

Tumor Suppressors

Major finding: The Hippo pathway blocks proliferation of tetraploid cells by inactivating YAP and stabilizing p53.

Mechanism: Extra centrosomes in tetraploid cells stimulate RAC1, which decreases active RHOA and activates LATS2.

Impact: This approach reveals mechanisms by which tetraploid cells bypass Hippo-mediated tumor suppression.

TETRAPLOIDY ACTIVATES THE HIPPO TUMOR SUPPRESSOR PATHWAY

Defects in mitosis or cytokinesis generate genetically unstable tetraploid cells, which may contribute to tumorigenesis and have been detected in numerous tumors. The proliferation of these cells is limited by p53-mediated cell-cycle arrest; however, the mechanisms underlying p53 activation in tetraploid cells are unknown. To address this question, Ganem, Cornils, and colleagues performed a genome-wide RNAi screen in tetraploid cells generated by induction of cytokinesis failure. This approach identified 98 proteins whose silencing enabled tetraploid cells to bypass p53-driven G₁ arrest, including many negative regulators of growth factor signaling. Depletion of large tumor suppressor kinase 2 (LATS2), a core component of the Hippo tumor suppressor pathway, increased tetraploid cell proliferation. Tetraploidy induced LATS2 activation, resulting in phosphorylation and inactivation of the transcriptional coactivators YAP and TAZ and stabilization of p53 via LATS2 interaction with MDM2. LATS2 activation was mediated, in part, by the presence of extra centrosomes in tetraploid

cells, which stimulated hyperactivation of the small G protein RAC1 and antagonized RHOA activity. Expression of constitutively active YAP, reactivation of RHOA, or attenuation of RAC activity in tetraploid cells was sufficient to overcome the Hippo pathway-dependent proliferative barrier. *In vivo* studies revealed that activation of the Hippo pathway suppressed the growth of naturally occurring tetraploid hepatocytes in a p53-dependent manner. Furthermore, *in vitro* evolution studies confirmed that the Hippo pathway was inactivated in proliferating tetraploid cells, as were many genes identified in the RNAi screen. These findings reveal a role for the Hippo pathway in suppressing the oncogenic potential of tetraploid cells and provide insight into how tumor cells can bypass Hippo-mediated growth inhibition. ■

Ganem NJ, Cornils H, Chiu SY, O'Rourke KP, Arnaud J, Yimlamai D, et al. Cytokinesis failure triggers Hippo tumor suppressor pathway activation. *Cell* 2014;158:833–48.

Drug Response

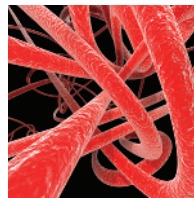
Major finding: Chloroquine mediates tumor vessel normalization in an autophagy-independent manner.

Mechanism: Chloroquine alters endosomal NOTCH1 trafficking and promotes NOTCH1 signaling in endothelial cells.

Impact: Chloroquine reduces tumor invasion and metastasis and enhances chemotherapy delivery and response.

THE ANTICANCER ACTIVITY OF CHLOROQUINE REQUIRES VESSEL NORMALIZATION

The anticancer properties of the antimalarial agent chloroquine are believed to result from the disruption of cancer cell autophagy, an essential nutrient-generating process in times of cellular stress. Maes, Kuchnio, and colleagues investigated whether the anticancer activity of chloroquine is also mediated by effects on stromal cells and whether these effects are dependent on autophagy. Chloroquine treatment reduced the growth and proliferation of melanoma cell lines in mice with concomitant decreases in the degradation of autophagy-selective substrates. Surprisingly, chloroquine treatment reduced tumor necrosis *in vivo*, but also resulted in less invasive tumors that exhibited impaired intravasation and diminished metastatic potential. Chloroquine-mediated effects on cancer cell invasion were not observed *in vitro*, suggesting an indirect effect on cancer cells through regulation of the tumor stroma. In support of this idea, chloroquine treatment induced normalization of tumor vasculature, including decreased hypoxia and improved tumor vessel structure and function, potentially contributing to a less stressed tumor microenvironment. In addition, increased vascular normalization in response to chloroquine enhanced the delivery and



efficacy of chemotherapy. Tumor vessel normalization occurred independently of autophagy in cancer cells and endothelial cells, and was instead mediated via modulation of endosomal degradation by chloroquine. Assessment of a panel of angiogenic molecules in endosomal compartments revealed altered trafficking of NOTCH1 to late endosomes after chloroquine treatment. Chloroquine induced the accumulation of NOTCH1 in late endosomes of endothelial cells, which resulted in increased enzymatic cleavage and formation of the transcriptionally active NOTCH1 intracellular domain. Inhibition of NOTCH1 signaling in endothelial cells eliminated chloroquine-induced tumor vessel normalization and its effects on metastasis and tumor necrosis. Taken together, these observations suggest that the anticancer activity of chloroquine is also mediated by autophagy-independent normalization of tumor vessels via sustained activation of NOTCH1 signaling in late endosomes. ■

Maes H, Kuchnio A, Peric A, Moens S, Nys K, De Bock K, et al. Tumor vessel normalization by chloroquine independent of autophagy. *Cancer Cell* 2014;26:190–206.

Note: Research Watch is written by Cancer Discovery Science Writers. Readers are encouraged to consult the original articles for full details. For more Research Watch, visit Cancer Discovery online at <http://CDnews.aacrjournals.org>.