Molecular & Cellular Oncology 2:4, e1002717; October/November/December 2015; Published with license by Taylor & Francis Group, LLC

Suppression of ectopic assembly of centriole proteins ensures mitotic spindle integrity

Gen Shiratsuchi and Daiju Kitagawa*

[1]Centrosome Biology Laboratory; Center for Frontier Research; National Institute of Genetics; Mishima, Shizuoka, Japan

Keywords: cartwheel, centriole, centrosome, chromosome segregation, genome integrity

Abbreviations: CLEM, correlative light electron microscopy; HsSAS-6, homo sapiens SAS-6; MTOC, microtubule organizing center; PCM, pericentriolar material; RBM14, RNA binding motif protein 14.

Abnormalities in maintaining the appropriate number of centrioles could be the origin of genome instability in tumor formation. Recently, we demonstrated that ectopic formation of aberrant centriole-related structures occurs even in the presence of pre-existing centrioles, leading to mitotic spindle defects and possibly contributing to tumorigenesis.

The centrosome is the major microtubule organizing center (MTOC) of animal cells and is composed of 2 centrioles and surrounding pericentriolar material (PCM). Precise regulation of the centriole number during the cell cycle is crucial for proper bipolar spindle formation and thus for genome integrity. Recent studies have indeed highlighted that centriole amplification can cause genome instability in tumor formation.[1] However, the underlying mechanisms for accurate control of centriole copy number remain incompletely understood.

Centriole formation starts with the assembly of a so-called cartwheel structure onto which microtubules are in turn added (**Fig. 1**, upper). The spindle assembly abnormal protein 6 (SAS-6) family of proteins have been shown to be evolutionarily essential for centriole formation, and were recently identified as crucial components of the inner part of the cartwheel.[2,3] A conserved binding partner of SAS-6 family proteins, SCL/TAL1 interrupting locus (STIL; also known as anastral spindle 2 [Ana2] in Drosophila or spindle assembly abnormal protein 5 [SAS-5] in C.elegans), also plays a critical role in centriole formation through physical interactions with the core centriolar components centromere protein J (CENPJ, also known as CPAP or spindle assembly abnormal protein 4 [SAS-4] in C.elegans) and polo-like kinase 4 (PLK4).[4-6] In addition to the canonical mode of centriole formation in which a new centriole assembles in the vicinity of the parental centriole, *de novo* assembly of centrioles in the cytoplasm can be induced in the case of natural or physical loss of pre-existing centrioles.[7] Although it is believed that this ectopic assembly of centrioles is normally suppressed in proliferating cells, how it is achieved remains unknown. Recently, we reported that RNA binding motif protein 14 (RBM14), a potential tumor suppressor, is a novel suppressor of ectopic assembly of centriole-related structures in human and mouse cells.[8]

It has been shown that RBM14 is a component of the nuclear paraspeckle, a small intranuclear structure that contains RNA and several ribonucleoproteins, and functions in transcriptional regulation.[9] Using mass spectrometry-based proteomic analysis, we identified RBM14 as a STIL-interacting protein. RNAi-mediated depletion of RBM14 in human cells resulted in dramatic amplification of structures containing several centriolar proteins in the cytoplasm. The ectopic formation of such "centriolar protein complexes" (**Fig. 1**, middle) occurred independently of reduction of known RBM14 function in transcriptional regulation. Moreover, we found that RBM14 in the cytoplasm normally suppresses complex formation between STIL and CPAP by direct association with STIL during interphase. We presume that, in the absence of RBM14, STIL forms excessive complexes with CPAP in the cytoplasm, leading to the ectopic assembly of centriole proteins. Our correlative light electron microscopy (CLEM) analysis showed that most of these protein complexes were unstructured, and seemed not to be genuine centrioles. Intriguingly, however, a microtubule renucleation assay demonstrated that some of them retained the ability to nucleate microtubules. Given that CPAP provides a scaffold for the assembly of PCM components in the cytoplasm, we assume that the ectopic STIL/CPAP complex could be a recruiting site for PCM components for microtubule nucleation.

Since the ectopic assembly of centriole proteins induced by RBM14 depletion

© Gen Shiratsuchi and Daiju Kitagawa
*Correspondence to: Daiju Kitagawa; Email: dkitagaw@nig.ac.jp
Submitted: 12/17/2014; Revised: 12/21/2014; Accepted: 12/22/2014
http://dx.doi.org/10.1080/23723556.2014.1002717
This is an Open Access article distributed under the terms of the Creative Commons Attribution-Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.

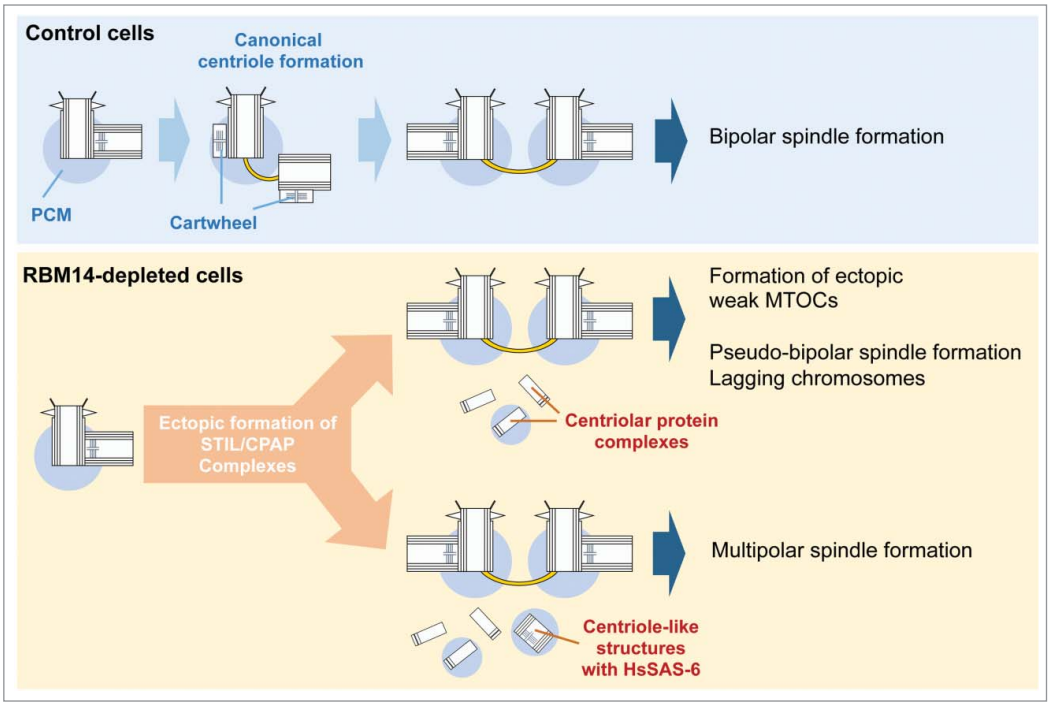

Figure 1. Putative mechanism whereby RBM14 depletion induces the ectopic formation of structures related to centrioles and affects mitotic spindle integrity. First, depletion of RNA binding motif protein 14 (RBM14) promotes ectopic formation of SCL/TAL1 interrupting locus (STIL)/centromere protein J (CENPJ, also known as CPAP) complexes, leading to ectopic assembly of the centriolar protein complexes in the cytoplasm. Although these protein complexes seem to contain pericentriolar material (PCM) and function as microtubule organizing centers (MTOCs), most of them probably form pseudo-bipolar spindles, sometimes accompanied by lagging chromosomes. A proportion of these protein complexes that further obtain other critical centriolar components such as human spindle assembly 6 homolog (HsSAS-6), a crucial component of the cartwheel, grow into structures that are more closely related to centrioles and form extra spindle poles.

was not dependent on human SAS-6 (HsSAS-6), it is likely that a cartwheel structure is dispensable for this process. In addition, live-cell imaging assays revealed that *de novo* formation of centriolar protein complexes took place in the cytoplasm and that these complexes gradually increased in size during S phase. Furthermore, our live CLEM analysis revealed that some of them subsequently incorporated other critical centriolar components such as HsSAS-6 and grew into more complete "centriole-like structures" (**Fig. 1**, bottom). Considering that *de novo* formation of premature centrioles induced by physical removal of pre-existing centrioles is known to occur during S phase,[7] there might be a close linkage between the 2 assembly mechanisms involving centriolar proteins.

As a consequence of these events the formation of bipolar spindles was significantly affected, which frequently resulted in the appearance of multipolar spindles and "pseudo-bipolar spindles", bipolar spindles with clustered centriolar protein complexes that were sometimes accompanied by lagging chromosomes. Notably, we also found a positive correlation between the frequency of HsSAS-6 recruitment to those ectopic structures and multipolar spindle formation, suggesting that the centriole-like structures with HsSAS-6 possess more robust MTOC activity than the unstructured centriolar protein complexes (**Fig. 1**, bottom).

In contrast to our understanding of canonical centriole formation, the mechanisms of ectopic assembly of centriole proteins have not been well characterized until recently. However, over the past several years, centrosomal protein 76kDa (CEP76), neutralized E3 ubiquitin protein ligase 4 (NEURL4), and lectin galactoside-binding soluble 3 binding protein (LGALS3BP) were reported to function in the ectopic formation of centriole-related structures. Intriguingly, the extent of MTOC activity and the components of the ectopic structures that are induced following depletion of these proteins are somewhat divergent. Further studies will therefore be needed to investigate such aberrant assembly of centriolar components and uncover the mechanisms by which they can become functional structures serving as MTOCs.

Considering these findings, in this study, we suggested a novel mechanism whereby centriolar proteins can assemble independently of a cartwheel structure in human cells. We also demonstrated that depletion of RBM14 in the early mouse embryo induced amplification of centriolar protein complexes in the same manner under physiological conditions *in vivo*. From the viewpoint of cancer biology, our study raises the possibility that formation of these aberrant structures related to centrioles could be a cause of genome instability in tumor formation. Although centriole amplification is frequently found in many types of cancer cells, the nature of such structures, which were recognized by only a limited number of centriolar markers, remains unclear. In this study, we found that cells containing the centriolar protein complexes, but not those containing the centriole-like structures with HsSAS-6, could form pseudo-bipolar spindles that were sometimes accompanied by lagging chromosomes, and could survive after cell division. Given that RBM14 has been reported as a potential tumor suppressor in human renal cancer cells,[10] accumulation of such kinds of minor errors might promote tumor formation in the long term.

Disclosure of Potential Conflict of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We are grateful to all the coauthors of our original paper for completing the study. We thank Pierre Gönczy for the mass spectrometry analysis, which was initiated in the Gönczy laboratory to identify STIL-interacting proteins in human cells. We also thank the people who gifted us the antibodies and reagents used in the study.

Funding

Our study on RBM14 was supported by JSPS, JST, The Ministry of Education, Science, Sports and Culture of Japan, The Uehara Memorial Foundation, Takeda Science Foundation, The Mitsubishi Foundation, Mochida Memorial Foundation, Astellas Foundation, The Sagawa Foundation, Novartis Foundation, The Center for the Promotion of Integrated Sciences of Sokendai, and The Transdisciplinary Research Integration center.

References

1. Nigg EA. Origins and consequences of centrosome aberrations in human cancers. Int J Cancer. 2006; 119: 2717-2723; PMID:17016823; http://dx.doi.org/10.1002/ijc.22245
2. Kitagawa D, Vakonakis I, Olieric N, Hilbert M, Keller D, Olieric V, Bortfeld M, Erat MC, Flückiger I, Gönczy P, Steinmetz MO. Structural basis of the 9-fold symmetry of centrioles. Cell 2011; 144: 364-375; PMID:21277013; http://dx.doi.org/10.1016/j.cell.2011.01.008
3. van Breugel M, Hirono M, Andreeva A, Yanagisawa HA, Yamaguchi S, Nakazawa Y, Morgner N, Petrovich M, Ebong IO, Robinson CV, Johnson CM, Veprintsev D, Zuber B. Structures of SAS-6 suggest its organization in centrioles. Science 2011; 331: 1196-1199; PMID:21273447; http://dx.doi.org/10.1126/science.1199325
4. Tang CJ, Lin SY, Hsu WB, Lin YN, Wu CT, Lin YC, Chang CW, Wu KS, Tang TK. The human microcephaly protein STIL interacts with CPAP and is required for procentriole formation. EMBO J 2011; 30: 4790-4804; PMID:22020124; http://dx.doi.org/10.1038/emboj.2011.378
5. Ohta M, Ashikawa T, Nozaki Y, Kozuka-Hata H, Goto H, Inagaki M, Oyama M, Kitagawa D. Direct interaction of Plk4 with STIL ensures formation of a single procentriole per parental centriole. Nat Commun. 2014; 5: 5267; PMID:25342035; http://dx.doi.org/10.1038/ncomms6267
6. Dzhindzhev NS, Tzolovsky G, Lipinszki Z, Schneider S, Lattao R, Fu J, Debski J, Dadlez M, Glover DM. Plk4 phosphorylates ana2 to trigger sas6 recruitment and procentriole formation. Curr Biol. 2014; 24: 2526-2532; PMID:12356862; http://dx.doi.org/10.1016/j.cub.2014.08.061
7. Khodjakov A, Rieder CL, Sluder G, Cassels G, Sibon O, Wang CL. De novo formation of centrosomes in vertebrate cells arrested during S phase. J Cell Biol 2002; 158:1171-1181; PMID:12356862; http://dx.doi.org/10.1083/jcb.200205102
8. Shiratsuchi G, Takaoka K, Ashikawa T, Hamada H, Kitagawa D. RBM14 prevents assembly of centriolar protein complexes and maintains mitotic spindle integrity. EMBO J. 2014; 34:97-114; "Epub ahead of print"; PMID:25385835; http://dx.doi.org/10.15252/embj.201488979
9. Iwasaki T, Chin WW, Ko L. Identification and characterization of RRM-containing coactivator activator (CoAA) as TRBP-interacting protein, and its splice variant as a coactivator modulator (CoAM). J Biol Chem 2001; 276:33375-33383; PMID:11443112; http://dx.doi.org/10.1074/jbc.M101517200
10. Kang YK, Schiff R, Ko L, Wang T, Tsai SY, Tsai MJ, O'Malley BW. Dual roles for coactivator activator and its counterbalancing isoform coactivator modulator in human kidney cell tumorigenesis. Cancer Res 2008; 68:7887-7896; PMID:18829545; http://dx.doi.org/10.1158/0008-5472.CAN-08-1734