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Cancer. A bull's eye for targeted lung cancer therapy

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Although a covalent bond is much stronger, precursors, acidic/basic conditions, and high temperatures are required for chemical synthesis. For MSAs, synthetic chemistry is used only to construct the basic building blocks (that is, the molecules), and weaker intermolecular bonds are involved in arranging and binding the blocks together into a structure. This weak bonding makes solution, and hence reversible, processing of MSAs possible.

The current top-down approach to nanotechnology, whereby nanostructures are created, manipulated, and modified by machine, is incapable of offering the complexity and economy of scale that MSA demonstrates in nature. Thus, solution processing and manufacturing of MSAs offer the enviable goal of mass production with the possibility of error correction at any stage of assembly. It is well recognized that this method could prove to be the most cost-effective way for the semiconductor electronics industry to produce functional nanodevices such as nanowires, nanotransistors, and nanosensors in large numbers (hundreds of billions at a time). Although these nanodevices will be the first to become available with MSA research, they are, in fact, the simpler cousins to the complex integrated nanostructures to come, in which many different molecules assemble (*3*).

Our ultimate goal is to understand how to form ordered functionality-tailored nanostructures via self-assembly in solution. These nano-sized novel structures, with unusual properties, will form by ordered assembly of different molecules. However, at present, the physics of a single molecule and its bulk molecular crystal is much better understood than that of the intermediate-size range of small molecular assemblies. Therefore, research has focused on ordered assemblies of identical molecules that self-assemble in solution. The strategy is to use conventional investigation techniques to determine the physical structure, electronic properties, and other interesting physical phenomena of simple MSAs, which will then pave the way for an understanding of more complex MSAs. In this respect, hexa-*peri*-hexabenzocoronene (HBC) derivative molecules offer a good molecular template from which to advance to complex MSAs (*4*).

HBCs are beautiful examples of molecular platelike structures. As shown in the figure, symmetrically substituted HBC-C8,2 molecules self-assemble in solution into nanowires several hundred nanometers in length and 3 nm in diameter. At high concentrations, these molecular nanowires assemble further into entropically favorable nanowire bundles several tens of micrometers in length and typically tens of nanometers in diameter (as shown in the SEM image). The ordered alignment of the molecules in the assembly ensures a homogeneous electronic coupling along the length of the nanowire. This in turn leads to the extended delocalization of the exciton eigenstates, which is confirmed by sharp peaks in their optical spectra.

Hill *et al*. (*1*) present an example of an asymmetrically substituted amphiphilic HBC molecule that self-assembles into remarkably defect-free large nanotubular objects, more than 14 nm wide and up to 10 μm long, with a clearly defined helicity, each involving around 50,000 HBC molecules.

At present, there is a huge global research effort targeted at carbon nanotubes. These structures are being investigated for applications ranging from actuators to reinforcement agents, nanoelectronic devices to controlled drug-release agents—with each application requiring a different, precisely defined physical and/or electronic structure (*5*). A major drawback of carbon nanotubes, however, is our apparent lack of structural control, which arises because they are formed by either a gas-phase or a plasma process. The route taken by Hill *et al.* demonstrates that precise control of intermolecular and environmental forces can lead to graphitic nanotubes with defined dimensions, helicity, and electronic properties—exactly as one frequently needs in molecular electronics and most other applications of carbon nanotubes.

Demonstrating that synthetic molecules can form ordered MSAs is a key step. However, attaining a small degree of functionality with simple MSAs will be very significant, as it may indeed open up new avenues for investigating complex MSAs and other nanosystems. Known and functional MSAs will be very useful as local probes to investigate more complex MSAs. In other words, MSAs could become the nanotools of the future.

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CANCER

A Bull's Eye for Targeted Lung Cancer Therapy

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The sequencing of the human genome has stirred public expectations of new and imminent treatments for human diseases, such as cancer. However, the pace at which molecular developments have "translated" into clinical applications has been frustratingly slow. Thus, it is refreshing to find a "bench" discovery that promises immediate clinical benefits. Such a discovery is reported by two Boston groups on page 2129 of this issue (*1*) and in a recent issue of the *New England Journal of Medicine*. Working with different populations of lung cancer patients, both groups discovered that certain somatically acquired mutations in the epidermal growth factor receptor gene (*EGFR*, *ERBB1*) in lung tumor tissue predict significant clinical responses to the drug gefitinib (Iressa), a tyrosine kinase inhibitor that targets EGFR. The new results help to explain puzzling clinical observations and will lead to the rational selection of a patient subpopulation that is most likely to benefit from gefitinib treatment. In addition, the new findings should accelerate work on targeted therapy for other common malignancies (*1*, *2*).

Lung cancer is a leading cause of death in both men and women in the United States and probably worldwide. Lung cancers are divided by histopathology into small cell lung cancers (~20%) and non–small cell lung cancers (~80%); the latter are further subdivided into adenocarcinomas (including bronchiolo-alveolar cancers), squamous, and large cell cancers. Of importance to the current work, the EGFR mutations were found in adenocarcinomas, which constitute ~50% of all U.S. lung cancer cases. Tobacco usage is the major cause of lung cancer, and in women

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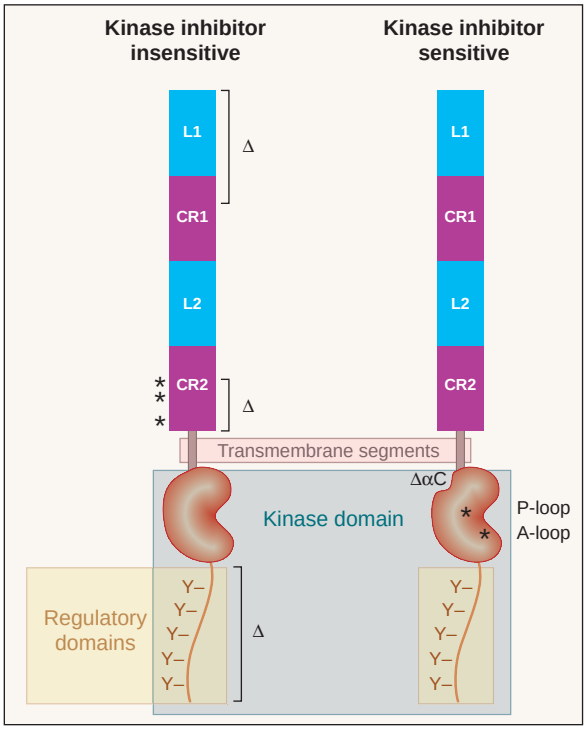
this has led to an epidemic of cases (*3*). Nevertheless, 15% of lung cancers arise in "never smokers" (an estimated 25,000 individuals per year in the U.S.), and 50% of newly diagnosed cases occur in people who quit smoking, often decades ago. This huge reservoir of former smokers, who are otherwise healthy but nevertheless remain at increased risk for lung cancer, is a major concern. In certain Pacific Rim countries, almost all lung cancers in women arise in never smokers, and the vast majority of these tumors are adenocarcinomas. Although the underlying genetic and environmental factors leading to lung cancer in never, light, or distant former smokers are unclear, recent clinical trials have shown that lung cancers arising in nonsmokers, particularly adenocarcinomas, are the most likely to respond to drugs such as gefitinib (Iressa) or erlotinib (Tarceva), tyrosine kinase inhibitors that target EGFR. Responses to these drugs have been unpredictable, but when they occur, they are often dramatic. As a result, the Food and Drug Administration has approved gefitinib for use in the U.S. For previously unexplained reasons, the clinical response rate to gefitinib is almost three times higher in Japanese patients than in American patients. Finally, in trials reported in press releases, erlotinib was shown to enhance survival and provide significant symptom relief compared with an inactive placebo when given to patients with advanced stage non–small cell lung cancer.

Increased EGFR expression is common in lung cancers, but neither EGFR expression levels nor phosphorylation state correlate with the response to gefitinib. Moreover, activating EGFR mutations frequently occur in glioblastomas (*4*), which invariably are unresponsive to gefitinib (see the figure). By contrast, a chimeric neutralizing antibody (cetuximab) that targets EGFR and prevents EGF-induced signaling is beneficial in the treatment of colon cancer, establishing EGFR as an effective drug target (*5*).

Paez *et al*. (*1*) reporting on page 1497 of this issue and Lynch and colleagues (*2*) reporting in the *New England Journal of Medicine* have solved the puzzle of the unpredictable responses to gefitinib. Both reports constitute a major advance toward the validation of EGFR as a target for cancer therapy. In addition, they provide a molecular basis for the mode of action of EGFR kinase inhibitors and the selective sensitivity of lung cancer patients to these drugs. Paez *et al*. initially screened Japanese and American patients with non–small cell lung cancer for mutations restricted to the activation loop of a large subset of all human membrane receptor tyrosine kinases. Lynch *et al*. used primary lung tumor samples from gefitinib responders versus nonresponders to search for mutations in *EGFR*. Both groups converged on the same molecular aberrations within the EGFR kinase domain of their lung tumor samples (see the figure). They identified two classes of somatic mutations in the lung tumors that all correlated with a clinical response to gefitinib. The first class comprises amino acid substitutions in the P-loop (exon 18) or in the activation domain (exon 21), and the second class comprises in-frame deletions within exon 19 that alter the structure and spatial orientation of the catalytically important αC helix.



EGFR mutations in tumors that are sensitive or insensitive to kinase inhibitors. Tumor mutations that are insensitive to kinase inhibitors, such as those found in human glioblastomas (*4*), include extensive deletions (Δ) or missense mutations (*) in the extracellular domain of EGFR, or deletions (Δ) of the regulatory intracellular domain (but not the kinase domain). In contrast, many non-small cell lung cancers carrying missense mutations (*) and deletions (Δ) in EGFR are sensitive to kinase inhibitors such as gefitinib (*1*, *2*). These mutations are found in three distinct sites in the EGFR kinase domain: the P-loop, the activation loop (A-loop), and the αC helix. Limited analysis of non–small cell lung cancer mutations suggests that tumor formation may require ligand binding to EGFR and receptor phosphorylation. The mutant EGFR allele also may be amplified (most notably with the ΔαC mutations). In kinase inhibitor–insensitive glioblastomas, tumors may be dependent or independent of ligand binding and the mutant EGFR locus is usually amplified. L1, L2: large (EGF binding) domain 1 and 2; CR 1, CR 2: cysteine-rich domain 1 and 2. Y, tyrosine residues in the regulatory domain that are available for phosphorylation.

All of these mutations involve amino acids that are either part of the ATP-binding pocket or that, when deleted, would be predicted to alter the structure of the catalytic cleft or the surface of the catalytic domain. These changes do not abrogate the need for ligand binding to induce receptor transphosphorylation, but substantially increase the amount and duration of EGF-dependent activation compared to wild-type receptors. Such structural changes may explain the ~10-fold–increased sensitivity of cultured mutant lung cancer cells to gefitinib, and the enhanced inhibition of mutant EGFR kinase activity by this drug in vitro (*1*, *2*). These EGFR kinase domain mutations are functionally distinct from those found in gefitinib-resistant gliomas. The glioma mutations do not affect the kinase domain, but rather alter the extracellular, juxtamembrane, or intracellular domains in ways leading to constitutively active EGFRs or to EGFRs that are resistant to endocytosis (*4*). In contrast, the kinase domain mutations that confer gefitinib sensitivity seem to enhance the catalytic response to paracrine or autocrine ligand-dependent stimulation (*1*). Ligand-induced receptor down-regulation by dephosphorylation or degradation is also impaired, resulting in mutant EGFRs that are in a persistent hyperactivated state. This finding may reflect an altered interaction of EGFRs with the cytoplasmic adaptor proteins and ubiquitin ligases that regulate EGFR endocytosis and lysosomal trafficking. The in-frame deletions, particularly those within exon 19, profoundly alter the structure and composition of an exposed surface loop that may participate in the interaction with cytoplasmic or membrane-associated proteins. Intriguingly, deletions in exon 19 are the most frequent mutations found by both groups, but they appear preferentially to affect different amino acid residues in the American and Japanese populations. It is possible that different genetic backgrounds and environmental factors account for the rise of lung cancer incidence in both American and Japanese nonsmokers. For women smokers, gender differences may also affect the exon 19 deletion interval.

As both groups indicate, there are additional questions and predictions that arise from their pioneering results. For instance, the enhanced response to ligand stimulation suggests that lung cancers bearing mutations in the EGFR kinase domain may not only be sensitive to kinase inhibitors, but should also respond synergistically to neutralizing antibodies (cetuximab) directed against the extracellular domain (*5*). Related to this issue are the molecular mechanisms by which kinase domain mutations affect EGFR activation. On the genomic level, sequence data presented by both groups show that point mutations in exon 18 and 21, respectively, are heterozygous. This contrasts with deletions in exon 19 where, in some of the sequencing traces presented, the normal allele is severely underrepresented or absent, indicating loss of heterozygosity or amplification of the mutant locus. Thus, the point mutations in exon 18 and 21, respectively, may be dominant in heterodimers consisting of a normal and a mutant EGFR or EGFR family member. In contrast, mutations in exon 19 may be functionally recessive and possibly require homodimerization for phenotypic penetrance. Alternatively, the stability of mRNAs bearing deletions may simply be reduced, requiring amplification of the mutant locus to achieve sufficient expression of the oncogenic variant.

The new studies go a long way toward establishing EGFR tyrosine kinase inhibitors as a valuable cancer treatment that may rival the success of another mutant kinase target therapy, imatinib (Gleevec). This well-known inhibitor of platelet-derived growth factor (PDGF) receptor, ABL, and c-KIT tyrosine kinases has already revolutionized the treatment of chronic myelogenous leukemia, and other tumors with activating mutations in these kinases (*6*). Inhibitors of EGFR tyrosine kinase may show similar successes in selected populations of lung cancer patients whose tumors bear these drug-responsive mutations.

The Paez and Lynch findings will galvanize the search for, use of, and design of clinical trials to test targeted therapies for common epithelial cancers. As regards lung cancer, clinical trials are being designed and preclinical studies are under way to determine if combining EGFR-targeted therapy with the best available chemotherapies will be beneficial to patients whose tumors carry EGFR mutations. Do other tumors carry these EGFR mutations, and are they sensitive to these drugs? Are similar mutations present in other members of the EGFR family that form heterodimers with EGFR, and would these or related designer drugs be effective? How do we move this finding into general clinical practice to allow rapid testing of tumor samples for mutations? Do these mutations occur in lung cancers arising in very light smokers, persons exposed to second-hand smoke, or smokers who quit many years ago? Are there mutations in other components of the EGFR signaling pathway that could also be drug targets? We predict an inverse relation between the incidence of *EGFR* and *K-RAS* mutations. This would suggest reopening the search for drugs that could target mutant Ras or other components of the Ras signaling cascade. A genome-wide search for mutations in kinases and related "druggable" targets has revealed frequent mutations in other kinases, and these efforts will undoubtedly be intensified. Large numbers of lung cancer patients were treated in randomized trials in which the combination of gefitinib or erlotinib with or without chemotherapy was compared. Despite initial disappointing results, these trials should be reexamined to analyze the responses of that subset of patients with *EGFR* mutations. Finally, because EGFR activation is often seen in preneoplastic lung lesions, do these mutations occur as a field effect that would identify the light, former, or never smokers most likely to develop lung cancer? Could these preneoplastic lung lesions be removed by early treatment with gefitinib? We eagerly await answers to these critical questions, which link laboratory and clinical studies and hold the promise of targeted lung cancer therapy.

References and Notes

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PLANT SCIENCES

Yoda Would Be Proud: Valves for Land Plants

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Stomata are structures in the plant epidermis that regulate the exchange of gases between a plant and its environment. Stomata comprise two guard cells on either side of a pore through which carbon dioxide required for photosynthesis passes. The stomata also reduce loss of water vapor to the environment. These structures are critical for plant productivity and were crucial to the evolution of land plants some half a billion years ago. Each leaf contains thousands of stomata, a number influenced by environmental factors during shoot development (*1*). The stomata are evenly spaced, one cell apart, to optimize gas exchange between the cell and its environment. The, as yet unidentified, genes that specify a stomatal fate are likely to be key targets of signaling pathways that control where and how often stomata form. On page 1494 of this issue, Bergmann *et al.* (*2*) identify a mitogen-activated protein kinase kinase (MAPKK) kinase called YODA as an important regulator of stomatal cell fate in the model plant *Arabidopsis*.

The unequal division of a protodermal cell results in the formation of a smaller cell, a precursor to the stomatal guard cell, and a larger cell that becomes a pavement cell and does not generate a stomatal cell unless it too divides unequally (see the first figure) (*3*). Such "piggyback" divisions place the smaller precursor cell at a distance from the original parent cell, implicating signaling between cells in the control of asymmetric cell division. Previous work has shown that two *Arabidopsis* genes—*TOO MANY MOUTHS* (*TMM*) and *STOMATAL DENSITY AND DISTRIBUTION1* (*SDD1*)—regulate early events in asymmetric cell division (*3*, *4*). Mutations in either gene induce production of too many stomata, which are often misplaced, suggesting that proteins encoded by normal versions of these genes are important for limiting unequal divisions and for ensuring that stomata are spaced one-cell apart. TMM is expressed in both daughter cells of the unequal division (*5*); SDD1 is expressed in just the smaller one (*6*). SDD1 is a predicted subtilisin-like protease that is probably secreted. Similar pro-

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