A brief review of the monopolarspindle (MPS1) dysfunction in $BRAF^{V600E}$-driven melanoma

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Melanoma is an aggressive skin cancer arising from melanocytes, which accounts for less than 2% of all skin cancer cases, but more than 70% of skin cancer-related deaths [1]. In 2015, an estimated 73,870 new cases of melanoma will be diagnosed and an estimated 9,940 deaths from melanoma will occur in the US [1]. 50%~70% of malignant melanoma patients possess mutations in *BRAF*, 90% of which are $BRAF^{V600E}$ [2,3]. This activating mutation leads to constitutively active RAF/MEK/ERK signaling [4]. ERK regulates gene expression, cell invasion and metabolism, the over-activation of which contributes to rapid cell proliferation and tumorigenesis [4]. Besides $BRAF^{V600E}$, 15%~18% melanoma cases contain mutations in *NRAS*, which also commonly leads to hyperactive RAF/MEK/ERK pathway [5]. Targeting $BRAF^{V600E}$ shows strong anticancer efficacy both *in vitro* and *in vivo* [6-8], and the use of Vemurafenib (a BRAF^V600E inhibitor) has received FDA approval to treat late-stage $BRAF^{V600E}$-driven melanoma. However, most patients develop drug resistance in approximately 6 months [9]. Combinatorial targeting of BRAF and MEK improves patient response, although acquired resistance still occurs frequently [9]. Under the circumstance of resistance, ERK is often reactivated [10,11]. However, the underlying molecular mechanisms are not well understood [10,11]. The current ERK inhibitor candidate has recently reached phase I clinical trials. However, no convincing data in relation to toxicity and patient response are available. Therefore, it is essential to search for new targets and investigate different approaches to treat melanoma.
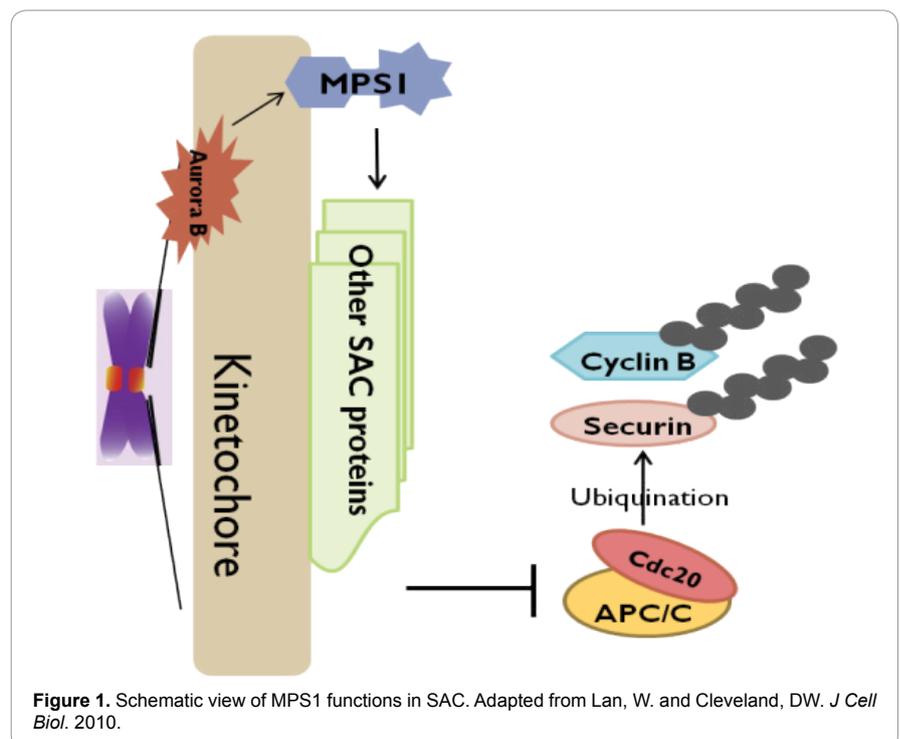
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Figure 1. Schematic view of MPS1 functions in SAC. Adapted from Lan, W. and Cleveland, DW. *J Cell Biol*. 2010.

MPS1, monopolar spindle, is a conserved mitotic kinase that controls progressions through cell mitosis. One role of MPS1 in human cells is to control centrosome duplication at the spindle pole, while the primary function of MPS1 is in mitotic spindle assembly checkpoint (SAC) [12]. SAC prevents the cell cycle progression from metaphase to anaphase before the proper bipolar attachment of all sister chromatids to the mitotic spindle [13]. See Figure 1 for detailed processes (reviewed by Reference [13]). The localization of MPS1 to the kinetochore is required to recruit other SAC proteins, such as Mad1, Mad2, Bub1 and BubR1. The overall effect is the inhibition of the anaphase-promoting complex/ cyclostomes (APC/C) by inactivating APC/C's activator, Cdc20. Therefore, securin and Cyclin B escape from APC/C-induced ubiquitination, which prevents cell cycle progression. Overexpression of MPS1 in normal budding yeast leads to constitutively activated SAC and cell cycle arrest without disturbing the mitotic spindle [14]. The depletion or inhibition of MPS1, on the other hand, causes severe mitotic defects [15-19]. Thus, MPS1 is crucial in maintaining proper spindle checkpoint and ensuring correct chromosomal segregation.

Cui's group is dedicated to investigate the role of MPS1 in human melanoma [20,21]. They showed that MPS1 protein level is two to three fold higher in melanoma cell lines with $BRAF^{V600E}$ compared to that in cells with $BRAF^{WT}$ [20]. Meanwhile, MPS1-associated myelin basic protein (MBP) kinase activity is approximately tenfold higher *in vitro*, suggesting the increase of MPS1 kinase activity led by $BRAF^{V600E}$ besides the protein level change [20]. Later, Cui's studies demonstrated that elevated MPS1 contributes to $BRAF^{V600E}$-induced centrosome amplification and spindle abnormalities, which results in aneuploidy in melanoma [21]. Furthermore, $BRAF^{V600E}$-mediated MPS1 elevation delays M-phase cell cycle and prolongs spindle checkpoint [21], indicating the abnormalities in SAC.

One interesting question is what are the mechanisms that lead to MPS1 elevation in $BRAF^{V600E}$-driven melanoma?

The primary regulatory mechanisms of MPS1 function are via posttranslational phosphorylation and degradation (reviewed by Reference [12]). For instance, phosphorylation at T676 and T686 are essential for MPS1 kinase activity [12]. In regards to MPS1 degradation, APC/C-Cdc20 and APC/C-Cdh1 ubiquitin ligase complex are key regulators of MPS1 degradation during cell cycle and the D-box motif in MPS1 is required for the ubiquitination [22]. Cui's studies showed that the elevation of MPS1 in melanoma is regulated by the post-transcriptional hyperphosphorylation, and $BRAF^{V600E}$-induced p-S281 is essential for MPS1 protein stabilization and increased MPS1 protein level [20,21]. S281 is close to the D-box of human MPS1. Thus, p-S281 may impair the ubiquitination of MPS1 [21].

Cui's studies also suggested that the contribution of $BRAF^{V600E}$ to MPS1 elevation is through ERK signaling [20,21]. MEK inhibitor U0126 impairs both MPS1 protein level and hyperphosphorylation of MPS1 within melanoma cells possessing $BRAF^{V600E}$ [20,21]. The tissue microarray of human melanoma clinical samples showed significant correlation between p-ERK and p-S281 in MPS1 [22]. However, the off-target effects of U0126 have been reported in various studies [23-25]. Furthermore, the co-immunoprecipitation study was done between MPS1 and

$BRAF^{V600E}$ rather than with ERK [20]. Therefore, more physical evidence is needed to elucidate the molecular mechanisms underlying ERK-mediated MPS1 regulation. In addition, the phosphorylation of Mps1 by MAPK (ERK) was examined in *Xenopus* extract [26]. p-S844 in the *Xenopus* Mps1 (XIMps1) is required for its kinetochore localization [26]. However, the *in vitro* phosphorylation assay was conducted with MAPK and truncated XIMps1, where the XIMps1 construct lacked protein sequences 1 to 755 [26]. Therefore, a majority of the potential phosphorylation sites in XIMPS1 were not examined. Taking together, it is unclear whether the elevated MPS1 in $BRAF^{V600E}$-driven melanoma is dependent upon the direct phosphorylation by ERK. As a future direction, it is important to further elucidate the interactions between ERK and MPS1 in $BRAF^{V600E}$-driven melanoma, which will greatly advance our knowledge of the ERK functions in regulating cancer cell mitosis.

Another question is, will MPS1 serve as a promising target in the treatment of human melanoma?

Based on the studies at the present stage, MPS1 is a promising target in various cancer types. MPS1 is upregulated at the mRNA level, protein level and kinase activity in many tumors, including bladder, breast, lung, esophagus and prostate cancer [27]. As suggested in Janssen's study [28], the reduction of SAC function could increase the frequency of chromosome mis-segregation, which serves as a strategy to eliminate cancer cells. The attempts to selectively inhibit MPS1 to induce SAC inactivation and promote cancer cell death were tested in various studies (reviewed by Reference [12]). Colombo's group identified a small-molecule MPS1 inhibitor, NMS-P715, which has antiproliferation effects and promotes cell death in many cancer cells, including colon, breast, renal, lung and lymphoma lines [27]. Oral administration of NMS-P715 in xenograft mouse models showed anticancer effects as well [28]. In 2014, studies in pancreatic ductal adenocarcinoma (PDAC) demonstrated the correlation between the overexpression of MPS1 and poor survival [29]. The treatment of NMS-P715 on both human and murine PDAC cell lines showed antiproliferative and proapoptotic effects *in vitro* [29]. In addition, Jemaa and colleagues demonstrated the antineoplastic activity of three different MPS1 inhibitors in human colorectal and cervical carcinoma cells, both *in vitro* and *in vivo* [30]. Significantly, MPS1 inhibitors are suggested to have specificities against cancer cells, as various human normal cells showed resistance to NMS-P715-induced chromosomal instability and growth inhibition [27,29].

In $BRAF^{V600E}$-driven melanoma, NMS-P715 has shown anticancer effect both *in vitro* and *in vivo* [27]. However, it remains unclear whether targeting MPS1 possesses anticancer efficacy in Vemurafenib resistant melanoma. Since MPS1 is suggested to be downstream of ERK signaling [20,21] and ERK is frequently reactivated while developing Vemurafenib resistance [10,11], it is possible that MPS1 is elevated in Vemurafenib resistant melanoma and serves as a potential drug target. Therefore, as a future direction, it is urgent to demonstrate the status of MPS1 in Vemurafenib resistant melanoma as well as its contributions towards the occurrence of resistance. Targeting MPS1 may provide a new therapeutic strategy for Vemurafenib resistant melanoma patients, as a second-line drug treatment.

References

1. Cancer facts & figures 2015. American Cancer Society.

2. Dhomen N, Reis-Filho JS, da Rocha Dias S, et al. Oncogenic Braf induces melanocyte senescence and melanoma in mice. *Cancer Cell*. 2009,15(4):294-303.

3. Zhang L, Shi R, He C, et al. Oncogenic B-Raf(V600E) abrogates the AKT/B-Raf/Mps1 interaction in melanoma cells. *CancerLett.* 2013,337(1):125-132.

4. Garnett MJ, Marais R. Guilty as charged: B-RAF is a human oncogene. *Cancer Cell*. 2004,6(4):313-319.

5. Bello DM, Ariyan CE, Carvajal RD. Melanoma mutagenesis and aberrant cell signaling. *Cancer Control*. 2013,20(4):261-281.

6. Flaherty KT, Igor Puzanov, Kevin B Kim, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med*. 2010,363:809-819.

7. Bollag G, Hirth P, Tsai J, et al. Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. *Nature*. 2010,467(7315):596-599.

8. Hooijkaas AI, Gadiot J, van der Valk M, Mooi WJ, Blank CU. Targeting BRAFV600E in an inducible murine model of melanoma. *Am J Pathol*. 2012,181(3):785-794.

9. Morris EJ, Jha S, Restaino CR, et al. Discovery of a novel ERK inhibitor with activity in models of acquired resistance to BRAF and MEK inhibitors. *Cancer Discov*. 2013,3(7):742-750.

10. Wellbrock C. MAPK pathway inhibition in melanoma: resistance three ways. *BiochemSoc Trans*. 2014,42(4):727-732.

11. Lidsky M, Antoun G, Speicher P, et al. MAP kinase hyper-activation and enhanced NRAS expression drive acquired vemurafenib resistance in V600E BRAF melanoma cells. *J Biol Chem*. 2014,289(40):27714-27726.

12. Liu X, Winey M. The MPS1 family of protein kinases. *Annu Rev Biochem*. 2012,81:561-585.

13. Lan W, Cleveland DW. A chemical tool box defines mitotic and interphase roles for Mps1 kinase. *J Cell Biol*. 2010,190(1):21-24.

14. Hardwick KG, Weiss E, Luca FC, Winey M, Murray AW. Activation of the budding yeast spindle assembly checkpoint without mitotic spindle disruption. *Science*. 1996,273(5277):953-956.

15. Maciejowski J, George KA, Terret ME. Mps1 directs the assembly of Cdc20 inhibitory complexes during interphase and mitosis to control M phase timing and spindle check point signaling. *J CellBiol*. 2010,190(1):89-100.

16. Santaguida S, Tighe A, D'Alise AM, Taylor SS, Musacchio A. Dissecting the role of MPS1 in chromosome biorientation and the spindle checkpoint through the small molecule inhibitor reversine. *J CellBiol*. 2010,190(1):73-87.

17. Tighe A, Staples O, Taylor S. Mps1 kinase activity restrains anaphase during an unperturbed mitosis and targets Mad2 to kinetochores. *J CellBiol*. 2008,181(6):893-901.

18. Schmidt M, YemimaBudirahardja, Rob Klompmaker, René H Medema. Ablation of the spindle assembly checkpoint by a compound targeting Mps1. *EMBO Rep*. 2005,6(9):866-872.

19. Kwiatkowski N, Jelluma N, Filippakopoulos P, et al. Small-molecule kinase inhibitors provide insight into Mps1 cell cycle function. *Nat Chem Biol.*2010,6(5):359-368.

20. Cui Y,Guadagno TM. B-Raf(V600E) signaling deregulates the mitotic spindle checkpoint through stabilizing Mps1 levels in melanoma cells. *Oncogene*. 2008,27(22):3122-3133.

21. Liu J, Cheng X, Zhang Y, et al. Phosphorylation of Mps1 by BRAFV600E prevents Mps1 degradation and contributes to chromosome instability in melanoma. *Oncogene*. 2013,32(6):713-723.

22. Cui Y, Cheng X, Zhang C, et al. Degradation of the human mitotic checkpoint kinase Mps1 is cell cycle-regulated by APC-cCdc20 and APC-cCdh1 ubiquitin ligases. *J Biol Chem*. 2010,285(43): 32988-32998.

23. Wauson EM, Guerra ML, Barylko B, Albanesi JP, Cobb MH. Off-target effects of MEK inhibitors. *Biochemistry*. 2013,52(31):5164-5166.

24. Evans C, Cook SJ, Coleman MP, Gilley J. MEK inhibitor U0126 reverses protection of axons from Wallerian degeneration independently of MEK-ERK signaling. *PLoS One*. 2013,8(10): e76505.

25. Mills CN, Joshi SS, Niles RM. Expression and function of hypoxia inducible factor-1 alpha in human melanoma under non-hypoxic conditions. *Mol Cancer*. 2009,8:104.

26. Zhao Y, Chen RH. Mps1 phosphorylation by MAP kinase is required for kinetochore localization of spindle-checkpoint proteins. *Curr Biol*. 2006,16(17):1764-1769.

27. Colombo R, Caldarelli M, Mennecozzi M, et al. Targeting the mitotic checkpoint for cancer therapy with NMS-P715, an inhibitor of MPS1 kinase. *Cancer Res*. 2010,70(24):10255-10264.

28. Janssen A, Kops GJ,Medema RH. Elevating the frequency of chromosomemis-segregation as a strategy to kill tumor cells. *ProcNatlAcadSci U S A*. 2009,106(45):19108-191013.

29. Slee RB, Grimes BR, Bansal R, et al. Selective inhibition of pancreatic ductal adenocarcinoma cell growth by the mitotic MPS1 kinase inhibitor NMS-P715. *Mol Cancer Ther*. 2014,13(2):307-315.

30. Jemaà M, Galluzzi L, Kepp O, et al. Characterization of novel MPS1 inhibitors with preclinical anticancer activity. *Cell Death Differ*. 2013,20(11):1532-1545.