JOURNAL WATCH

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Our panel of experts highlight the most important research articles across the spectrum of topics relevant to the field of breast cancer management

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Bhola, NE, Balko JM, Dugger TC *et al.* TGF- β inhibition enhances chemotherapy action against triple-negative breast cancer. *J. Clin. Invest.* 123, 1348–1358 (2013).

Drug-resistant metastatic disease is one of the major clinical problems with triple-negative breast cancer. Pathway analysis of genes differentially expressed in pre- and post-treatment biopsies in a neoadjuvant setting can provide clues to the mechanisms of resistance. Using NanoString® (NanoString Technologies, WA, USA) technology, these authors measured breast cancer cell line-derived TGF- β signature scores (101 upregulated and 51 down-regulated genes) in 17 breast tumor biopsies collected pre- and post-chemotherapy (anthracyclines and paclitaxel or docetaxel). Post-treatment biopsies had higher TGF- β signature scores compared with pretreatment biopsies. In cell line models, paclitaxel activated the TGF- β pathway as measured by SMAD2 phosphorylation both *in vitro* and *in vivo*, which consequently increased the number of cells with a stem cell phenotype. Pharmacological inhibitors of TGF- β , including LY2157299, reduced the tumor-initiating potential of paclitaxel-treated cells. Both paclitaxel and TGF- β increased the levels of the chemokine IL-8, a well-known chemokine involved in the self-renewal of cancer stem cells. Interestingly, while a neutralizing antibody against IL-8 reduced basal but not TGF- β -induced mammosphere formation, knockdown of its receptors, CXCR1 or CXCR2, reduced both basal and TGF- β -induced

mammospheres. The data presented in this manuscript have two major implications. First, this illustrates the power of recently developed techniques, which enable analysis of limited primary tumor materials first, and then functional studies using cell lines. Since pathways for the analysis can be preselected, there is no need for extensive and expensive sequencing and bioinformatics support. Second, there are efforts to develop IL-8 inhibitors to reduce cancer stem cells and to increase chemosensitivity. However, IL-8-independent signaling from its receptors CXCR1/CXCR2 appears to be involved in the cancer stem cell phenotype of paclitaxel- or TGF- β -treated cells. As evidence for noncanonical ligands of chemokine receptors continues to emerge, we may see new players in the CXCR1/CXCR2 signaling network in the near future. As LY2157299 is slowly moving into the clinic (there are currently Phase Ib/IIa trials for malignant glioma), the next few years may see clinical trials in triple-negative breast cancers with TGF- β inhibitors and chemotherapy. One omission of the current study is the lack of immunohistochemical analysis of phospho-SMAD2 in pre- and post-treatment biopsies to further demonstrate activation of TGF- β pathways upon chemotherapy. Since data generated using core needle biopsies can be biased because of tumor heterogeneity, simplified assays that can measure TGF- β pathway activation in multiple regions of residual tumors through surrogate markers will be of practical use.

– Written by Harikrishna Nakshatri

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Pal B, Bouras T, Shi W et al. Global changes in the mammary epigenome are induced by hormonal cues and coordinated EZH2. *Cell Rep.* 3, 411–426 (2013).

Estrogen has always received more attention than progesterone in the breast cancer field. However, several recent studies on the normal breast cellular hierarchy clearly demonstrate an important role for progesterone in maintaining the normal stem–progenitor–mature luminal cell hierarchy. This report reveals how progesterone utilizes epigenetic machinery to influence gene-expression patterns in specific cell types during normal mammary development and during pregnancy. EZH2, a histone methyltransferase that is overexpressed or mutated in many cancers, is the primary target of progesterone. Progesterone and prolactin govern the majority of gene-expression changes associated with pregnancy. Mammary stem cell (MaSC) populations expand dramatically during pregnancy, and hormonal cues direct these stem cells towards alveolar differentiation, accompanied by increased expression of genes required for lipid biosynthesis and lipid catabolism. Naturally, significant epigenetic changes are expected during this differentiation process. The authors first determined the genome-wide histone H3K4 trimethylation (H3K4me3), H3K9 dimethylation (H3K9me2) and H3K27 trimethylation (H3K27me3) patterns and correlated these methylation patterns with gene expression in MaSCs, luminal progenitor cells and mature luminal cells. As expected, transcription start sites of transcribed genes were enriched for H3K4me3, whereas genes with lower or no expression showed elevated H3K27me3 markers. When a similar analysis was carried out with MaSCs and total luminal cells isolated from the mammary glands of pregnant mice, H3K27me3 markers and gene-expression levels displayed an inverse relationship, and these markers were dramatically lower in genes required for milk production. Overall, the total number of luminal genes with H3K27me3 was dramatically higher in cells isolated from

the mammary glands of pregnant mice, but significantly lower in the mammary glands of ovariectomized mice. These results provide a strong indication for the role of pregnancy hormones in global H3K27me3. EZH2 is the principal component of the protein complex that mediates H3K27me3. Consistent with its role in pregnancy-associated epigenetic changes, EZH2 levels were intense in the ducts and alveoli of pregnant mice, and declined in the lactating and involuting gland. This increase in the level of EZH2 is likely due to post-translational modification, particularly Thr487 phosphorylation. It appears that progesterone activates the protein kinases Cdk1/Cdk2 through the paracrine RANKL/RANK system, which phosphorylate and stabilize EZH2. Since the majority of prior clinical studies attempted to associate EZH2 mRNA levels with breast cancer outcome, the data presented in this report highlight the need to measure phospho-EZH2 levels as a surrogate marker of EZH2 activity in cancer. In addition, as results of conditional knockout mouse models demonstrated the need for EZH2 in lactation, the next question is what effects EZH2 overexpression/mutation will have on lactation and the behavior of breast cancers diagnosed in pregnant women. One can imagine a perfect collaboration between aberrantly expressed EZH2 and increased progesterone signaling during pregnancy. This collaboration may lead to the expansion of the MaSC pool with cancer stem cell activity and aberrant alveolar differentiation. In this respect, previous studies have demonstrated a role for EZH2 in breast cancer stem cells. Most of our understanding of epigenetic changes during lineage commitment and differentiation is primarily derived from studies using embryonic stem cells and induced pluripotent stem cells. We are just beginning to unlock the secrets of epigenetics in the stem cell hierarchy of adult organs, which will impact our understanding of the origins of cancer.

– Written by Harikrishna Nakshatri

Yu M, Bardia A, Wittner BS et al. Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. *Science* 339, 580–584 (2013).

The significance of epithelial to mesenchymal transition (EMT) observed in cultured cancer cells on the metastatic progression of cancer *in vivo* has been a matter of debate for a number of years. The issue is even more complicated because there is recent preclinical evidence of cancer cells with EMT regaining epithelial features after embedding in distant organs. Thus, capturing cells with the EMT phenotype *in vivo*, which may be a transient phenotype, has been technically challenging. This report provides strong evidence for the role of EMT in blood-borne breast cancer metastasis. The authors developed a quantifiable RNA *in situ* hybridization technique that measures seven epithelial and three mesenchymal transcripts in tumor cells. Keratins 5, 7, 8, 18 and 19, EpCAM and E-cadherins were the epithelial transcripts (E-transcripts), whereas fibronectin, N-cadherin and SERPINE1 were the mesenchymal transcripts (M-transcripts). First, the authors examined the expression pattern of these transcripts in primary ductal carcinoma *in situ*, ER⁺/PR⁺, HER2⁺ and triple-negative breast cancers. Epithelial cells of the tumors were predominantly positive for the E-transcripts, whereas stromal cells were positive for the M-transcripts. The presence of a small population of cells that were positive for both E- and M-transcripts caught the authors' attention. These biphenotypic cells were present mostly in triple-negative breast cancers and were detected in draining lymph nodes. The authors then collected circulating tumor cells (CTCs) from patients with metastatic disease using a microfluidic herringbone chip, which isolates tumor cells based on cell surface EpCAM, EGF receptor and HER2 proteins. CTCs of triple-negative and HER2⁺ breast cancer patients were predominantly positive for M-transcripts, whereas CTCs from ER⁺/PR⁺ patients were predominantly positive for E-transcripts. The most intriguing aspect was the effect of chemotherapy on

the epithelial and mesenchymal features of CTCs. Patients who responded to treatment showed decline in CTCs positive for M-transcripts, whereas patients who progressed while on treatment displayed progressive increases in CTCs positive for M-transcripts. However, the sample size is too small to generalize these results or to use this measurement as a marker of treatment response. CTCs from one patient, who showed transient response to various therapies, were subjected to additional analysis, including RNA-sequencing of CTCs at serial time points. These studies revealed the enrichment of the transcription factor FOXC1 in CTCs positive for M-transcripts. FOXC1 is a known inducer of EMT and is associated with the cancer stem cell phenotype. Whether immunohistochemical analysis of CTCs for FOXC1 will be sufficient to identify CTCs positive for M-transcripts and utilize this information for assessing treatment effects remain to be determined. Despite the major breakthroughs highlighted in this report, one concern regarding CTCs still remains – can EpCAM antibody capture all CTCs? EMT *in vitro* is associated with a decline in EpCAM expression and the

most aggressive CTCs expressing M-transcripts alone may have escaped isolation using techniques that rely on EpCAM. Therefore, CTC isolation methods need further improvements before the routine use of CTCs for diagnostic, prognostic and predictive purposes.

– Written by Harikrishna Nakshatri

Barbashina V, Corben AD, Akram M et al. Mucinous micropapillary carcinoma of the breast: an aggressive counterpart to conventional pure mucinous tumors. *Hum. Pathol.* doi:10.1016/j.humpath.2013.01.003 (2013) (Epub ahead of print).

Pure mucinous carcinoma (PMC) is classified as a luminal subtype and considered to be at a low risk of recurrence in the National Comprehensive Cancer Network guidelines or the St Gallen consensus recommendations. However, it is also known that a minor subset of patients with PMC suffer metastases and recurrences after surgical resection. Focusing on histopathological features, in this study, the clinical significance of mucinous micropapillary carcinoma (MUNPC) as a subset of PMC was studied.

The MUNPC was histologically characterized by invasive micropapillary architecture arranged in floret-like and pseudoacinar structures surrounded by stromal spaces filled with mucin, psammoma bodies and intermediate to high nuclear grade. Compared with classic PMC, MUNPC tended to show higher rates of axillary lymph node metastasis and lymphovascular invasion. The authors reviewed a total of 60 cases of MUNPC, and suggested a correlation with more aggressive clinical behavior. Although the results are not conclusive, routine identification of MUNPC might be useful in the future for further individualization of the treatment of patients with PMC.

– Written by Hitoshi Tsuda

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