

Breast Cancer

Major finding: Patients with ER⁺/HER2⁻ breast cancer have interconverting HER2⁺ and HER2⁻ circulating tumor cells.

Concept: Circulating breast tumor cells switch between HER2⁺ proliferative and HER2⁻ drug-resistant states.

Impact: Combination therapy may be warranted to target both HER2⁺ and HER2⁻ subpopulations.

CIRCULATING BREAST CANCER CELLS INTERCONVERT BETWEEN DISTINCT STATES

In patients with advanced estrogen receptor (ER)⁺/HER2⁻ breast cancer, a subpopulation of HER2⁺ circulating tumor cells (CTC) has been observed to emerge after multiple courses of therapy, but the role of acquired HER2 heterogeneity in disease progression is unclear. Jordan and colleagues found that 16 of 19 patients with ER⁺/HER2⁻ recurrent metastatic breast cancer acquired HER2⁺ CTCs after multiple courses of therapy. Single-cell RNA sequencing revealed a bimodal distribution of HER2 expression in CTCs indicative of distinct HER2⁺ and HER2⁻ subpopulations. HER2⁺ CTCs had an enhanced proliferation rate and generated larger tumors and more frequent lung metastases in mice. However, despite the enhanced proliferation rate of HER2⁺ cells, both populations coexisted, suggesting the possibility of interconversion between subpopulations. Indeed, spontaneous interconversion between subpopulations was observed *in vitro* and *in vivo*, with HER2⁺ and HER2⁻ cells producing cells of the opposite type within four doublings. The subpopulations exhibited distinct expression profiles with HER2⁺ CTCs having increased expression of pro-growth genes and phosphorylation of receptor tyrosine kinases (RTK), and HER2⁻ CTCs having increased expression of proteins involved in Notch

signaling and DNA damage. A drug screen revealed that HER2⁺ CTCs were not dependent on HER2 but sensitive to combined inhibition of multiple RTKs, whereas HER2⁻ cells were specifically sensitive to inhibitors of Notch activity and resistant to chemotherapeutic agents. Consistent with these findings, suppression of HER2 induced a rapid conversion from a HER2⁺ to a HER2⁻ state, as did chemotherapeutic agents or oxidative stress, indicating that the switch to a less proliferative state promotes a more drug-resistant phenotype. Moreover, in orthotopic mammary xenografts generated from a mixture of HER2⁺ and HER2⁻ CTCs, combined chemotherapy and Notch inhibition delayed tumor recurrence compared with chemotherapy alone. The finding that HER2⁺ and HER2⁻ cells can spontaneously interconvert to switch between proliferative and drug-resistant states suggests that combination therapy targeting both subpopulations may be required to eliminate these metastatic tumors. ■

Jordan NV, Bardia A, Wittner BS, Benes C, Ligorio M, Zheng Y, et al. HER2 expression identifies dynamic functional states within circulating breast cancer cells. *Nature* 2016 Aug 24 [Epub ahead of print].

Signaling

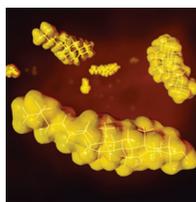
Major finding: Cholesterol is the cellular sterol that drives Hedgehog signaling through PTCH.

Mechanism: Cholesterol binds the cysteine-rich domain of SMO to induce a conformational change activating SMO.

Impact: Cholesterol is the second messenger that links PTCH and SMO to activate Hedgehog signaling.

ENDOGENOUS CHOLESTEROL INDUCES SMOOTHENED TO DRIVE HEDGEHOG SIGNALING

The Hedgehog signaling pathway is a developmental pathway that is activated when Hedgehog ligands bind to the Patched (PTCH) receptor, subsequently relieving PTCH-mediated repression of the transmembrane oncoprotein Smoothened (SMO). PTCH1 contains a sterol-sensing domain that is critical for inhibiting SMO and has homology to bacterial transporters of small lipophilic molecules. Because oxysterols, which are oxidized cholesterol derivatives, have been shown to activate SMO, it has been proposed that PTCH removes oxysterols from SMO to suppress SMO activity. However, endogenous oxysterols are present at low levels that are insufficient to activate Hedgehog signaling, while also activating SMO in a PTCH-independent manner. Huang and colleagues sought to identify the cellular sterol that mediates SMO activation and define the mechanism underlying its interaction with and activation of SMO. Crystallization of the extracellular cysteine-rich domain of SMO (SMOCD), alone or in complex with oxysterol, identified an



oxysterol-induced conformational change, which was sufficient for SMO activation, suggesting that sterol binding is integral for Hedgehog signaling. Cholesterol, which is the most abundant sterol present in cells, was shown to bind SMOCD and to activate Hedgehog signaling in a dose-dependent manner. Further, cholesterol, but not an oxysterol, synergized with sonic hedgehog (SHH) to activate Hedgehog signaling. Consistent with these findings, SMO mutants defective in binding and responding to oxysterols were still responsive to cholesterol and to SHH. Taken together, these findings describe a sterol-SMOCD interaction-dependent mechanism underlying SMO activation, and identify cholesterol as the sterol critical for SMO activation by PTCH-mediated Hedgehog signaling. ■

Huang P, Nedelcu D, Watanabe M, Jao C, Kim Y, Liu J, et al. Cellular cholesterol directly activates smoothened in hedgehog signaling. *Cell* 2016;166:1176–87.e14.