Innovations

Subverting the cell cycle
Mitotix, Inc.

For many years, cell cycle researchers have promised cancer cures on grant applications, while cancer researchers have turned up a growing list of proto-oncogenes with only tenuous links to the cell cycle. More recently, however, the number of known cell cycle proteins has shot up, and a number have been identified as culprits in cancer development. Companies such as Mitotix, Inc., of Cambridge, Massachusetts, are hoping to attack these new molecular targets, and so replace the present generation of toxic cancer therapeutics.

Of cyclins, CDKs and cancer

A group of protein kinases called the CDKs, and their obligatory cyclin partners, drive progression through the cell cycle (Figure 1). The father of all CDKs is Cdc2, which acts primarily in G2 and mitosis. But the cyclin/CDK pairs involved in cancer are clustered just before the G1- to S-phase transition, at the so-called restriction point. It is here that growth factors are needed to commit the cell to another round of DNA replication and cell division.

The protein that has been most spectacularly implicated in cancer is neither a cyclin nor a CDK. p16 is one of a family of small CDK inhibitors: it's specific target is the Cdk4/cyclin D complex. Initial reports of frequent p16 deletions were criticized, as they were based on the inspection of tumor cell lines, not primary tumors. But p16 has recovered its position of importance. Perhaps only the venerable p53 is deleted in more cancers, and almost all cancers have defects in one component of the p16 pathway, which includes p16, cyclin D, Cdk4, and the substrate of Cdk4 kinase activity, Rb. This is the most popular pathway for drug companies, including Mitotix.

The cell cycle heads for S phase when Rb, now phosphorylated by Cdk4, releases members of the E2F family of transcription factors, so they are free to activate the transcription of a number of genes related to DNA synthesis. As cyclin D levels dwindle, Cdk2/cyclin E takes over the job of phosphorylating Rb and other substrates, putting the cell cycle past the point of mitogen dependence. Entry into S phase is complete once E2F activates the gene for cyclin A, which pairs with Cdk2 to keep DNA replication going. Elevated levels of cyclins A and E have also been detected in various cancers.

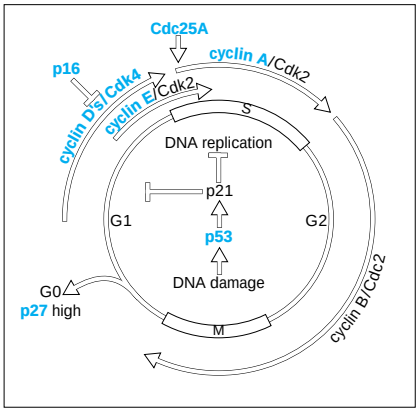
Two CDK inhibitors can stop the orderly progression from cyclin D to E to A. p27, which is present at high levels in quiescent cells, controls the decision to enter or exit the cycle in the first place. And p21 is at least partly responsible for cell cycle arrest after DNA damage. The damage is somehow sensed by p53, which activates p21 and other proteins.

Gentlemen, select your targets

Mitotix is working on most of the cell cycle proteins commonly altered in cancer: Cdk4/cyclin D, p16, Cdk2/cyclin E, p27, p53, and Cdc25A, which removes an inhibitory phosphate from human Cdk2. It has patents and license agreements covering a number of these proteins.

"You want a target that drives the pathology of the disease," explains Muzammil Mansuri, vice president of drug discovery at Mitotix. But this is a young field, and there are no rules for choosing drug targets. "If a molecule is altered [in cancer], does that necessarily mean it's a good target?" asks Jim Roberts of the Fred Hutchinson Cancer Research Center in Seattle. "It makes it easier to understand what you're going after: you're going after the thing that is wrong in the cell. On the other hand, you may be inhibiting an essential process. From that perspective, it's a less attractive target."

Figure 1



Cell cycle targets involved in cancer. Proteins shown in blue are found at levels higher or lower than normal in cancer cells. Such proteins include all components of the p16/Cdk4/cyclin D pathway, including their downstream target, Rb. See text for further details.

Selectivity — shutting down cancer cells more than healthy cells — is the key to cancer chemotherapy, and an area where current therapies do poorly. If CDK inhibitors block a process essential to all cells, will they be any better than DNA synthesis inhibitors? According to David Morgan of the University of California, San Francisco, "that question is often brushed aside."

The Seattle project, directed by Lee Hartwell and Steve Friend at the Fred Hutchinson Cancer Research Center, hopes to get around this problem by identifying genes that only become essential once other genes — those commonly deleted in cancer — are gone. Non-malignant cells, with the redundant genes A and B, would not be affected by a drug targeting gene A.

But both Roberts and Morgan are hopeful that the Mitotix approach will also yield improved therapies. Mitotix should be encouraged, says Morgan, by the relatively minor problems experienced by mice deleted for one of the three cyclin D genes, a result that suggests that

many cells may get along just fine without these proteins. Other target proteins may be present in some tissues and not others, and so be uniquely good targets for certain cancers. But in the end, says Roberts, "you just can't predict the outcome until you have a good inhibitor."

The Mitotix approach

What is needed is a proof-of-concept experiment. Mitotix, and their collaborators at DuPont-Merck, are working on a mouse model of tumorigenesis in which p16 production by the tumor can be turned on or off. "Such an animal could model the effect a Cdk4/cyclin D1 inhibitor may have on tumor cells," says Mansuri. He says that the preliminary results obtained after inducing p16 expression in the tumor "appear to be quite positive."

The mouse is part of a collaboration between DuPont and Mitotix, which covers small molecule inhibitors of Cdk4/cyclin D and Cdk2/cyclin E, and mimetics of p16 and p27. Mitotix is concentrating on the biology and assay development, and leaving large-scale chemistry and screening to DuPont. Mitotix has recently patented various chromone derivatives, ATP-competitive ligands that reportedly inhibit cell proliferation.

A similar agreement with BASF Pharma covers inhibitors of Cdc25 for the treatment of cancer. David Beach of Cold Spring Harbor Laboratory in New York, who in 1992 founded Mitotix with Giulio Draetta (now at the European Institute of Oncology in Milan), found that CDC25A is an oncogene that is overproduced in many breast cancers. Mitotix has developed *in vitro* assays for the phosphatase activity of Cdc25A, and *in vivo* assays based on the ability of Cdc25A to induce the premature and lethal entry of fission yeast into mitosis. They have also isolated CDC25 homologs from *Candida albicans*. The low level of similarity between human and fungal CDC25 homologs make this gene a good target for anti-fungal therapies.

Another cell proliferation disorder is restenosis, the regrowth of cells lining blood vessels after balloon angioplasty. Mitotix plans to stop this growth with localized gene therapy, delivering either p16 or p27.

An alternative to slowing cell division is inducing cell suicide, or apoptosis. p53 stimulates apoptosis, especially once the p16/Rb pathway is gone. But the cells of ~90 % of cervical cancers fail to apoptose because E6, a papillomavirus protein, helps degrade p53. When E6 is present, a cellular protein called E6-AP selects p53 as the target for ubiquitination and subsequent destruction. As proof-of-concept, Mitotix researchers have shown that p53 levels are raised through the use of antisense inhibitors of E6-AP. They have also isolated other proteins that bind in the E6/E6-AP complex, and are screening for inhibitors of p53 ubiquitination. p27 degradation is also an interest, as Mitotix researchers discovered that p27 protein levels are controlled by modulating ubiquitin-mediated destruction.

Cell cycle competitors

Mitotix has chosen to straddle two of the most competitive fields: cancer therapeutics and cell cycle research. Not surprisingly, Mitotix has plenty of company. "There are hordes of them out there right now," says Morgan.

"Practically every pharmaceutical company has a CDK screen going on," says Roberts. Cdk4 is the most popular anti-cancer target. It is the only enzyme in the vital p16 pathway, which is the only pathway that is clearly before the restriction point. And Cdk4 is currently free from patent protection, as it was originally cloned as a PCR fragment and its importance not appreciated. Companies with screens for Cdk4/cyclin D inhibitors include Onyx (Richmond, California, with Parke-Davis), Novartis, Glaxo-Wellcome, Inc., Agouron Pharmaceuticals, Inc. (La Jolla, California), and Pharmacopeia, Inc. (Princeton, New Jersey).

Although Cdk4 is in the public domain, Mitotix does have exclusive rights to cyclin D for drug discovery through its association with Beach and license agreements. Along with groups led by Andrew Arnold of Harvard's Massachussetts General Hospital and Charles Sherr of St. Jude Children's Research Hospital in Memphis, Beach's group discovered cyclin D in 1991. Given that conventional Cdk4 activity assays require cyclin D, and that the cyclin D patent is yet to be isssued, the patent implications in this field are unresolved.

Other companies are taking less conventional approaches. Onyx has removed a gene from adenovirus to target the virus to $p53^-$ cancer cells. The viral gene normally puts p53 out of action, allowing the virus to grow in any cell. But the new virus can only grow in $p53^-$ cells. In human tumors grafted into nude mice, the new virus replicated in and lysed the tumor cells.

David Lane of the University of Dundee has a new company, Cyclacel, which is focussing on his recent work on small peptides that disrupt protein–protein interactions in G1/S. Finally, Bob Kerbel, of Sunnybrook Health Science Centre in Toronto, has worked with researchers at Gilead Sciences in Foster City, California, on a back-to-front strategy. They have shown that antisense inhibition of p27, by accelerating the division of some slowly dividing cancer cells, can potentiate the killing of these cells by conventional anti-cancer drugs.

More variations will follow. "By no means would I consider the CDKs the pinnacle of our capabilities in cell cycle research," says Duncan Walker of Glaxo-Wellcome. "Within a year or so," he says, there will be action aplenty.

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