Unraveling the Complexity of Endocrine Resistance in Breast Cancer by Functional Genomics

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Despite the proven benefit of antiestrogen drugs in breast cancer treatment, resistant disease ultimately develops in advanced breast cancer. In this issue of *Cancer Cell*, Iorns et al. find that loss of CDK10 expression promotes resistance of cells to tamoxifen and is associated with poor outcome in breast cancer patients treated with the drug. CDK10 loss increases the activity of the transcription factor ETS2 on the promoter of the *RAF1* gene, elevating ERK/MAPK kinase pathway activity and relieving tamoxifen-induced G1 arrest. CDK10 is thus a potential biomarker for sensitivity in prospective clinical trials of patients treated with endocrine therapies.

Tamoxifen is one of the most successful agents used in the management of hormone receptor-positive breast cancer. In trials of adjuvant treatment with tamoxifen in early breast cancer following surgery, the absolute improvements in 10-year survival were 10.9% for node-positive and 5.6% for node-negative patients (EBCTCG, 2005). Its use improves quality of life in patients with estrogen receptor (ER)-positive advanced (metastatic) breast cancer, often delaying the need for cytotoxic chemotherapy. Despite its success and status as one of the most active agents available for patients with this disease, the development of tamoxifen resistance is almost inevitable in patients with advanced disease, and up to 40% of patients treated with tamoxifen in the adjuvant setting suffer disease relapse.

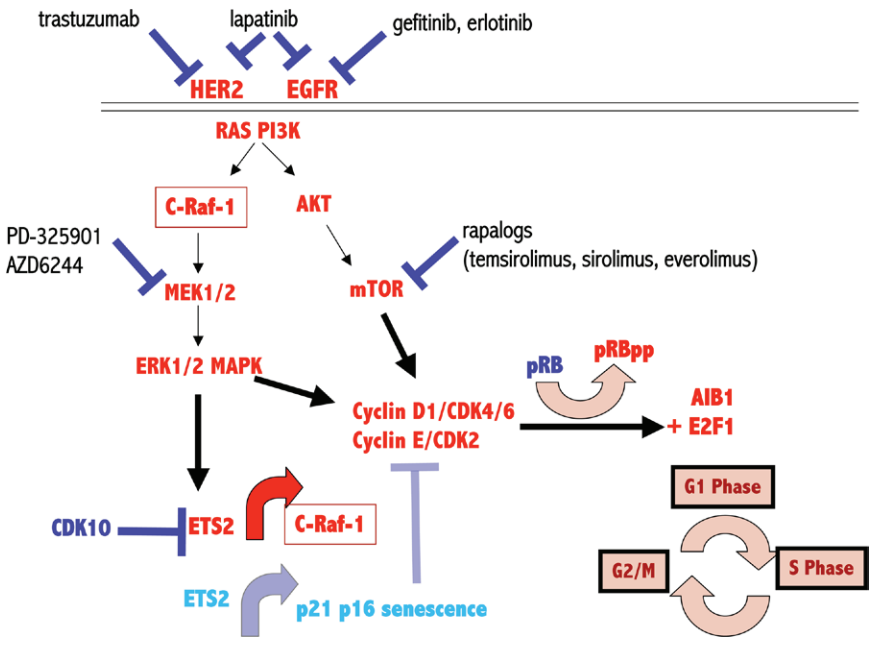

Figure 1. Endocrine Resistance Mediated by Signal Transduction Cascades
Tamoxifen induces a G1 cell cycle arrest. Activation of signal transduction molecules associated with resistance to endocrine therapies (red) or sensitivity (blue) affects progression through the G1/S phase of the cell cycle. CDK10 loss drives ERK pathway activity through transcriptional upregulation by ETS2 of c-Raf-1. ETS2 also induces senescence through $p16^{INK4A}$. STIs targeting this pathway (black) may enhance endocrine response.

Laboratory studies have demonstrated that activation of EGF receptor family and the ERK/MAPK pathway promote estrogen-independent growth (see Figure 1), leading to several clinical trials using signal transduction inhibitors (STIs) to enhance endocrine sensitivity (Johnston et al., 2007). These trials have met with limited success, emphasizing the requirement to define novel endocrine resistance pathways and establish mechanisms through which established signaling axes may be activated in order to optimize the selection of patients for future trials. This may lead to the development of improved approaches to prolong benefit from endocrine agents and to select patients who may benefit from the use of alternative treatment strategies targeting the endocrine axis.

With this in mind, Ashworth and colleagues (Iorns et al., 2008) have developed an RNA interference (RNAi) screening approach to identify kinases involved in the acquisition of tamoxifen resistance in a well-described ER-positive breast cancer cell line, MCF-7. The authors transfect these cells with synthetic small interfering (si) RNAs target-

ing the expression of some 779 kinases and related proteins, assaying the effect on cell proliferation over a week in the presence or absence of tamoxifen. Iorns et al. have identified three genes whose knockdown reproducibly caused resistance to tamoxifen treatment: cyclin-dependent kinase 10 (CDK10), CDC2-related protein kinase 7 (CRK7) and mitogen-activated protein kinase kinase 7 (MAP2K7).

Concentrating on a thorough examination of the role of CDK10 in cellular responsiveness to tamoxifen, the authors show that effective silencing of this kinase induces resistance to ERα signaling and overcomes a G1 cell cycle arrest in response to tamoxifen treatment by subverting cyclin D1 expression. Importantly, Iorns et al. provide evidence that tamoxifen resistance conferred by CDK10 suppression does not result in increased expression or activation of ERα and that expression of ER target genes are not induced, so tamoxifen resistance is not conferred by ligand-independent ERα activation. Furthermore, CDK10 silencing also confers resistance to a relatively new antiestrogen agent used in clinical practice, fulvestrant, or ICI 182,780, which induces ERα degradation.

The authors proceed to define the pathway of resistance to tamoxifen mediated through CDK10 suppression. A likely suspect in the hunt to connect tamoxifen resistance with cyclin D1 expression was the ERK pathway, activation of which has been shown to result in increased cyclin D1 expression (Lavoie et al., 1996). Activation of the pathway components ERK1/2 and MEK1/2 was observed following CDK10 silencing, while suppression of ERK pathway signaling at the same time as CDK10 inhibition restored sensitivity to tamoxifen. As for proteins upstream of MEK, both the level and activity of the upstream kinase c-Raf-1 were increased following CDK10 silencing.

Given the increased c-Raf-1 mRNA witnessed in cells lacking CDK10, an obvious suspect that might be responsible for these aberrant transcriptional effects was the ETS2 transcription factor, implicated by its previously documented association with CDK10 (Kasten and Giordano, 2001) and with tamoxifen resistance in breast cancer through ERK1/2 signaling (Svensson et al., 2005). CDK10 has been shown to inhibit the transactivation capacity of ETS2 (Kasten and Giordano, 2001), so CDK10 loss would be predicted to potentiate ETS2-mediated gene transcription. Again, resistance could be partially overcome following cosuppression of ETS2 and CDK10, and the authors provide evidence confirming their association in MCF-7 cells. Are the couple guilty by association, or is ETS2 directly implicated in c-Raf-1 transcriptional activation? Chromatin immunoprecipitation experiments here place CDK10 and ETS2 on the *RAF1* gene promoter, while loss of CDK10 potentiates ETS2 interaction with the promoter.

This presents a compelling model (see Figure 1). However, ETS2 is a relatively promiscuous transcription factor and may also potentiate growth arrest through the induction of the broad CDK inhibitor $p21^{CIP1}$ (Beier et al., 1999) and senescence through the induction of the CDK4/6 inhibitor $p16^{INK4A}$ (Ohtani et al., 2001). This is particularly relevant as the MCF-7 cells used here display biallelic loss of CDKN2A, the gene encoding $p16^{INK4A}$, which has been shown to confer resistance to ETS1- or ETS2-induced senescence (Huot et al., 2002). Furthermore, recent evidence implicates pRb as a nodal regulator of antiestrogen responsiveness (Varma et al., 2007) as pRb inactivation results in antiestrogen resistance, and expression of AIB1, a nuclear receptor coactivator, impairs tamoxifen-induced cell cycle arrest by potentiating E2F1 activity (a target of pRb-mediated repression) (Louie et al., 2004). Does CDK10 influence ETS2 interaction with the CDKN2A promoter and $p16^{INK4A}$ expression in a manner similar to what is seen in this study with c-Raf-1? Similar experiments characterizing the effects of CDK10 silencing in estrogen-responsive breast cancer cell lines with wild-type $p16^{INK4A}$ will be interesting; a targeted approach toward ETS2 inactivation in order to potentiate tamoxifen responsiveness could in principle induce a paradoxical effect, limiting oncogene-induced senescence and conceivably potentiating endocrine resistance.

Nevertheless, in two separate clinical data sets of breast cancer patients treated with adjuvant tamoxifen, Ashworth and colleagues demonstrate that low CDK10 expression is associated with a statistically significant shorter time to distant relapse and, in one data set, poorer overall survival. These data are compelling, particularly as CDK10 expression appears to have no association with HER2 status or level of ERα expression, and may represent a new biomarker of endocrine responsiveness worthy of follow-up in prospective studies. It will be interesting to assess whether CDK10 expression affects disease-free survival in estrogen receptor-positive patients only (as might be predicted from this study), or whether CDK10 loss confers poorer prognosis regardless of ER status. It might be predicted that CDK10 loss in tumors (potentiating ETS2-mediated transactivation) could segregate with biallelic mutations in CDKN2A, which ablate a cellular defense mechanism against oncogene-induced senescence. Could CDK10 loss play a role in the acquisition of hormone-refractory prostate cancer, a disease that displays altered regulation of the ETS family of transcription factors including ETS2, potentially driving c-Raf-1 expression and androgen independence (Mukherjee et al., 2005)?

This study provides a compelling connection between a previously known signal transduction pathway associated with resistance to endocrine therapies and a poorly studied cyclin-dependent kinase associated with worse outcome following tamoxifen treatment in vivo. It demonstrates once again the power of RNA interference screens as a means to deciphering the basic biological mechanisms underlying the behavior of cancer cells. A remaining challenge is to identify the significance of ETS pathway activation in prospective clinical studies and to identify molecules capable of effectively limiting pathway activity to prolong endocrine responsiveness.

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Modeling Multiple Myeloma by AID-Dependent Conditional Activation of MYC

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Efforts to create a mouse model that provides even a phenocopy of human multiple myeloma (MM) have been unsuccessful. In this issue of *Cancer Cell*, Bergsagel and colleagues describe an apparent solution to this problem by creating a model in which a MYC transgene containing a stop codon and flanking Igκ regulatory sequences is activated sporadically in germinal center B cells by AID-dependent somatic hypermutation that reverts the stop codon. Although much remains to be done to fully characterize this model, this approach is likely to impact the creation of sporadic models for other kinds of germinal center B cell tumors.

The term "multiple myeloma" was suggested by von Rustizky in 1873, when he found during autopsy *multiple* separate bone marrow (*myel*-) tumors (-*oma*) (Malpas et al., 2004). Multiple myeloma (MM) is a post-germinal center tumor of long-lived bone marrow (BM) plasmablasts/plasma cells (PC) that have undergone extensive somatic hypermutation of Ig heavy chain (IgH) and Ig light chain (IgL) genes, antigen selection, and productive IgH switch recombination. Most tumors produce IgG or IgA, with IgM produced by only 1% of tumors. MM usually is preceded by a similarly age-dependent premalignant tumor called monoclonal gammopathy of undetermined significance (MGUS), the most common lymphoid tumor in humans, occurring in nearly 3% of individuals over the age of 50. For MGUS, the serum monoclonal Ig (M-Ig) typically is 5–30 g/l (normal polyclonal Ig is 7–15 g/l), with the tumor cells comprising no more than 10% of BM mononuclear cells. Although MGUS usually remains stable for many years, there can be sporadic progression to frankly malignant MM expressing the same M-Ig at a rate of 1% per year. Smoldering MM, with a stable BM content of 10%–30% tumor cells, has a higher rate of sporadic progression. Frankly malignant MM usually is progressive and is associated with secondary pathologies, sometimes including osteoporosis, osteolytic lesions, anemia, immunodeficiency, and decreased kidney function. Similar to long-lived PC, MGUS and MM have a strong dependence on the BM microenvironment for survival and growth, although some individuals develop extramedullary MM, or PC leukemia, most often as a very late event. In contrast to long-lived PC, MGUS and MM have the ability to proliferate at a low rate, usually with only a few percent of cycling cells until advanced stages of MM.

Early oncogenic events that are shared by MGUS and MM include primary IgH translocations (40%) and hyperdiploidy (50%), with dysregulation of a CYCLIN D gene being a unifying event for all tumors (Chng et al., 2007). Based on these early events, it has been proposed that MGUS and MM can be classified into at least five distinct molecular groups (diseases) that are associated with different biological and clinical characteristics. No genetic abnormalities distinguish MM and MGUS, although activating RAS mutations occur in 30%–40% of MM tumors but only 5% of MGUS tumors. Among shared genetic events that occur during progression of MM, rearrangements of *MYC* (rarely *MYCN*) represent a very late progression event that appears to be associated with increased proliferation and decreased stromal cell dependence.

The development of animal models for MM, including both de novo and syngeneic (Vanderkerken et al., 2003) or xenogenic (human MM into SCID-Hu, SCID-Rab, or NOD-SCID mice) (Matsui et al., 2008; Yata and Yaccoby, 2004) mouse transplant models