Keren *et al.* increase the complexity of such devices by using yet another biomolecule, the RecA protein, which plays a major role in genetic recombination of the cell. Multiple RecA proteins polymerize at a single-stranded DNA (ssDNA) molecule. By homologous recombination, the resulting DNA-protein complex recognizes and binds to the complementary target sequence present in double-stranded DNA (dsDNA). Keren *et al.* treat the DNA-protein complex with silver ions, which are reduced by aldehyde groups previously generated in the dsDNA target. The resulting small silver grains are later used for the wet-chemical deposition of gold.

This procedure leads to the formation of a conductive wire, with an insulating gap precisely at the position where RecA was bound (see panel A in the figure). The information encoded in the DNA molecules thus replaces the masks used in conventional lithography, while the RecA protein serves as the resist. This approach should, in principle, work with high resolution over a broad range of length scales from nanometers to many micrometers.

Molecular lithography also enables the generation of branchpoints (three-way junctions) in linear DNA fragments. This is accomplished with the use of a dsDNA fragment containing a single-stranded end in the initial RecA polymerization step (see panel B). Treatment of the DNA-protein complex with polymerase leads to the sequence-specific formation of a stable three-way junction, which can later be used for the fabrication of more complex components, such as three-terminal electronic devices. This clear demonstration of the feasibility of biomolecular construction is likely to trigger further efforts to design advanced host systems required for efficient in situ generation of nanostructured DNA networks, and for their site-specific attachment and connection with the macroscopic world.

Molecular lithography can also be used for the sequence-specific positioning of molecular objects (see panel C). To this end, the ssDNA used for polymerization of the RecA monomers is modified with molecular entities that are functional devices or allow for the binding of such devices. To illustrate this possibility, Keren *et al.* introduced biotin groups in the ssDNA, enabling the specific docking of streptavidin-coated gold nanoparticles that were later used for the growth of a metal island. This feature of molecular lithography should stimulate extensive follow-ups, taking advantage of previous work on the DNA-directed assembly of metal and semiconductor nanoparticles (*7*) and/or proteins (*8*).

Additional perspectives for molecular lithography may be opened by incorporating novel proteins obtained by molecular biotechnology techniques, such as directed evolution and phage-display. These approaches have already allowed the in vitro production of protein linker units that recognize semiconductor quantum dot surfaces (*9*). The linkers can thus be used to assemble individual nanometer-size inorganic particles into two- and three-dimensional superstructures.

The combination of molecular lithography and protein bioengineering should open up ways to generate and integrate supramolecular nanoparticle networks and microelectronic devices with biomaterials that possess distinct functionality with respect to enzymatic activity and redox properties (*10*). The highly evolved catalytic turnover of many enzymes and the mechanical or electronic transduction properties of, for example, motor proteins, oxidoreductases, and ion channels, should enable new applications in the areas of sensing, catalysis, and electronics. The joint venture of biotechnology and electronic engineering promises plenty of excitement from future developments.

References

1. G. E. Moore, *Electronics* **38**, 114 (1965).
2. G. M. Whitesides, J. P. Mathias, C. T. Seto, *Science* **254**, 1312 (1991).
3. C. M. Niemeyer, *Angew. Chem. Int. Ed.* **40**, 4128 (2001).
4. N. C. Seeman, *Nano Lett.* **1**, 22 (2001).
5. K. Keren *et al.*, *Science* **297**, 72 (2002).
6. E. Braun, Y. Eichen, U. Sivan, G. Ben-Yoseph, *Nature* **391**, 775 (1998).
7. J. J. Storhoff, C. A. Mirkin, *Chem. Rev.* **99**, 1849 (1999).
8. C. M. Niemeyer, W. Bürger, J. Peplies, *Angew. Chem. Int. Ed. Engl.* **37**, 2265 (1998).
9. S. W. Lee, C. Mao, C. E. Flynn, A. M. Belcher, *Science* **296**, 892 (2002).
10. I. Willner, B. Willner, *Trends Biotechnol.* **19**, 222 (2001).

PERSPECTIVES: CANCER

Addiction to Oncogenes—the Achilles Heal of Cancer

I. Bernard Weinstein

A single cancer cell frequently contains mutations in multiple genes, gross chromosomal abnormalities, and widespread changes in its gene expression profile. An axiom in cancer research is that the multistage process of tumor formation (*1*) is driven by progressive acquisition of activating mutations in dominant growth-enhancing genes (oncogenes) and inactivating mutations in recessive growth-inhibitory genes (tumor suppressor genes) (*2*). Epigenetic (nonmutational) abnormalities leading to increased or decreased expression of these genes, respectively, are also important for tumorigenesis (*2–4*). Since the discovery of oncogenes about 20 years ago, more than 100 oncogenes and at least 15 tumor suppressor genes have been identified, and the list keeps growing. Oncogenes and tumor suppressor genes are important not only for cell proliferation but also for cell fate determination (differentiation, senescence, and apoptosis), their effects often depending on the type of cell in which they are expressed. Thus, overexpression of a given oncogene can enhance growth in one cell type but inhibit growth or induce apoptosis in another (*2–4*).

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A tantalizing question still under debate is whether an oncogene that is crucial for the initial development of a specific tumor is required for maintaining the malignant phenotype of that tumor. The study by Jain *et al.* on page 102 of this issue (*5*) addresses this question. By unraveling the molecular circuitry that maintains the biologic properties of cancer cells, we will be better able to predict selective molecular targets for cancer therapy. Jain and colleagues (*5*) engineered a conditional transgenic mouse to overexpress the *myc* oncogene, which induced formation of highly malignant osteogenic sarcoma. They discovered that brief loss of *myc* overexpression caused the tumor cells to differentiate into mature osteocytes that formed histologically normal bone. It is also intriguing that subsequent reactivation of *myc*, rather than restoring tumor growth as would be predicted, instead induced apoptosis of the tumor cells.

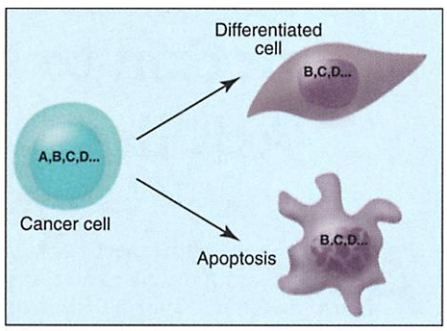
These findings are consistent with other data showing that cancer cells are often "addicted to" (that is, physiologically dependent on) the continued activity of specific activated or overexpressed oncogenes for maintenance of their malignant phenotype. For example, Felsher and Bishop (*6*) showed that transgenic mice expressing the *myc* oncogene in hematopoietic cells developed malignant T cell leukemias and acute myeloid leukemias. However, when this gene was switched off the leukemic cells underwent proliferative arrest, differentiation, and apoptosis. Pelengaris *et al.* (*7*) targeted expression of an activatable

form of the c-Myc protein to the epidermis of mice and observed formation of angiogenic premalignant skin lesions, which regressed when the c-Myc protein was deactivated. These investigators also discovered that sustained activation of c-Myc is required for maintaining invasive tumors of pancreatic β cells in a transgenic mouse model (*8*). Transgenic mice expressing an inducible form of the H-*ras* oncogene readily developed melanomas; when the *ras* gene was switched off the melanomas rapidly underwent apoptosis and regressed (*9*). In another transgenic model, conditional expression of a *Bcr-Abl* fusion gene resulted in the development of leukemia that eventually killed all of the mice. Yet, when the expression of this gene was switched off, even at advanced stages of disease, the leukemic cells underwent rapid and extensive apoptosis and the mice survived (*10*). This finding is relevant to recent encouraging clinical trials of a drug (that blocks the tyrosine kinase activity of the BCR-ABL protein) for treating patients with chronic myeloid leukemia (CML), who carry the *Bcr-Abl* translocation.

It could be argued that all of these results are peculiar to transgenic mice with leukemia or cancer, because in these models the engineered oncogene plays an unusually potent role in the neoplastic process. Furthermore, in mice there may be fewer additional genetic changes during tumor progression than in humans, since in mice the entire carcinogenic process occurs within months rather than decades. However, evidence is accumulating that the phenotype of human cancer cells is also frequently dependent on the continued expression of a single mutated or overexpressed oncogene. Growth of a human pancreatic cancer cell line carrying a mutant K-*ras* oncogene, but not one carrying normal K-*ras*, was inhibited by an antisense K-*ras* oligonucleotide (*11*). Moreover, *Her-2/neu* antisense oligonucleotides prevented proliferation of breast cancer cells with amplified *Her-2/neu*, but had no effect on breast cancer cells that did not overexpress this gene (*12*).

The cell cycle control gene *cyclin D1* is frequently overexpressed in a variety of human cancers, often during the early stages of tumor development (*3*). The continued overexpression of cyclin D1 is critical for maintaining the phenotype of cancer cells. Introduction of antisense cyclin D1 into human esophageal, colon, pancreatic, or squamous carcinoma cells reverted their phenotype toward normal and prevented tumor formation in mice (*12*). Furthermore, antisense-treated pancreatic cancer cells showed increased sensitivity to chemotherapeutic agents. Intriguingly, the revertant esophageal cancer cell line still expressed moderately high levels of cyclin D1. Thus, the parental cells were "addicted" to cyclin D1, that is, they required a greater amount of cyclin D1 to maintain their malignant phenotype than did other cancer cells that never overexpressed cyclin D1.

Despite these examples, if an activated or overexpressed oncogene exerts its effects by causing genomic instability (thus leading to other critical mutations), then blocking its expression may not reverse the cancer phenotype (*5*). This appears to be the case in *myc*-transformed (*13*) or SV40-transformed (*14*) fibroblasts. The need for continued activity of a specific oncogene to maintain the cancer phenotype may depend on its functions, the type of cell, and other factors involved in tumor formation (*3*, *5*). For example, in transgenic mice where overexpression of *c-myc* initiates and maintains invasive mammary carcinomas, a subset of these tumors apparently escape c-Myc dependence by activating endogenous *ras* oncogenes (*15*).



A one-step remedy. Cancer cells acquire abnormalities in multiple oncogenes and tumor suppressor genes (A, B, C, and D). Inactivation of a single critical oncogene (A) can induce cancer cells to differentiate into cells with a normal phenotype or to undergo apoptosis. This dependence on (addiction to) A for maintaining the cancer phenotype provides an Achilles heel for tumors that can be exploited in cancer therapy.

Reintroducing a wild-type tumor suppressor gene (encoding, for example, p53, Rb, or APC) into human cancer cells where the respective endogenous gene is inactive usually promotes marked inhibition of growth, induction of apoptosis, and/or inhibition of tumorigenesis in mice (*3*). These results are also unexpected because, if these cancer cells evolved simply through the stepwise acquisition of several mutations and altered gene expression, then correction of just one mutation should have only a modest inhibitory effect. Thus, some cancer cells seem to be "hypersensitive" to the growth-inhibitory effects of specific tumor suppressor genes (*3*).

The phenomena of oncogene addiction and tumor suppressor gene hypersensitivity suggest that the multistage process of carcinogenesis is not simply a summation of the individual effects of oncogene activation and tumor suppressor gene inactivation. This is consistent with the fact that the proteins encoded by these genes often play multiple roles in complex and interacting networks that display both positive and negative feedback control. Furthermore, throughout the multistage process, the evolving cancer cell must maintain a state of homeostasis between positive- and negative-acting factors in order to maintain structural integrity, viability, and normal replication. For these reasons, the intracellular circuitry or "wiring diagram" that regulates signal transduction and gene expression in cancer cells is very different, even bizarre, when compared to that of normal cells (*3*). Because of their bizarre circuitry, cancer cells may be more dependent on the activity of specific oncogenes and more sensitive to the growth-inhibitory effects of specific tumor suppressor genes than normal cells.

The Jain *et al.* paper and other recent studies present an optimistic message with respect to new approaches for treating cancer. Clinical evidence that oncogene addiction exists is provided by specific antibodies against the Her-2/neu receptor (*16*) that are being used to successfully treat breast cancer, and the striking therapeutic effects of the drug imatinib mesylate (STI571) that targets the *Bcr-Abl* oncogene in CML (*17*). Likewise, the antitumor effects of viral vectors encoding the *p53* tumor suppressor gene (*18*) may be due to tumor suppressor hypersensitivity.

The notions of oncogene addiction and tumor suppressor hypersensitivity should help in identifying new cancer drug targets among the myriad changes in gene expression revealed by microarray analyses of human cancers (*19*). However, pinpointing the most critical targets will also require a more dynamic understanding of cancer cell circuitry. Combinations of drugs will probably still be required for effective cancer therapy. It is likely that administering a single drug will lead to the emergence of drug-resistant mutations (*20*) or of cell variants whose circuitry is no longer addicted to a specific oncogene or sensitive to a specific tumor suppressor (*3*).

References

1. P. C. Nowell, *Science* **194**, 23 (1976).
2. B. Vogelstein, K. W. Kinzler, Eds., *Genetic Basis of Human Cancer* (McGraw-Hill, New York, 1998).
3. I. B. Weinstein, *Carcinogenesis* **21**, 857 (2000).
4. D. Hanahan, R. A. Weinberg, *Cell* **100**, 57 (2000).
5. M. Jain *et al.*, *Science* **297**, 102 (2002).
6. D. W. Felsher, J. M. Bishop, *Mol. Cell* **4**, 199 (1999).
7. S. Pelengaris *et al.*, *Mol. Cell* **3**, 565 (1999).
8. S. Pelengaris *et al.*, *Cell* **109**, 321 (2002).
9. L. Chin *et al.*, *Nature* **400**, 468 (1999).
10. C. S. Huettner *et al.*, *Nature Genet.* **24**, 57 (2000).
11. K. Aoki, *Mol. Carcinog.* **20**, 251 (1997).
12. R. Colomer, *Br. J. Cancer* **70**, 819 (1994).
13. D. W. Felsher, J. M. Bishop, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 3940 (1999).
14. D. Ewald *et al.*, *Science* **273**, 1384 (1996).
15. C. C. Cruz *et al.*, *Nature Med.* **7**, 235 (2001).
16. C. L. Vogel *et al.*, *J. Clin. Oncol.* **20**, 719 (2002).
17. H. Kantarjian, *N. Engl. J. Med.* **346**, 645 (2002).
18. F. McCormick, *Nature Rev. Cancer* **1**, 130 (2001).
19. S. L. Pomeroy *et al.*, *Nature* **415**, 436 (2002).
20. M. Gorre *et al.*, *Science* **293**, 876 (2001).

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