caspase-3 showed it to be a tetramer of two 10-kD and two 20-kD subunits. With this knowledge, the investigators wisely chose to fuse the DNA binding domain of the LexA protein to the relatively exposed NH2-terminus of the p10 subunit. The GAL4 activating domain was fused to the NH2-terminus of the cDNA library clones. In addition, the p20 subunit was made as a native protein. All were expressed at high concentrations by the yeast ADH1 gene.

Next, the authors chose to modify the active site of the caspase-3 protease to create an inactive complex. The idea was to use a defective enzyme to trap the enzyme substrate at the precleavage step. As do many proteases, caspase-3 has an active-site cysteine that is essential for its enzymatic activity. They substituted a serine residue for the cysteine.

The test experiment consisted of transforming the fusion and native components of caspase-3 into yeast and assaying its binding to a known substrate, CrmA. By a "readout" β-galactosidase assay, the authors showed that only a yeast cell with all components of the system was functional. Next, they moved on to screen a library (mouse embryo cDNA). They isolated 69 clones that could be divided into 13 different clone groups. In vitro assays of the cloned potential targets confirmed that 10 were bona fide substrates for purified caspase-3 enzyme. Sequencing them showed that three clones were parts of the same gene that encodes gelsolin. Additional screens of a mouse thymus library picked out gelsolin clones as well.

Further studies in this report were focused on fleshing out the functional significance of the gelsolin connection to apoptosis. In one series of experiments, they transfected a gelsolin-overexpressing clone into Jurkat cells that are sensitive to apoptotic death. In this cell type, apoptosis can be triggered by adding an antibody to the membrane protein Fas. Amazingly, clones that overexpressed gelsolin were largely immune to the deadly effects of the antibody to Fas. Furthermore, biochemical changes in the gelsolin-overexpressing clones were reflected in a blunting of the apoptotic cascade.

The "two-hybridzyme" approach to finding enzyme substrates might have a broad applicability to other polypeptide-modifying enzymes, such as acetylases, isomerases, and carboxylases. The combination of good enzymatic data, crystallographic coordinates, and yeast genetics provides a new tool for molecular enzymologists.

—ROBERT SIKORSKI AND RICHARD PETERS

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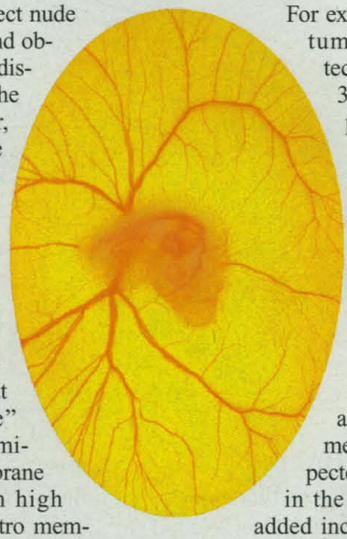
TECHSIGHTING
TUMOR GROWTH ASSAY
Mailbox:www.sciencemag.org/dmail.cgi?53842b

Metastasis in Eggs

Studies of the molecular mechanisms of cancer depend on the use of appropriate model systems. Systems are chosen to emphasize different aspects of the cancer "life cycle." Cell culture systems are used to assay the transformation ability of an oncogene or the blocking effect of a tumor suppressor. Transgenic mice are often used to study multistage genetic changes in a tumor, but how do you study one of the earliest phases of tumor formation, such as intravasation—when a neoplastic cell gains the ability to enter the blood stream and metastasize?

For a simple, in vivo intravasation system, one can inject nude mice with tumor cells and observe the appearance of distant site metastases in the lungs or liver; however, the "readout" of the mouse system is not very sensitive, and the expense of the assay is prohibitive for large-scale drug screening efforts. For an in vitro assay, one could use a cell culture with multichamber assay wells that employ a "digestible" membrane. Cells that migrate through the membrane can be measured with high sensitivity, but the in vitro membranes are no match for the complexity found in natural blood vessels and basement membranes.



PHOTOTAKE/PNI

A system that uses real tissue barriers and affords high sensitivity measurements of intravasation has just been developed by investigators at the Mount Sinai School of Medicine in New York (*1*). They combined chicken eggs and the polymerase chain reaction (PCR) to uncover new details about the process of cancer cell metastasis.

The underlying anatomy of the chicken egg is key to how the new assay works. The egg's hard shell is lined by a membrane called the chorioallantoic membrane (CAM). The embryo itself lies wrapped in a sac created by the CAM. Separating the CAM and the embryo proper are layers of connective tissue and blood vessels. Because of the asymmetric nature of the CAM, experiments can be designed that involve the upper and lower CAM (polar ends of the egg).

The authors began their investigations by creating an artificial air sac to separate the upper CAM from the egg shell. They then injected human tumor cells or tissues into this air sac and monitored the movement of these neoplastic cells to the lower CAM. Because the only way that cells can get to the lower CAM is by way of blood vessels, the assay measures the foreign cells' intravasation.

To assay metastasized cells in the lower CAM, the authors designed a PCR strategy to amplify human Alu sequences. Alu DNA sequences are plentiful in even one cell, so there are many target copies. In mock mixing experiments, they were able to detect as few as one human tumor cell in 2×10^6 normal chicken cells.

Armed with this powerful assay system, they performed a series of experiments to test the time course of intravasation in the egg and the effect of genetic and chemical modifications on the process. They also looked at different tumor types as well. For example, they found that human tumor cells (Hep3) were detectable in the lower CAM about 32 hours after seeding the upper CAM. This delay argues for an active entry process and against a simple entry of the cells into leaky blood vessels. Tumor cells detected in the lower CAM soon after the CAM has been seeded would more likely indicate the latter process.

To examine specific molecules, they attempted to block a class of proteases, known as metalloproteinases (MMPs), suspected of playing an essential role in the intravasation process. They added increasing amounts of an MMP inhibitor, marimastat, and showed a dose-dependent block in intravasation potential. In addition, they manipulated the intracellular levels of the receptor urokinase plasminogen activator (uPA) with antisense constructs. By decreasing the level of uPA as much as 70%, they could almost completely block intravasation in the chick system.

These studies are clearly only a hint of what will likely be a wealth of information about the metastatic process that will come out of work with the chicken egg system. The list of obvious future experiments is long and includes cataloging the intravasation potential of existing cell lines, determining the inhibitory effect of potential drugs, and examining the effects of genetic alterations in oncogenes and tumor suppressors.

—ROBERT SIKORSKI AND RICHARD PETERS

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