ante for those betting types who strive to predict the phenotype of mice lacking the E type cyclins or cdk2, or of normal human cells treated to lack these molecules. Based on current dogma, many would place their money on an absolute requirement for cdk2 or E cyclins in normal cell cycles. Given the work presented by Tetsu and McCormick, the payoff may go to those betting on less profound phenotypes, and such experiments will likely force the preparation of a new set of model slides for all those with interest in cdk2's role in mammalian cell cycle control.

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Connecting estrogen receptor function, transcriptional repression, and E-cadherin expression in breast cancer

A recent paper in *Cell* (Fujita et al., 2003) demonstrates that MTA3, a novel component of the Mi-2/NuRD transcriptional repression complex, is an estrogen receptor-regulated inhibitor of the Snail zinc finger transcription factor in breast cancer. Given the important role of Snail in repressing E-cadherin transcription and the function of E-cadherin as a tumor suppressor protein and regulator of epithelial architecture, the findings offer potentially significant new insights into cancer pathogenesis.

In the United States and much of the Western world, breast cancer rivals lung cancer as the most frequent cause of cancer-related death in women, with upwards of 12 percent of women diagnosed with breast cancer during their lifetimes. While breast cancer mortality appears to have shown some encouraging decreases in the recent past, presently, about 25% of women diagnosed with breast cancer will die of the disease (Baselga and Norton, 2002). Hormonal factors play a key role in normal breast development and in growth and progression of breast cancer.

Perhaps chief among the hormonal factors involved in breast cancer is the ovarian steroid hormone estrogen. The biological actions of estrogen are dependent on the cellular function of a high-affinity estrogen receptor (ER) (McDonnell and Norris, 2002). Two estrogen receptors—ERα and ERβ—have been identified, but most estrogenic responses appear to require ERα. Upon binding estrogen or other ligands, ER is released from its inhibition by a large heat shock protein complex. Following dimerization, ER activates transcription of specific cellular genes via direct and indirect interactions with their regulatory regions. In breast cancer cells expressing ER, estrogen has potent effects on cell proliferation, differentiation, and survival, perhaps in part via estrogen's ability to affect the cellular response to various growth factors and other cues from surrounding extracellular matrix and stromal cells. While ER expression in breast cancer is generally associated with a better clinical outcome, the clinical utility of ER as a prognostic marker is modest. Rather, the principal value of defining the ER status (and the progesterone receptor status) of a breast cancer is for prediction of the patient's likely response to systemic therapy, particularly adjuvant therapy with tamoxifen, a selective estrogen receptor modifier and antiestrogenic agent in the breast (Baselga and Norton, 2002).

Besides hormonal and other environmental factors, germline and somatic mutations and gene expression changes play key roles in breast cancer initiation and progression. There is great interest in defining how the various mutations and gene expression changes contribute to breast cancer development and its aggressive behavior, especially because recent studies have indicated that particular gene expression signatures in breast cancer are associated with good prognosis and other signatures are associated with poor prognosis (van de Vijver et al., 2002). A major challenge for workers pursuing gene expression

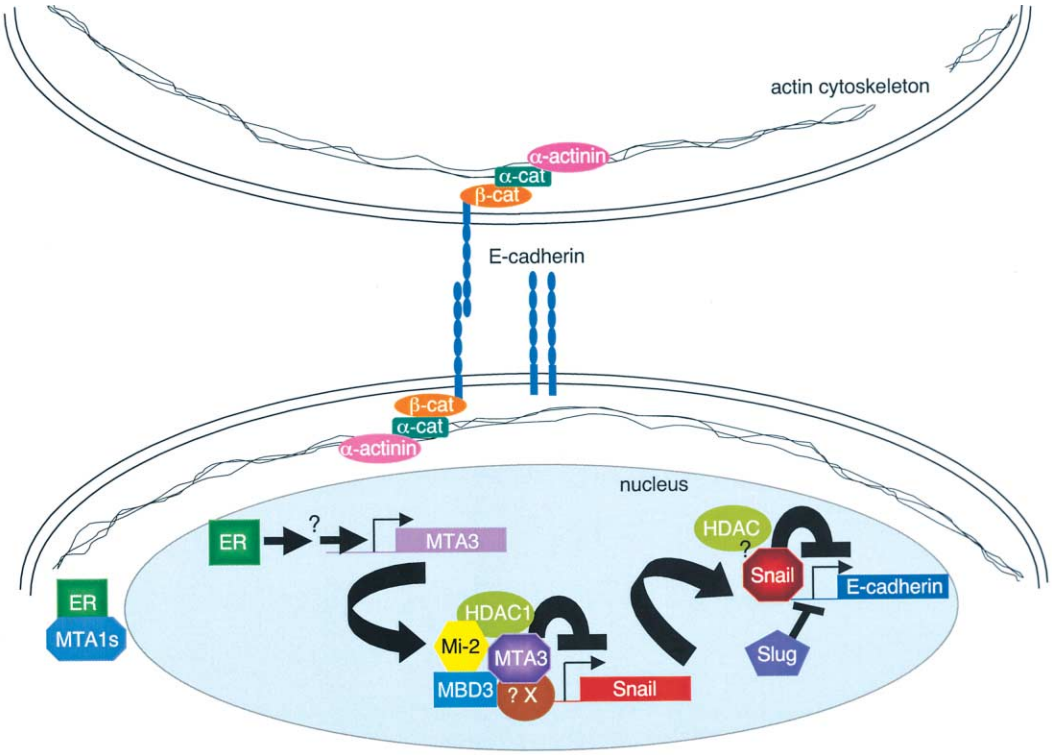

Figure 1. Model of factors and presumed mechanisms contributing to estrogen receptor-mediated activation of MTA3; MTA3's repression of Snail; and Snail's repression of E-cadherin

The model is based largely on the data presented in the article by Fujita et al. (2003). E-cadherin functions as a cell-cell adhesion molecule at adheren junctions of epithelial cells via homotypic interactions with E-cadherin molecules on neighboring cells and cytoplasmic interactions with the catenins, α-actinin (or vinculin), and the actin cortical cytoskeleton. Expression of the MTA3 (metastatic tumor antigen 3) gene is regulated indirectly by ER function. MTA3 is a component of the Mi-2/NuRD transcriptional repression complex, and other components include the nucleosome-stimulated ATPase Mi-2, the methyl CpG binding protein-related protein MBD3, histone deacetylase 1 (HDAC1), and potentially other unknown proteins (e.g., X). The MTA3-containing complex represses transcription of the gene encoding the Snail zinc finger DNA binding protein via binding to Snail promoter elements. The Snail protein binds to multiple E box elements in the E-cadherin promoter and likely mediates repression through interactions with other proteins, such as HDACs. The Snail-related protein Slug also functions to repress E-cadherin transcription, but Slug is not regulated by the MTA3 complex. A metastatic tumor antigen 1 isoform (termed MTA1s) can sequester ER in the cytoplasm, but the relationship of MTA1s action to expression of MTA3, Snail, and E-cadherin remains unknown.

analyses in breast cancer is to define key signaling pathways and transcriptional regulators impacting on expression of critical target genes, such as protooncogenes or tumor suppressor genes. A recent paper in *Cell* (Fujita et al., 2003) appears to offer new and exciting insights into mechanisms by which expression of the E-cadherin tumor suppressor gene may be silenced in some breast and other cancers.

E-cadherin is the prototypic member of the classic cadherin family of single-pass transmembrane glycoproteins mediating Ca^{2+}-dependent cell-cell adhesion, and it plays essential roles in development, cell polarity, and tissue morphology (Takeichi, 1995). E-cadherin's extracellular domain interacts in a homotypic fashion with E-cadherin molecules on an opposing cell, and its cytoplasmic domain is linked directly and/or indirectly to the actin cytoskeleton at the adherens junction via interactions with catenins (Figure 1). Disruption of the cadherin-catenin complex has been seen in cancers arising in many tissues and has been correlated with pathological and clinical features, such as tumor dedifferentiation, infiltrative growth, lymph node metastasis, and a worse patient prognosis. Maybe the strongest evidence in support of a causal role for cadherin alterations in cancer pathogenesis is the observation that germline mutations in the gene encoding E-cadherin (known as *CDH1*) strongly predispose affected individuals to diffuse-type gastric cancer and more modestly to breast carcinoma (Pharoah et al., 2001). *CDH1* inactivation seems to adhere to the Knudson two-hit model for tumor suppressor gene inactivation, with biallelic *CDH1* defects in cancers arising in those carrying germline *CDH1* mutations. Further evidence of a causal role for E-cadherin defects in cancer has been offered by the identification of somatic inactivating *CDH1* gene mutations in upwards of 50% of diffuse-type gastric and infiltrative lobular breast carcinomas and in subsets of other malignancies (Hajra and Fearon, 2002).

While immunohistochemical studies have demonstrated that reduced or absent E-cadherin expression is common in many types of carcinomas, in the majority of cancers where expression is lost, *CDH1* mutations are rare or absent (Hajra and Fearon, 2002). Proposed epigenetic mechanisms for E-cadherin inactivation include alterations in expression and/or function of *trans*-acting factors that regulate *CDH1* gene transcription, hyper-

methylation of the *CDH1* promoter, and chromatin-mediated effects. Consistent with a role for *trans*-acting factors, analysis of breast cancer somatic cell hybrids suggests that a dominant repression pathway extinguishes *CDH1* transcription via effects on its proximal promoter (Hajra and Fearon, 2002), and three E box elements in this region have been proposed to be critical in silencing of *CDH1* transcription in cancer (Hajra et al., 2002). A number of transcription factors may bind to the *CDH1* E box elements to repress transcription, including the zinc finger transcription factor Snail (Cano et al., 2000; Batlle et al., 2000), the Snail-related factor Slug (Hajra et al., 2002), and SIP1 (ZEB-2) (Comijn et al., 2001). Increased expression of Snail and/or Slug has been correlated with loss of E-cadherin expression in cancers of various types (Batlle et al., 2000; Poser et al., 2001; Hajra et al., 2002; Blanco et al., 2002).

While the prior results offered evidence of an association between expression of Snail and/or other transcriptional repressors and loss of E-cadherin expression in cancer, knowledge was lacking on the specific mechanisms contributing to aberrant expression of Snail and other transcriptional repressors in cancer. In their article, Fujita and coworkers provide compelling evidence that MTA3, a novel component of the Mi-2/NuRD (for *nu*cleosome *r*emodeling and *d*eacylation) transcription repression complex, can directly repress Snail transcription. Various genetic and biochemical studies had previously implicated the Mi-2/NuRD complex in transcriptional repression via its intrinsic histone deacetylase (HDAC) and nucleosome-stimulated ATPase activities and its ability to interact with DNA binding proteins (Becker and Horz, 2002). Although some prior studies had implicated overexpression of the roughly 80 kDa *m*etastatic *t*umor *a*ntigen *1* (MTA1) protein in invasive and metastatic growth (Toh et al., 1997), Fujita and colleagues focused attention on the related factor MTA3. After demonstrating MTA3 was a bona fide component of the Mi-2/NuRD complex and not present in complexes with MTA1 or MTA2, the authors established that the approximately 60 kDa MTA3 protein could function in model assays to repress gene expression through HDAC-dependent mechanisms. Of some interest, in breast cancer cells, expression of endogenous MTA3 transcripts and protein was dependent on activation of ER, though the MTA3 gene did not appear to be directly activated by ER.

In large part because of the connection between ER function and MTA3 expression, Fujita and colleagues speculated that changes in epithelial architecture seen in ER-negative breast cancer might result from a loss of MTA3 function. Moreover, because of E-cadherin's role in epithelial architecture, the authors wondered whether MTA3 might play a role in regulation of E-cadherin, perhaps via MTA3's ability to repress expression of factors that might themselves repress CDH1 gene transcription, such as Snail or Slug. Remarkably, MTA3, but not the related protein MTA1, had potent inhibitory effects via HDAC-dependent mechanisms on expression of Snail but not Slug (Figure 1). Using chromatin immunoprecipitation approaches, the MTA3 complex was found to associate with Snail promoter elements. MBD3, a protein closely related to the methyl CpG binding protein MBD2, was also present in the MTA3 complex. In the cell lines studied, the authors used both overexpression and RNA interference approaches to buttress their claims about the role of MTA3 in repression of Snail and the role of Snail in repression of E-cadherin. To establish the relevance of their claims for primary breast cancers, the authors pursued studies of primary breast carcinoma specimens, demonstrating MTA3 expression was well correlated with that of ER and E-cadherin. Finally, analysis of published microarray gene expression data from a large panel of primary breast carcinomas for which ER status was known yielded mixed but generally supportive data for the authors' claims about the relationships of MTA3 expression to ER, Snail, and E-cadherin expression.

Overall, the data in the Fujita et al. article are compelling, and the findings enhance understanding of mechanisms contributing to loss of E-cadherin function in cancer. The results also implicate intact ER function as a potentially crucial factor in differentiation and maintenance of normal epithelial architecture in breast epithelium. In spite of the advances offered by the work, gaps in our knowledge remain. Because ER did not appear to activate MTA3 expression directly and ER and MTA3 expression were not tightly correlated in primary breast cancer specimens, it seems that critical intermediary factors responsible for ER's apparent role in regulating MTA3 gene expression remain to be defined. A curious, but potentially interesting, side note with respect to the ER-MTA3 connection is the observation by Kumar et al. (2002) that an alternatively spliced form of MTA1 present in some breast cancer cells, termed MTA1s, sequesters ERα in the cytoplasm and inhibits its function. Besides the uncertainties in the ER-MTA3 connection, the specific DNA binding factors and cofactors responsible for targeting MTA3 and other Mi/NuRD repression complex components to the Snail promoter are unknown. Nor is it yet clear what determines the specificity of the action of MTA3, but not MTA1 or MTA2, on Snail gene expression. Because Snail and E-cadherin show at best a rather modest inverse correlation in their expression in breast cancers (Blanco et al., 2002; Hajra et al., 2002), uncertainties remain regarding the relative contribution of Snail versus other potential repressors of E-cadherin, such as Slug or SIP1, in E-cadherin silencing in breast cancer. Also, the ability of Snail and other repressors to extinguish E-cadherin transcription in cancer cells presumably depends on other factors, including likely HDACs and possibly other histone-modifying factors, as well as perhaps DNA methyltransferases to insure that E-cadherin transcription is fully "locked off." Moreover, while E-cadherin is often suggested to be an "invasion suppressor," definitive data on the biochemical and phenotypic consequences of its reduced or absent expression in cancer cells are largely lacking. Nonetheless, in spite of the uncertainties and unanswered questions, the findings of Fujita et al. highlight important avenues for further investigation. Indeed, their encouraging results suggest that we can expect much progress in the relatively near future in advancing understanding of the various transcription factors and chromatin remodeling proteins that play crucial roles in regulating expression of key genes in the cancer process.

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