Cell

Hippo Pathway Key to Ploidy Checkpoint

Bin Zhao[1] and Kun-Liang Guan[2,*]
[1]Life Sciences Institute and Innovation Center for Cell Biology, Zhejiang University, Hangzhou, Zhejiang 310058, China
[2]Department of Pharmacology and Moores Cancer Center, University of California San Diego, La Jolla, CA 92093, USA
*Correspondence: kuguan@ucsd.edu
http://dx.doi.org/10.1016/j.cell.2014.07.041

Tetraploid cells generated by abnormal cell division are often arrested during the cell cycle or cleared by apoptosis. Evasion of these defense mechanisms leads to genomic instability and tumorigenesis. In this issue, Ganem et al. report that extra centrosome-induced activation of the Hippo pathway kinase LATS2 is a key mechanism of tetraploidy-induced cell-cycle arrest.

Tetraploidy (having four sets of chromosomes) can result from failed cell division. For example, chromosome nondisjunction and telomere crisis can result in cytokinesis failure, thus generating binucleated cells. Alternatively, cells under prolonged spindle assembly checkpoint arrest can become tetraploid with a single large nucleus due to mitotic slippage, a slow progression through mitosis. Tetraploid or aneuploid cells are commonly found in cancer. It has been shown that p53-inactive tetraploid, but not p53-inactive diploid, mammary epithelial cells produce malignant tumors in nude mice (Fujiwara et al., 2005). Thus, tetraploidy is proposed to be causal to tumorigenesis. The tumorigenic potential of tetraploid cells could be due to the genomic instability caused by chromosome mis-segregation (Ganem et al., 2009). Tetraploid cells are normally arrested in the G1 phase of the cell cycle in a p53-dependent manner, thus limiting the risk of genomic instability, aneuploidy, and tumorigenesis. However, the mechanism of tetraploidy-induced G1 arrest is not completely understood.

In this issue of *Cell*, Ganem et al. (2014) identify essential genes specific for tetraploidy-induced cell cycle arrest by RNAi screening and gene expression profiling (Figure 1). Importantly, the Hippo tumor suppressor pathway kinase LATS2 specifically functions in tetraploidy-induced, but not DNA damage-induced, G1 arrest. LATS2 is activated in tetraploid cells, and its knockdown induces binucleated cell mitosis almost as potently as knockdown of p53. LATS2 is reported to activate p53 by inhibiting Mdm2 (Aylon et al., 2006), and this phenomenon is confirmed in the context of tetraploidy. In the Hippo pathway, LATS2 phosphorylates the transcription coactivator YAP to promote YAP cytoplasmic retention and degradation (Yu and Guan, 2013). Interestingly, expression of active YAP dramatically increases the ploidy of hepatocytes in vivo, suggesting that YAP may be an effector downstream of LATS2 in ploidy regulation.

Tetraploid cells are different from diploid cells in many ways. For example, tetraploid cells have double the amount of DNA, a larger cell volume, and in most cases, extra centrosomes. Although the idea that there is a checkpoint directly counting chromosome or centrosome number is fascinating, this notion is not supported by experimental evidence. In addition, DNA damage or other off-target effects of cytoskeleton poisons used to induce tetraploidy could not be the major reason of G1 arrest. Thus, the source of the signal triggering tetraploidy-induced cell-cycle arrest had not been pinpointed. The study by Ganem et al. (2014) shows that tetraploid cells have lower Rho activity, which is at least partially due to increased Rac activation in the presence of excess microtubules nucleated by extra centrosomes. Restoring Rho activity enables the cell to bypass G1 arrest. Interestingly, Rho is also known to indirectly but potently suppress LATS1/2 activity in the Hippo pathway. Consistently, enhanced cell-matrix adhesion, which activates Rho, is reported to reduce G1 arrest of tetraploid cells. The current study also demonstrates that lysophosphatidic acid stimulation, which is known to activate Rho and inhibit LATS2 (Yu and Guan, 2013), alleviates tetraploidy-induced G1 arrest. Thus, it is reasonable to speculate that Rho integrates various upstream signals to control G1 arrest of tetraploid cells, which may explain the leakiness of the hypothesized "tetraploidy checkpoint." Previous reports have demonstrated that LATS2 translocates from the cytoplasm to the nucleus following microtubule disruption, which correlates with p53 activation (Aylon et al., 2006). Whether LATS2 translocation is involved in Rho-induced inhibition is an interesting open question. It is of note that, although naturally evolved proliferating tetraploid cells lose their extra centrosomes, the accompanied inactivation of LATS2 is not simply mediated by reactivation of Rho but is instead transcriptionally regulated. This would be an important consideration when designing strategy to target tetraploid cells.

This study, together with a previous one, demonstrates the critical role of LATS2 in both cytokinesis failure and mitotic slippage-induced G1 arrest (Figure 1). Importantly, LATS2 is also involved in oncogene-induced cell-cycle arrest. Decreased LATS2 expression was observed in cells that evaded H-RasV12-induced cell-cycle arrest (Aylon et al., 2009). Thus, tetraploid cells and oncogene-expressing cells adopt a similar strategy to evade cell-cycle arrest. In addition, knockdown of LATS2 also leads to bypass of senescence such as that induced by Rb (Tschöp et al., 2011).

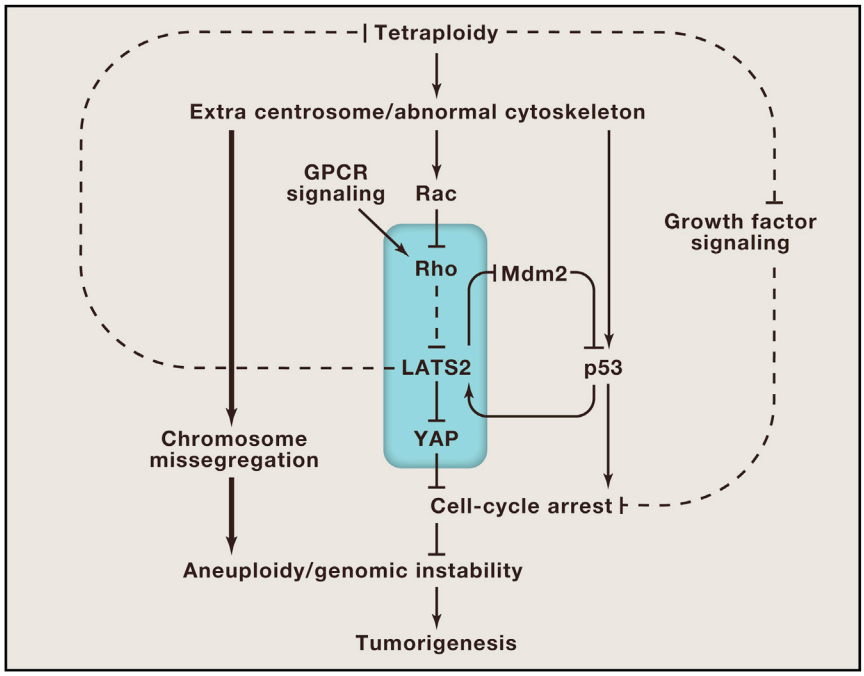

Figure 1. LATS2 in Tetraploidy-Induced Cell-Cycle Arrest

Most tetraploid cells have extra centrosomes and abnormal cytoskeleton, which lead to genomic instability. On the other hand, tetraploid cells are prevented from erotic cell divisions by cell-cycle arrest, largely due to p53 activation, although activation of growth factor signaling can bypass cell-cycle arrest in a p53-independent manner. A model is proposed that extra centrosomes initiate G1 arrest via a Rac-Rho-LATS2-YAP pathway. Activation of certain GPCR signaling inhibits LATS2 and enhances evasion of cell-cycle arrest. LATS2 also promotes proper cytokinesis and inhibits centrosome overduplication, thus inhibiting tetraploidy. The Hippo pathway is highlighted in blue.

Therefore, an intriguing question is how generally LATS2 activation is involved in cell-cycle arrest induced by various insults. It was recently found that the gain of YAP activity is an important mechanism during relapse of K-Ras-dependent cancers after Ras inhibition (Kapoor et al., 2014; Shao et al., 2014). Therefore, YAP activation may be a substitute for LATS2 inhibition in overriding growth arrest.

LATS2 and its homolog, LATS1, are known to function in cytokinesis. Inactivation of LATS1 abrogates cytokinesis in HeLa cells, and LATS1 knockout mouse fibroblast (MEF) cells have an elevated rate of multinucleation. LATS2 knockout MEF cells have an increased failure of cytokinesis, centrosome amplification, polyploidy, and aneuploidy (McPherson et al., 2004). However, caution should be taken in interpreting these data because the accumulation of multinucleated cells could be due to either cytokinesis failure or the bypass of cell-cycle arrest. In budding and fission yeasts, the LATS homologs Dbf2p and Sid2p are central to the mitotic-exit network and septation-initiation network, respectively. Thus, the function of LATS2 in later phases of mitosis may be conserved to some extent through evolution, although the mechanisms are likely different.

The study by Pellman and colleagues reveals a critical role of the Hippo pathway, particularly LATS2 and YAP, in tetraploidy-induced cell-cycle arrest. Given the known function of the Hippo pathway in regulating apoptosis, one may speculate that LATS2 activation also contributes to apoptotic elimination of tetraploid cells that are accidentally produced in normal tissues. However, some important questions remain. For example, the underlying mechanism of LATS2 inhibition by Rho remains elusive. The mechanism of LATS2 in p53 and cell-cycle regulation is also not completely understood. Current evidence suggests that tetraploidy activates LATS2, which then inhibits Mdm2. However, the mechanism of LATS2 in Mdm2 regulation is unclear. In addition, although expression of active YAP breaches tetraploidy-induced cell-cycle arrest, the role of endogenous YAP in this process has not been directly tested. Other effectors downstream of LATS2 mediating tetraploidy-induced G1 arrest possibly exist. Nevertheless, this study suggests that arresting the cell cycle of tetraploid cells, and thus maintaining genomic stability, might be a new mechanism for the tumor suppressor function of the Hippo pathway.

ACKNOWLEDGMENTS

We thank Qi Zhou for assistance in preparation of the figure. B.Z. is supported by grants from the National Natural Science Foundation of China (31271508), the State Key Development Program for Basic Research of China (2013CB945303), and the Natural Science Foundation of Zhejiang (LR12C07001). K.-L.G. is supported by grants from the NIH (EY022611 and CA132809).

REFERENCES

Aylon, Y., Michael, D., Shmueli, A., Yabuta, N., Nojima, H., and Oren, M. (2006). Genes Dev. *20*, 2687–2700.

Aylon, Y., Yabuta, N., Besserglick, H., Buganim, Y., Rotter, V., Nojima, H., and Oren, M. (2009). Oncogene *28*, 4469–4479.

Fujiwara, T., Bandi, M., Nitta, M., Ivanova, E.V., Bronson, R.T., and Pellman, D. (2005). Nature *437*, 1043–1047.

Ganem, N.J., Godinho, S.A., and Pellman, D. (2009). Nature *460*, 278–282.

Ganem, N.J., Cornils, H., Chiu, S., O'Rourke, K.P., Arnaud, J., Yimlamai, D., Théry, M., Camargo, F.D., and Pellman, D. (2014). Cell *158*, this issue, 833–848.

Kapoor, A., Yao, W., Ying, H., Hua, S., Liewen, A., Wang, Q., Zhong, Y., Wu, C.J., Sadanandam, A., Hu, B., et al. (2014). Cell *158*, 185–197.

McPherson, J.P., Tamblyn, L., Elia, A., Migon, E., Shehabeldin, A., Matysiak-Zablocki, E., Lemmers, B., Salmena, L., Hakem, A., Fish, J., et al. (2004). EMBO J. *23*, 3677–3688.

Shao, D.D., Xue, W., Krall, E.B., Bhutkar, A., Piccioni, F., Wang, X., Schinzel, A.C., Sood, S., Rosenbluh, J., Kim, J.W., et al. (2014). Cell *158*, 171–184.

Tschöp, K., Conery, A.R., Litovchick, L., Decaprio, J.A., Settleman, J., Harlow, E., and Dyson, N. (2011). Genes Dev. *25*, 814–830.

Yu, F.-X., and Guan, K.-L. (2013). Genes Dev. *27*, 355–371.