Of Snail, mice, and women

Mechanisms for breast cancer recurrence and metastases are poorly understood. New evidence from a transgenic mouse mammary tumor model suggests that the transcriptional repressor, Snail, may play a role in recurrence by downregulating E-cadherin and inducing an epithelial-to-mesenchymal transition. Preliminary information from expression microarray data sets from primary human breast cancers suggests that high levels of Snail are correlated with poor clinical outcome for women with early breast cancer. The identification of a molecular pathway involved in mammary tumor recurrence in a mouse model offers both opportunity and challenge to confirm, extend, and exploit these findings in the clinic.

Although breast cancer is a highly prevalent disease, breast cancer mortality has dropped in the United States and Western Europe in recent years. This improvement has been attributed to early detection, optimal use of surgery and radiotherapy, and use of adjuvant systemic therapy like the selective estrogen receptor modulator, tamoxifen, and combination chemotherapy (EBCTCG, 2005). Despite these advances, women with a new diagnosis of early stage breast cancer face three potential threats: recurrence of the original cancer in the same breast, recurrence of the original cancer at a distant site, and development of a new primary breast cancer in the same or opposite breast. Of these possibilities, development of distant metastases from the original cancer is the most feared, as it is generally a highly treatable but ultimately incurable illness. A baffling feature is that the time course of distant recurrence is unpredictable; although some patients sustain a recurrence within the first few years after diagnosis, others suffer such an event many years or even decades after initial diagnosis. Efforts to bring clarity to our understanding clinically have led to the identification of prognostic factors (those factors, like axillary lymph node involvement and tumor size, that predict the natural history of the breast cancer in the absence of treatment), and predictive factors (those factors, like expression of estrogen receptor α, progesterone receptor, and HER-2/neu proteins, that predict response to a specific treatment) (Cianfrocca and Goldstein, 2004). Although useful for counseling in the clinic, these factors shed less light than we might like on the underlying mechanisms for recurrence.

In this issue of *Cancer Cell*, Moody et al. (2005) tackle the vexing question of mechanisms for breast cancer recurrence using a doxycycline-inducible bitransgenic mouse model for HER-2/neu induced mammary carcinogenesis. Doxycycline induction of HER-2/neu expression commonly led to development of invasive mammary adenocarcinomas. Doxycycline withdrawal then resulted in initial regression in 97% of tumors, but over a one-year observation period, 86% of animals developed recurrent tumors in the absence of doxycycline. In a rigorous set of studies, the investigators show that: (1) these are true recurrences of preexisting disease rather than de novo tumors, (2) recurrences occur independent of HER-2/neu expression, (3) recurrent tumors demonstrate an altered morphology suggestive of an epithelial-to-mesenchymal transition (EMT), and (4) upregulation of expression of the zinc finger transcription factor, Snail, a key regulator of EMT in other model systems, appears to play a causative role. In a creative application of in silico translational science, Moody et al. (2005) queried four published data sets of expression microarrays of primary human breast cancers about the relationship between Snail expression and clinical outcomes and suggest that Snail expression predicts poorer relapse-free survival independently of other prognostic markers.

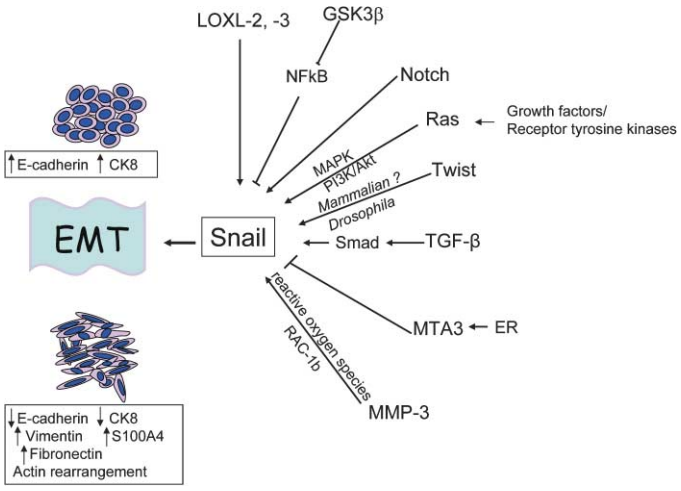

Figure 1. The central role of Snail in epithelial-to-mesenchymal transition

Snail is situated at the hub of multiple pathways leading to epithelial-to-mesenchymal transition (EMT). A zinc finger-containing transcription factor, Snail represses E-cadherin expression and mediates EMT transition in mouse and human cells. In turn, multiple signal transduction pathways regulate Snail expression.

How should we view these results? Certainly they are biologically plausible. The Snail family of zinc-finger transcription factors plays a central role in mesoderm formation and promotion of cell motion across species. Key to this discussion, Snail superfamily members contribute to phenomena such as the EMT, a means by which epithelial cells from one region can dissociate and migrate to a new location (Nieto, 2002). This process is fundamental to both normal development and malignant progression. Functionally, Snail is known to repress E-cadherin, desmoplakin, muc-1, and cytokeratin 18, either directly or indirectly, while its expression is associated with enhanced vimentin and fibronectin expression. Recently, brisk progress has been made in understanding how Snail itself is regulated and how it mediates transcriptional repression of targets like E-cadherin (Figure 1). Indeed, it appears that Snail is located at the hub of multiple signaling pathways leading to EMT (Figure 1). Snail is directly induced by TGFβ1 and RAS, a process dependent on both MAPK and PI3K activities (Peinado et al., 2003). Two members of the lysyl-oxidase gene family, LOXL-2 and -3, stabilize Snail, and

functionally cooperate with Snail to downregulate E-cadherin expression (Peinado et al., 2005). Highlighting the importance of the microenvironment, expression of the stromal matrix metalloproteinase MMP-3, through generation of Rac1b, causes an increase in cellular reactive oxygen species, which stimulates expression of Snail (Radisky et al. 2005). Snail's ability to induce EMT is also determined by the GSKβ3 glycogen synthase kinase that prevents Snail degradation and promotes Snail localization to the nucleus (Zhou et al., 2004). A negative regulator of Snail expression is MTA3 (metastasis associated gene-3), a Mi-2/NuRD histone deacetylase subunit, which is responsive to estrogen signaling (Fujita et al. 2003). More speculatively, in *Drosophila*, another inducer of the EMT, the Twist helix-loop-helix transcription factor, induces expression of Snail and represses expression of E-cadherin (Castanon and Baylies, 2002); the existence of such a connection has remained elusive in mammals. Finally, in addition to its effects in epithelial cells, Snail may also play an important role in tumor stromal cells. Serial analysis of gene expression showed that Snail was one of the most abundantly expressed genes in both tumor endothelial and epithelial cells of human breast carcinomas, but was undetectable in normal breast (Parker et al., 2004).

However, there are puzzles and challenges that remain when we consider the implications of these murine studies of Moody et al. (2005) for human breast cancer. In the lab, it is not known if Snail expression in human breast cancer cells leads to altered morphology or EMT. In the clinic, in the strictest sense, these results apply only to the scenario of local recurrence within the operated breast. Histologically, such recurrences in women usually resemble the original tumor rather than demonstrating the morphologic transition to a spindle cell morphology observed in the mouse mammary cancer recurrences; whether they might demonstrate altered expression of Snail, E-cadherin, vimentin, fibronectin, and cytokeratin 8 in comparison to the original tumor is not clearly known. Whether the Snail-associated EMT contributes to formation of distant metastatic deposits from the recurrent mouse tumors or primary breast cancers in women is also not known. Biopsy of metastatic breast cancer is not routinely performed, leading to a paucity of tissues for this type of analysis, but spindle cell morphology is rarely seen in those metastatic tissues that are obtained. Because of the long disease-free interval that patients may enjoy, it is not always possible to recover archived specimens from the original cancer at time of recurrence for simultaneous study of primary and metastatic lesions. Translational breast cancer researchers are beginning to address these limitations by considering a more routine role for biopsy of metastatic lesions as well as rapid autopsy programs to acquire metastatic tissues to facilitate research and ultimately improve care. In addition, this transgenic mouse model does not take into account the impact of therapy on mechanisms and patterns of recurrence. Virtually all breast cancer recurrences at any site in women occur in the context of previous systemic drug administration and/or breast radiotherapy whose impact on the biology of the recurrence is poorly understood. Finally, an important challenge is whether the provocative but hypothesis-generating results about Snail mRNA expression in human breast cancers from the microarray studies presented here and in small studies using in situ hybridization published elsewhere (Blanco et al., 2002) can be confirmed by immunohistochemical analysis of Snail protein expression in larger independent tumor banks that are linked to clinical outcomes. Only then will we understand whether the connection between Snail, EMT, and recurrence in the HER-2/neu model in mice also exists in women.

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Selected reading

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