self-administration rather than the failure of a discrete action (lever press) to produce the expected results (cocaine). Importantly, Sutton *et al.*[3] were able to mimic critical aspects of extinction training by implanting extra copies of the gene encoding GluR1 into the nucleus accumbens using viral-mediated gene transfer[6].

Although we are far from understanding the molecular mechanisms of the work of Sutton *et al.*[3], it has considerable significance. Foremost, it identifies a neuroadaptation that is not caused by the binding of a powerful drug to its receptor, but rather by the failure of an expected drug injection to result from an established drug-seeking act. It reflects a neuroadaptation that is the result of a powerful psychological experience—the frustration of an expectation—rather than a powerful pharmacological experience. One interpretation is that extinction-induced elevations in GluR1 and GluR2 within the nucleus accumbens reflect the memory of associations between the drug and the actions taken to get it in the past, and the effect of this memory on brain biology. The mechanisms underlying these elevations are not known, but they likely involve differences in post-transcriptional processes (protein redistribution, degradation), because they are not caused by elevated mRNA expression. They may involve midbrain dopamine systems, which are known to be affected by stress or the 'surprise' of not receiving what is expected[7]. Regardless, increases in these subunits would be expected to cause increased numbers of AMPA glutamate receptors within the nucleus accumbens, and subsequent increases in the sensitivity of nucleus accumbens neurons to the excitatory actions of glutamate.

Past work has led to the simple hypothesis that treatments that decrease the excitability of the nucleus accumbens are rewarding[5], whereas treatments that increase excitability are aversive[8] (Fig. 1). Elevated excitability and its accoutrements (including increased flux of calcium into nucleus accumbens neurons) may trigger the activation of transcription factors and genes that lead directly to aversive states[2,9]. At least in rats, treatments associated with aversive states such as severe drug withdrawal tend to decrease rather than increase the likelihood of drug-seeking behaviors, whereas administration of small amounts of drug tends to whet the appetite for more[10].

The findings of Sutton *et al.*[3] are consistent with what many addiction researchers have long suspected: behavioral approaches that incorporate extinction-like processes may have efficacy in the treatment of cocaine addiction, either on their own or as an adjunct to more traditional strategies involving pharmacotherapies. Theoretically, the current study may also spark interest in the development of pharmacotherapies that selectively regulate GluR1 levels in the nucleus accumbens, although such specificity is currently unprecedented. However, what students of the brain may find most intriguing about this work is that it shows an effect on brain biology that we can, for now, categorize as being the result of a psychological event: the frustration[11] resulting from unmet expectations. We already have many examples of how brain biology can affect behavior and mental function. The Sutton *et al.*[3] findings offer insight on the other side—the less-studied side—of the mind–brain interaction.

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Predicting the future of breast cancer

Two new studies suggest that tests based on Cyclin E or microarray analysis have the potential to outperform conventional criteria predicting the outcome of breast cancer.

ÅKE BORG, MÅRTEN FERNÖ & CARSTEN PETERSON

Breast cancer continues to be a major cause of premature death in women of Western societies, despite progress in early detection and treatment and advances in our understanding of cancer's molecular basis. Besides the obvious need to develop even more efficient and cancer-cell–specific drugs, a particular concern has been to accurately predict the outcome of primary treatment among patients with early stage disease, like lymph-node–negative status. Which patients can be cured by surgery alone and which require additional (adjuvant) endocrine or cytotoxic systemic drug treatment such as tamoxifen, antracyclins or taxanes? Also, many node-positive patients are at low risk—if they only could be correctly identified, they could be spared unnecessary and costly treatment.

Two recent papers in the *New England Journal of Medicine* describe strong correlations between biological factors of tumors and clinical outcome of breast cancer, but use quite different strategies to accomplish this important task. Keyomarsi *et al.*[1] determine the level of the cyclin E protein and its various iso-

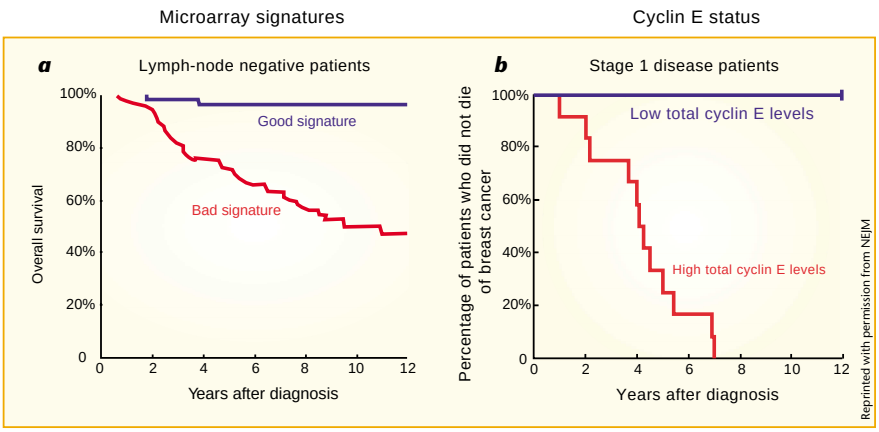

Fig. 1 Prognostic performance of microarray and cyclin E analyses. ***a***, Tumors with good and poor signatures are compared in lymph-node–negative stage I–II patients (tumor size 50 mm or less). ***b***, Low and high total cyclin E levels are compared in stage I patients (lymph-node–negative and tumor size 20 mm or less).

forms using traditional immunohistochemical assays or western blot analyses of tumor lysates, whereas van de Vijver *et al.*[2] use a global approach and dense DNA microarrays. Van de Vijver *et al.* analyze the transcript level of thousands of genes followed by state-of-the-art statistical methods to define gene-expression signatures of low- and high-risk tumors. Both studies take advantage of five- to ten-year-old tumor banks and associated patient data.

Cyclin E is a well-known cell-cycle regulator and key executor of growth-promoting stimuli. Cyclin E is induced in late G1 phase when cells decide whether to undergo DNA replication and subsequent division, or to fall into a dormant G0 phase. Cyclin E, in complex with its kinase partner CDK2, phosphorylates the retinoblastoma protein, thereby releasing transcription factors essential for programmed DNA synthesis, and is targeted by tumor-suppressor proteins such as p53, p21 and p27. Notably, Keyomarsi *et al.* refine the designation of high and low cyclin E levels by allowing detection of both full-length and low-molecular-weight isoforms of the protein, using gel electrophoresis and antibodies against carboxy-terminal epitopes retained in the smaller cyclin E isoforms or cleavage products. The authors report a very strong and independent correlation of cyclin E levels with survival in both node-negative and node-positive breast cancer. This correlation is most remarkable in their set of stage I (node-negative and size 20 mm or less) tumors, where none of 102 cyclin-E–negative, but all 12 cyclin-E–positive, cases died within five years of diagnosis (Fig. 1). Unfortunately, some standard prognostic factors were not used in the analysis. These include histological grade and markers of cell proliferation (Ki-67, TLI and S-phase fraction) or invasion (urokinase plasminogen activator).

The current work by van de Vijver *et al.* extends their recent study[3] of 78 young (<55 years) node-negative patients, selected to include both cases with early recurrence and cases with long, disease-free survival. The authors identified a set of 70 differentially expressed genes, which optimally predicted clinical outcome (Fig. 1). In their current extended study of 295 consecutive, young (<53 years), stage I–II breast cancer patients, the authors included both node-negative and node-positive cases. They used the predefined set of 70 marker genes to classify tumors according to a good- or poor-prognosis signature—and then analyzed patient outcome. Patients whose tumors had a good-prognosis signature were largely free of recurrence at the ten-year follow-up (85%) compared with patients whose tumors fell in the poor-prognosis category (50%).

The poor-prognosis signature also strongly correlated with high histological grade and negative estrogen receptor (ER) status. Nonetheless, the gene-expression signature outperformed both the NIH consensus and the St. Gallen criteria for high-risk breast cancer, which rely on more-traditional indicators.

Importantly, the prognosis signatures of van de Vijver *et al.* performed equally well in node-negative and node-positive patients. This indicates that the processes of dissemination of cancer cells by means of blood and lymph vessels are different; the former is dependent on critical genetic alterations that are early events in some tumors, whereas the latter is a passive process reflecting the chronologic status of the tumor[4].

How do these two studies compare with each other? Could the analysis of a single factor using traditional western blot technique outweigh a state-of-the-art holistic gene expression profiling approach and sophisticated statistical efforts? The answer must await confirmatory studies and analysis of the same set of tumors with both assays. However, the Keyomarsi *et al.* study casts light on the significance of taking post-translational protein modifications into account when evaluating the role of key cellular regulators, information that will not be available from transcript signatures.

Cyclin E expression is tightly connected to cell proliferation and its prognostic value may to a certain extent mirror the general adverse effects of fast-growing tumors. Moreover, the low-molecular-weight cyclin E isoforms can be surrogate markers of related cellular processes, reflecting upstream gene alterations such as protease activation[5] or loss of ubiquitin ligation[6]. More directly, high cyclin E levels, especially of the constitutively expressed isoforms, may cause chromosomal instability[7] and polyploidization by endoreplication[8].

Interestingly, 1 of the 70 classifiers used in the van de Vijver paper is the cyclin E2 gene, which has a pattern of expression and function that is both distinct and redundant to cyclin E(1) (ref. 9). The microarray used in the previous study by the same group[3] contains a cyclin E1 clone, but its signal did not pass the tests used for measurement and was hence not part of their analysis yielding the 70 classifying genes.

A major concern in retrospective studies such as these is whether the analysis reveals true prognostic factors or a combination of factors related to the adjuvant treatment. How appropriate are these new factors in a prospective setting and for the individual patient faced with diagnosis? Both studies fall somewhat short for these real-life questions as they are based on

materials from patients that underwent different types of therapy—either adjuvant anti-estrogen or chemotherapy. Obviously, future selection of patients that should be spared adjuvant treatment cannot be based entirely on studies in which treatment is confounding the results.

ER status is a weak prognostic factor *per se*, but a powerful predictor of response to anti-hormonal therapy[10]. ER status may also be related to the effect of chemotherapy-induced ovarian ablation in pre-menopausal women[11]. ER status has also been shown to be a major determinant of tumor phenotypes, broadly dividing breast cancer in two main subclasses with profoundly different gene-expression profiles[12,13]. Accordingly, the prognostic value of any factor closely related to ER status will also be influenced by adjuvant treatment. Indeed, cyclin-E–driven breast cancers are distinct from ER-positive tumors[14], and the good-prognosis signature described by van de Vijver *et al*. overlaps markedly with ER status. The prognostic performance of cyclin E and the microarray-based gene-expression signature must thus be critically evaluated, taking adjuvant treatment into account. However, as untreated retrospective tumor materials are scarce, an alternative solution would be to evaluate new prognostic markers on a uniformly treated patient cohort, or in hormone-dependent and -independent tumor subsets separately.

Both of the current studies have the potential of making a lasting impact on breast cancer treatment. The simplicity of the cyclin E assay is attractive and its future role will soon be confirmed or challenged. Whether the particular prognostic signatures defined in the current microarray-based study will survive, or fall into the large archives of other 'promising' factors, is somewhat less clear. In all likelihood, DNA microarray techniques will continue to have an important role in disease characterization. Breast cancer is a heterogeneous disease and thorough analysis of large sets of tumors will allow new molecular classification systems and drug targets to be defined (for instance, by activated signaling pathways, metabolic profiles and interactions with surrounding tissues). More-difficult tasks lie ahead in predicting therapy response to achieve the ultimate goal of 'individualized' treatment of cancer patients, as these algorithms must take additional parameters into account, such as variability in drug metabolism and other constitutional features.

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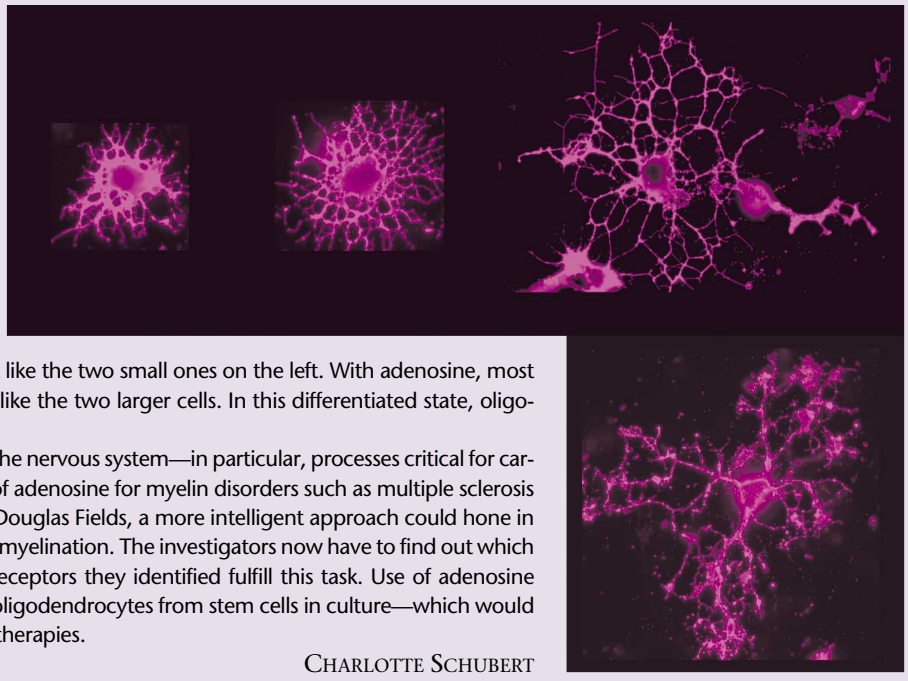
Myelin management



Neuronal activity activates myelin-producing cells in the central nervous system. Now, Beth Stevens *et al.* have zeroed in on how myelin-producing cells in the CNS, oligodendrocytes, respond to neuronal activity. In the December 5 *Neuron*, they report that the neuronal signaling molecule, adenosine, acts as the key mediator. Shown are oligodendrocytes in culture, treated with growth factor. Without adenosine, most of the cells look like the two small ones on the left. With adenosine, most oligodendrocytes sprout long processes, like the two larger cells. In this differentiated state, oligodendrocytes are primed to myelinate.

Adenosine mediates many activities in the nervous system—in particular, processes critical for cardiovascular health. So direct application of adenosine for myelin disorders such as multiple sclerosis seems remote. Instead, says lead author Douglas Fields, a more intelligent approach could hone in on the adenosine receptor that promotes myelination. The investigators now have to find out which of the four oligodendrocyte adenosine receptors they identified fulfill this task. Use of adenosine might also enable production of mature oligodendrocytes from stem cells in culture—which would boost hopes for effective transplantation therapies.

CHARLOTTE SCHUBERT

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