

p27 Shows Its Dark Side In Breast Cancer

March 16, 2015

A Neves

Cell division is critical for normal development, and is known to go awry in many different types of cancer. It is a highly regulated process involving two major classes of proteins: cyclin-dependent kinases (Cdks) that trigger cell cycle transitions, and cyclin-dependent kinase inhibitors (CKI) that keep Cdks in check. p27 is a CKI that is known to function in the nucleus as a tumor suppressor. Unlike other tumor suppressors, however, p27 is rarely mutated in human cancers, and tumors that develop in p27 heterozygous mice do not lose the remaining allele. Moreover, previous studies showed that mice harboring a homozygous mutation in p27 that prevents binding to Cdks are more tumor prone than are mice lacking p27 entirely. Together, these studies paint a complex picture and collectively suggest that p27 may have oncogenic functions when localized to the cytoplasm. Indeed, localization of p27 to the cytoplasm correlates with poor prognosis, tumor grade and survival in some tumor types, including breast and ovarian cancer. However, how cytoplasmic p27 becomes oncogenic and its relevance for therapy resistance had remained unclear.

A recent Fred Hutch study led by Research Associate Dr. Hui Zhao in the lab of Dr. Jim Roberts (Basic Sciences Division), in collaboration with the lab of Dr. Bruce Clurman, (Human Biology and Clinical Research Divisions) and published in *Oncotarget*, sought to shed some light on these questions. As a first step to investigate the role of p27 in human breast cancer, the authors compared p27 levels using immunoblotting and immunofluorescence in quiescent and proliferating breast cancer cell lines that were stratified by Her2 status. Her2 is an epidermal growth factor receptor family member that is known to be amplified in 15-20% of breast cancers. In non-transformed breast epithelial cells (MCF10A) or Her2- breast cancer cell lines (HCC38 and HCC1937), p27 levels markedly decreased following cell cycle entry. In contrast, the researchers found that p27 levels remained high in Her2+ cell lines (UACC893 and BT474) after cell cycle induction, but was located in the cytoplasm instead of the nucleus.

Next, the authors examined the link between p27 localization and cell proliferation and found that cytoplasmic p27 correlated with increased proliferation. Lapatinib is a FDA-approved drug that inhibits the kinase activity of Her2. Treatment of Her2+ cells with lapatinib halted cell cycle progression and resulted in nuclear rather than cytoplasmic expression of p27. To determine whether p27 was required for lapatinib-induced cell cycle arrest, the investigators knocked down p27

in Her2+ cells using short hairpin RNA (shRNA) and found that, in contrast to control cells, p27 knockdown cells failed to undergo efficient cell cycle arrest in response to lapatinib.

One outstanding question was whether localization of p27 to the cytoplasm is simply a mechanism for reducing nuclear p27 or if it has a more active, oncogenic role. To address this, the researchers knocked down p27 in Her2+ cells treated with lapatinib and assayed for viability. Strikingly, knockdown of p27 in Her2+ cells resulted in hypersensitivity to lapatinib and caused increased cell death, as visualized by staining with cleaved caspase-3. To corroborate these findings, the authors expressed a nuclear-excluded form of p27 (p27 Δ NLS) in Her2+ cells where endogenous p27 was knocked down. Notably, p27 Δ NLS expression reversed lapatinib sensitivity of Her2+ cells and reduced apoptosis.

Overall, this study demonstrated that cytoplasmic p27 promotes resistance of Her2+ breast cancer cells to lapatinib. "Our results suggest that p27 localization may be useful as a predictive biomarker of therapeutic response in patients with Her2+ breast cancers", said Dr. Zhao.

[Zhao H, Faltermeier CM, Mendelsohn L, Porter PL, Clurman BE, Roberts JM. 2014.](#) Mislocalization of p27 to the cytoplasm of breast cancer cells confers resistance to anti-HER2 targeted therapy. *Oncotarget*, 5(24), 12704-14.

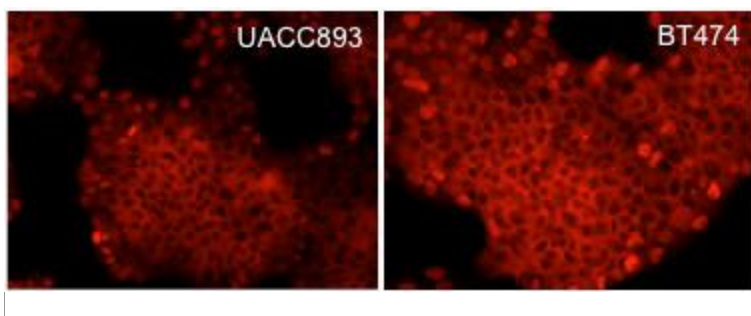


Image provided by Dr. Zhao

Immunofluorescence staining of p27 in Her2+ human breast cancer cell lines (UACC893 and BT474 cells). Cells were released into cell cycle for 72 hr after synchronization by serum starvation. The "honeycomb-like" staining of the cell sheet results from nuclear exclusion of p27.