EJMO

DOI: 10.14744/ejmo.2021.13872
EJMO 2021;5(3):205–208

Review

Mitotic Spindle as Therapeutic Target for Tetraploid Cancer Cells

Mohamed Jemaà

Department of Laboratory Medicine, Translational Cancer Research, Faculty of Medicine, Lund University, Sweden

Abstract

Tetraploidy constitutes a genomically metastable state that drives oncogenesis by leading aneuploidy. Tetraploid sub-population is frequently found in pre-neoplastic lesions. This particular population is relatively more resistant against DNA damaging agents and in consequence, it is important to selectively target tetraploid cancer cells. Here, we listed all the studies that targeted preferentially tetraploid tumors cells focusing on mitosis machinery, essentially the spindle pole apparatus and the spindle assembly checkpoint pathways.

Keywords: Centrosome Apparatus, Tetraploidy, Spindle Assembly Checkpoint, Mitotic Catastrophe

Cite This Article: *Jemaà M. Mitotic Spindle as Therapeutic Target for Tetraploid Cancer Cells. EJMO 2021;5(3):205–208.*

Chromosome instability (CIN) is the process when cells display chromosome mis-segregation during mitosis and leads to karyotype change and abnormal DNA content.[1, 2] CIN engender cell aneuploidy, with different chromosomes copy number in daughter cells.[3] Tetraploidy, an intermediate stage of CIN, is the accumulation of two sets of chromosomes, and tetraploid cells can be generated by mitotic slippage (endocycling or endoreplication), or by aborted mitosis (endomitosis or cytokinesis failure), or by cell-cell membrane fusion.[4, 5]

Excluding some physiological context such as hepatocytes, differentiating megakaryocytes or in the syncytiotrophoblast, striated muscle cells and osteoclasts with an irreversible tetraploid arrested G1 phase, normal cells do not live with chromosomes number perturbation.[6] Robust intrinsic programmed death pathways[7] or immunological surveillance mechanism normally prevents tetraploidy.[8] However, CIN and aneuploidization has been associated with cancer progression and aggressiveness,[9] therapeutic resistance,[10] metastasis[11] and poor patient prognosis.[12] Illicit tetraploidization is a major mechanism through which aneuploid cancer cells are generated.[3]

Due to the clear involvement of CIN and aneuploidy, and de facto tetraploidization, in oncogenesis and cancer progression, several strategies were developed to selectively eradicate tetraploid cancer sub-population. One of the main strategies was targeting several key mitotic effectors and exacerbating the chromosomal instability to death.

This review summarizes the studies that reported a higher sensitivity in tetraploid cells when mitotic effectors were inhibited.

A. Inhibition of the Centrosome Apparatus

Centrosomes are the main microtubule nucleating structures in the cells. They undergo duplication and maturation during the cell cycle and form the mitotic bipolar spindle assembly.[13] The deregulation of the mitotic bipolar spindle showed a highly toxicity in cancer cells.[14] Moreover, as most tetraploid cells have extra centrosomes, targeting the centrosomes associated molecules showed a selective effect on tetraploid cells. Figure 1 shows the different fates that could happen once a key centrosome kinase or kinesin is inhibited.

Address for correspondence: Mohamed Jemaà, MD. Department of Laboratory Medicine, Translational Cancer Research, Faculty of Medicine, Lund University, Sweden
Phone: +216 21 123 145 **E-mail:** jemaamohamed@gmail.com
Submitted Date: August 06, 2021 **Accepted Date:** September 04, 2021 **Available Online Date:** September 24, 2021

A.1. Eg5

The kinesin Eg5, also called kinesin spindle protein (KSP) or KIF11, is a molecular motor that separates the microtubules that are attached to the two centrosomes and thus contribute to the arrangement of the bipolar arrangement of the spindle.[15] The knockdown or the pharmacological inhibition of Eg5 provokes a non-separation of the centrosomes and mono-astral mitosis figures and consequent blockage in mitosis.

The mitotic blockage is followed by a reversion from the metaphase to the prophase G1-state in diploid cells. However, in tetraploid cancer cells it has been shown that the metaphase arrest was significantly shortened and cells undergo karyokinesis and illicit cell division, and ultimately, mitotic catastrophe. Tetraploid cells reduced the metaphase arrest by nearly 50% and advanced to karyokinesis and aneuploid daughter cells while diploid cells reverse to G1 mainly. This difference in cell cycle fate kills preferentially the tetraploid cancer cells.[6]

A.2. HSET

The kinesin-like protein HSET or KIFC1 is a minus end-directed motor protein that promotes microtubule cross-linking, sliding, bundling and spindle pole focusing. HSET and Eg5 act antagonistically and the simultaneous inhibition of HSET and Eg5 restores centrosome separation. In diploid cells, HSET is non-essential protein, however; in tetraploid cancer cells, HSET drives supernumerary centrosomes clustering. Disruption of such a mechanism selectively kills tetraploid cancer cells. Indeed, HSET inhibition induces multipolar anaphases figures and consequent abnormal mitosis and non-viable daughter cells due to the activation of mitotic catastrophe.[16]

A.3. PLK1

The Polo-like kinase 1 PLK1 is a serine/threonine protein kinase that plays essential roles during the cell cycle. PLK1 kinase has a variety of functions including in the early mitosis stage where PLK1 is localized at the centrosome and facilitates γ-tubulin recruitment and centrosome maturation and separation.[17] The depletion or inhibition of PLK1 blocks the cells in G2/M cell cycle phase and while the diploid cells reverse to G1 phase, +/- 25% of the tetraploid cells undergo mitotic slippage and acquire extended ploidy and become more unstable. This leads to the activation of the mitotic catastrophe preferentially in tetraploid cells. This particular fate can be explained by the important role of PLK1 in the Spindle Assembly Checkpoint (SAC). Indeed, PLK1 is located at the kinetochores during the metaphase and regulates the chromosome segregation at the spindle midzone. This function could introduce the SAC as therapeutic target for tetraploid cancer cells.[18]

B. Abrogation of the Spindle Assembly Checkpoint SAC

The SAC controls the correct attachment between kinetochores and microtubules during prometaphase. It monitors the metaphase-to-anaphase transition until all chromosomes are completely attached and bioriented at the metaphase plate. This ensures correct segregation of sister chromatids, which is conditio sine qua non for normal cell division.[19, 20] The abrogation of the SAC provokes a mitotic defaults accumulation, a cascade of polyploidy/aneuploidy and eventually cell death.[21] The inhibition of several key components of the SAC showed a high selectivity to tetraploid cancer cells. Figure 1 shows the various cell fates that might undergo after SAC kinases inhibition.

B.1. MPS1

MPS1 is an essential dual-specificity protein kinase that phosphorylates serines/threonines and tyrosines. MPS1 kinase is evolutionarily conserved and has multiple roles essentially in mitosis, and its most important function is ensuring proper biorientation of sister chromatids on the mitotic spindle at kinetochores.[22] During mitosis, PLK1 and MPS1 cooperatively regulate the SAC.[23] Similar to the inhibition of PLK1, the abrogation of MPS1 kills preferentially

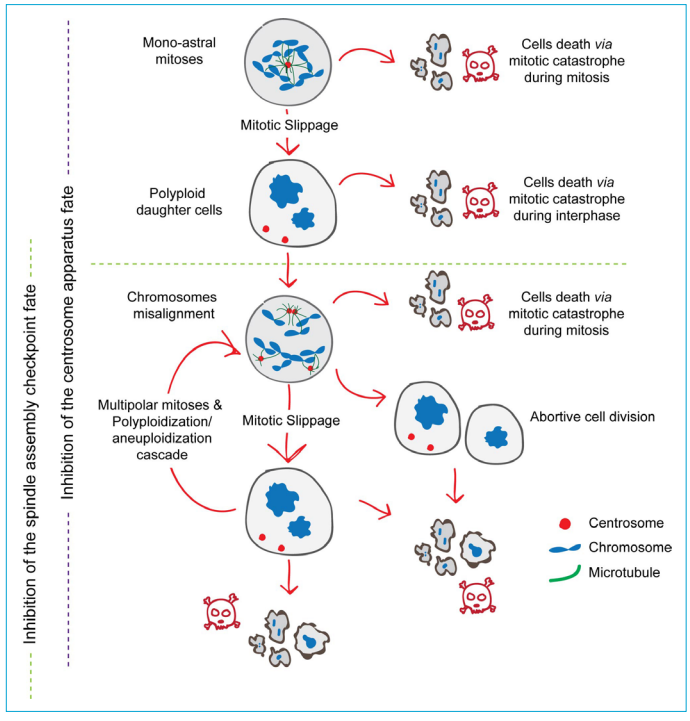

Figure 1. Different fates possibilities leading to mitotic catastrophe when inhibiting the centrosome apparatus or the spindle assembly checkpoint in tetraploid cancer cells.

tetraploid cancer cells. The MPS1 inhibition abolishes the SAC function, shortened the mitosis and induced a polyploidization/aneuploidization program and daughter cells executed mitotic catastrophe both during mitosis or the interphase. The preferential cytotoxic effect on tetraploid cells came from the elevated dependency of polyploid cells to the SAC activity for their survival and thus it becomes an Achilles's heel when abrogated. This effect is even more present when the polyploid status is increased in absence of SAC functions.[24, 25]

B.2. MAD2 & BUBR1

MAD2 and BUBR1 are one of the main effectors of the spindle assembly checkpoint.[19] The inhibition or the depletion of MAD2 or BUBR1 engenders severe chromosome missegregation that induce cell toxicity. Moreover, both MAD2 and BUBR1 are required for centrosome clustering and their inhibition enhances multipolar mitosis. Targeting these kinases showed a preferential cytotoxic effect in polyploid and tetraploid cancer cells.[16]

B.3. Aurora B

Aurora B is a member of the Aurora family of serine/threonine kinases. Aurora B forms the chromosomal passenger complex (CPC) in association with the centromere protein INCENP, the Survivin and the Borealin. The Aurora B controls the chromosome segregation and the SAC in upstream.[20] Indeed, when inhibited, Aurora B prevents kinetochore recruitment of all other SAC components. This leads to a mitotic slippage and excessive genome reduplication that, at term, provokes mitotic catastrophe preferentially in tetraploid cells.[26] We should however signal that the inhibition of Aurora A, the other main kinase of the Aurora family and an important kinase for centrosome maturation, mitotic spindle formation and cytokinesis doesn't kill preferentially tetraploid carcinoma cells.[26, 27]

B.4. CHK1

The checkpoint kinase-1 CHK1 is a conserved protein kinase involved in the DNA damage response (DDR) and the cell cycle checkpoints to preserve genome integrity.[28] The CHK1 delays the entry of cells with damaged DNA into mitosis. CHK1 is not a component of the Spindle Assembly Checkpoint, however, CHK1 phosphorylate a number of SAC components and is required for metaphase arrest. The pharmacological inhibition of CHK1, use of dominant-negative mutant or silencing with siRNA, kills preferentially the tetraploid cancer cells. This cytotoxicity occurs after a SAC delay, P53 activation and mitotic catastrophe.[29]

Concluding Remarks

As an intermediate and unstable karyotype status between diploid and aneuploid cancer cells, Tetraploidy was correlated with oncogenesis and found at early stages of multiple cancer cell types. Targeting this particular sub-population is a highly relevant strategy in cancer treatment and may be an approach to achieve a significantly improved clinical outcome and also to overcome resistance. In this short review, we summarized the published studies that demonstrated a preferential effect on tetraploid cells compared to diploid when targeting the mitotic apparatus. One of the challenges for future studies will be the identification of efficient synergistic co-treatment that target microtubules during mitosis and increase the treatment efficiency with lowest dose and side effect. The co-treatment of the tetraploid cells in vitro with MPS1 inhibitors and microtubule depolymerization inhibitor paclitaxel,[24] or the PLK1 inhibitor and the microtubule polymerization inhibitor vincristine or colchicine[18] showed already interesting synergy. This effort needs to be largely conducted with the other mitotic effectors listed in this review and confirmed with suitable in vivo and preclinical models as well as in clinical trials.

Acknowledgments

M. J. is financed by the Wenner-Gren Foundation (UPD2018-0055) and by funding from Per-Eric and Ulla Schyberg's Foundation (39178), Gunnar Nilssons Cancerstiftelse (GN-2019-2-137), The Crafoord Foundation (20190584), the Sigurd och Elsa Goljes Minne Foundation (LA2018-0168) and the Royal Physiographic Society of Lund (39448).

Disclosures

Ethics Committee Approval: Ethics committee approval was not requested for this study.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

References

1. Weaver BA, Cleveland DW. Does aneuploidy cause cancer? Curr Opin Cell Biol 2006;18:658–67. [CrossRef]
2. van Jaarsveld RH, Kops GJPL. Difference Makers: Chromosomal Instability versus Aneuploidy in Cancer. Trends Cancer 2016;2:561–71. [CrossRef]
3. Vitale I, Galluzzi L, Senovilla L, Criollo A, Jemaa M, Castedo M, et al. Illicit survival of cancer cells during polyploidization and depolyploidization. Cell Death Differ 2011;18:1403–13. [CrossRef]
4. Potapova TA, Zhu J, Li R. Aneuploidy and chromosomal instability: a vicious cycle driving cellular evolution and cancer genome chaos. Cancer Metastasis Rev 2013;32:377–89. [CrossRef]
5. Storchova Z, Kuffer C. The consequences of tetraploidy and an-

euploidy. J Cell Sci 2008;121:3859–66. [CrossRef]
6. Rello-Varona S, Vitale I, Kepp O, Senovilla L, Jemaá M, Métivier D, et al. Preferential killing of tetraploid tumor cells by targeting the mitotic kinesin Eg5. Cell Cycle 2009;8:1030–5. [CrossRef]
7. Vitale I, Galluzzi L, Castedo M, Kroemer G. Mitotic catastrophe: a mechanism for avoiding genomic instability. Nat Rev Mol Cell Biol 2011;12:385–92. [CrossRef]
8. Senovilla L, Vitale I, Martins I, Tailler M, Pailleret C, Michaud M, et al. An immunosurveillance mechanism controls cancer cell ploidy. Science 2012;337:1678–84. [CrossRef]
9. Chunduri NK, Storchová Z. The diverse consequences of aneuploidy. Nat Cell Biol 2019;21:54–62. [CrossRef]
10. Lee AJ, Endesfelder D, Rowan AJ, Walther A, Birkbak NJ, Futreal PA, et al. Chromosomal instability confers intrinsic multidrug resistance. Cancer Res 2011;71:1858–70. [CrossRef]
11. Bakhoum SF, Ngo B, Laughney AM, Cavallo JA, Murphy CJ, Ly P, et al. Chromosomal instability drives metastasis through a cytosolic DNA response. Nature 2018;553:467–72. [CrossRef]
12. Carter SL, Eklund AC, Kohane IS, Harris LN, Szallasi Z. A signature of chromosomal instability inferred from gene expression profiles predicts clinical outcome in multiple human cancers. Nat Genet 2006;38:1043–8. [CrossRef]
13. Fu J, Hagan IM, Glover DM. The centrosome and its duplication cycle. Cold Spring Harb Perspect Biol 2015;7:a015800. [CrossRef]
14. Rivera-Rivera Y, Saavedra HI. Centrosome - a promising anticancer target. Biologics 2016;10:167–76. [CrossRef]
15. Wojcik EJ, Buckley RS, Richard J, Liu L, Huckaba TM, Kim S. Kinesin-5: cross-bridging mechanism to targeted clinical therapy. Gene 2013;531:133–49. [CrossRef]
16. Kwon M, Godinho SA, Chandhok NS, Ganem NJ, Azioune A, Thery M, et al. Mechanisms to suppress multipolar divisions in cancer cells with extra centrosomes. Genes Dev 2008;22:2189–203. [CrossRef]
17. Zitouni S, Nabais C, Jana SC, Guerrero A, Bettencourt-Dias M. Polo-like kinases: structural variations lead to multiple functions. Nat Rev Mol Cell Biol 2014;15:433–52. [CrossRef]
18. Jemaà M, Kifagi C, Serrano SS, Massoumi R. Preferential killing of tetraploid colon cancer cells by targeting the mitotic kinase PLK1. Cell Physiol Biochem 2020;54:303–20. [CrossRef]
19. Lara-Gonzalez P, Westhorpe FG, Taylor SS. The spindle assembly checkpoint. Curr Biol 2012;22:R966–80. [CrossRef]
20. Manic G, Corradi F, Sistigu A, Siteni S, Vitale I. Molecular regulation of the spindle assembly checkpoint by kinases and phosphatases. Int Rev Cell Mol Biol 2017;328:105–61. [CrossRef]
21. Jemaà M, Galluzzi L, Kepp O, Senovilla L, Brands M, Boemer U, et al. Characterization of novel MPS1 inhibitors with preclinical anticancer activity. Cell Death Differ 2013;20:1532–45. [CrossRef]
22. Liu X, Winey M. The MPS1 family of protein kinases. Annu Rev Biochem 2012;81:561–85. [CrossRef]
23. von Schubert C, Cubizolles F, Bracher JM, Sliedrecht T, Kops GJPL, Nigg EA. Plk1 and Mps1 cooperatively regulate the spindle assembly checkpoint in human cells. Cell Rep 2015;12:66–78. [CrossRef]
24. Jemaà M, Manic G, Lledo G, Lissa D, Reynes C, Morin N, et al. Whole-genome duplication increases tumor cell sensitivity to MPS1 inhibition. Oncotarget 2016;7:885–901. [CrossRef]
25. Libouban MAA, de Roos JADM, Uitdehaag JCM, Willemsen-Seegers N, Mainardi S, Dylus J, et al. Stable aneuploid tumors cells are more sensitive to TTK inhibition than chromosomally unstable cell lines. Oncotarget 2017;8:38309–25. [CrossRef]
26. Marxer M, Foucar CE, Man WY, Chen Y, Ma HT, Poon RY. Tetraploidization increases sensitivity to Aurora B kinase inhibition. Cell Cycle 2012;11:2567–77. [CrossRef]
27. Yan M, Wang C, He B, Yang M, Tong M, Long Z, et al. Aurora-A kinase: a potent oncogene and target for cancer therapy. Med Res Rev 2016;36:1036–79. [CrossRef]
28. Patil M, Pabla N, Dong Z. Checkpoint kinase 1 in DNA damage response and cell cycle regulation. Cell Mol Life Sci 2013;70:4009–21. [CrossRef]
29. Vitale I, Galluzzi L, Vivet S, Nanty L, Dessen P, Senovilla L, et al. Inhibition of Chk1 kills tetraploid tumor cells through a p53-dependent pathway. PLoS One 2007;2:e1337.