the availability of high-resolution atomic structure of shugoshin will help in designing or screening for small molecules with applications in cancer therapy and other diseases. Apart from this, availability of structure will help in better understanding shugoshin localization and its interaction with other cellular proteins or complexes. The availability of high-resolution structure will also be important in better understanding the way shugoshin is involved in different diseases. The diverse and big size of shugoshin pose a big challenge for structure biologist [24,77]. Apart from this, innately disordered regions in most shugoshin proteins make it difficult to get a high-resolution structure. The presence of different splicing isoforms of shugoshin in higher eukaryotes poses another hurdle in structural studies. Furthermore, we are still not aware of the cellular abundance of shugoshin, how it is regulated, and what protein factors and signaling is involved in maintaining the optimum level of shugoshin in a cell. Therefore, future research on shugoshin will be important and rewarding from point of basic science as well as from prospective of medical science.

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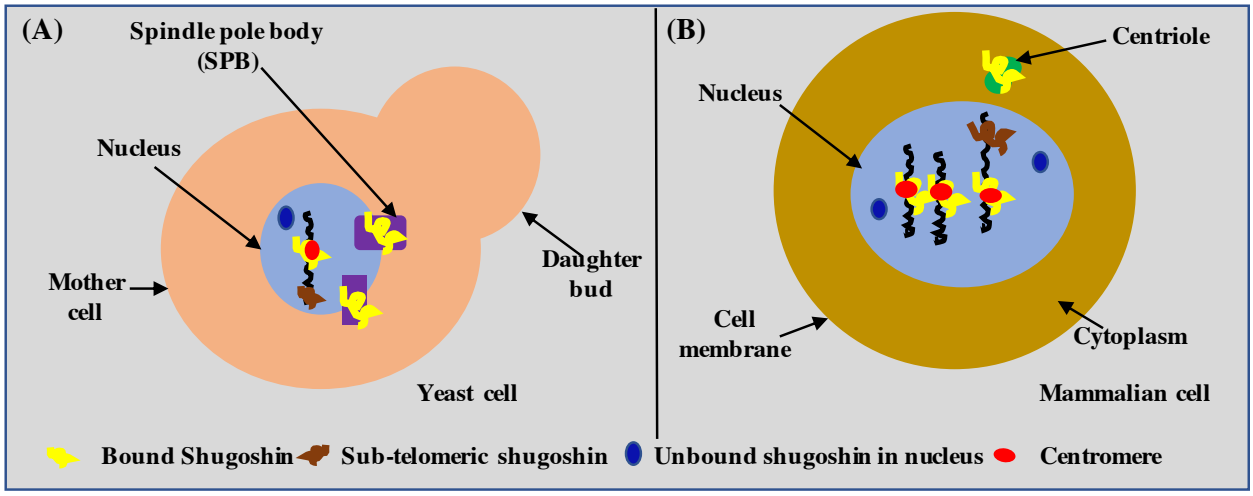

Figure 1. Cellular localization of shugoshin. Localization of shugoshin in (A) budding yeast and (B) mammalian cell. Note: Diagrams are for demonstration purpose only.

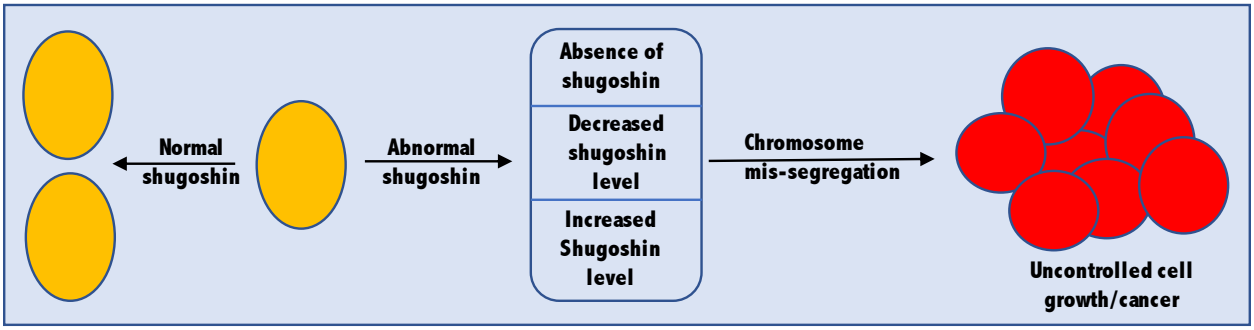

Figure 2. Shugoshin as tumour associated gene. Complete absence as well as increased or decreased level of shugoshin was found to be associated with different type of cancers.

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Table 1. Number of genes coding for shugoshin in different species.

Species	No. of Genes	Kingdom	References
Saccharomyces cerevisiae	1	Fungi	*
Schizosaccharomyces pombe	2	Fungi	*
Mus masculus	2	Animalia	Uniprot
Arabidopsis thialana	2	Plantae	Uniprot
§Homo sapiens	2	Animalia	Uniprot
Drosophila melanogaster	1	Animalia	Uniprot
Caenorhabditis elegence	1	Animalia	Uniprot
Oryza sativa	1	Plantae	Uniprot
Xenopus laevis	1	Animalia	Uniprot
Neurospora crassa	1	Fungi	Uniprot
Danio rerio	1	Animalia	Uniprot
Zea mays	1	Plantae	Uniprot
Rattus norvegicus	1	Animalia	Uniprot
Candida glabrata	1	Fungi	*
Kluyveromyces lactis	1	Fungi	*
Aphis gossypii	1	Fungi	*
Pristionchus pacificus	1	Animalia	Uniprot
Oryzias latipes	1	Animalia	Uniprot
Candida albicans	1	Fungi	*

*https://portals.broadinstitute.org/cgibin/regev/orthogroups/show_orthogroup.cgi?orf=YOR073W.
Uniprot (www.uniprot.org/uniprot).
§ Total ten isoforms have been reported in humans.

Table 2. Size, molecular mass of different isoforms of human SGO1 and SGO2

Shugoshin	Isoform	Number of amino acid residues	Mol. Mass (in kDa)	Identifier
*SGO1	Isoform 1	561	64.19	Q5FBB7-1
	Isoform 2	309	35.344	Q5FBB7-2
	Isoform 3	292	33.501	Q5FBB7-3
	Isoform 4	275	31.276	Q5FBB7-4
	Isoform 5	258	29.433	Q5FBB7-5
	Isoform 6	527	60.122	Q5FBB7-6
	Isoform 7	215	24.646	Q5FBB7-7
!SGO2	Isoform 1	1265	144.739	Q562F6-1
	Isoform 2	1261	144.181	Q562F6-2
	Isoform 3	247	28.23	Q562F6-3

*https://www.uniprot.org/uniprot/Q5FBB7
! https://www.uniprot.org/uniprot/Q562F6