THE RIPENING FRUITS OF RECEPTOR BIOLOGY

NEW ORLEANS—How can one extract rational drug design from insights into the basic biology and specific molecular interactions governing receptor expression and ligand binding? That was a major theme at this year's *Bio/Technology* symposium.

Michael Brown (University of Texas, Southwest Medical School, Dallas, TX) provided a background—his Nobel-laureate work showed the LDL-receptor could be partially restored, in some familial hypercholesterolemias, by manipulating an alternative pathway for cholesterol biosynthesis. Against this background, Mark Greene (University of Pennsylvania Medical School, Philadelphia, PA) and Ira Pastan (NCI, Bethesda, MD) demonstrated receptor-based approaches to pharmaceutical intervention in neoplastic diseases displaying over-expressed or inappropriately expressed receptors, rather than diseases characterized by receptor deficiencies.

Greene's laboratory has been using monoclonal antibodies directed against the receptor encoded by the *neu* oncogene to probe its domain structure and to modulate its surface expression. When pregnant rodents are exposed to the carcinogen ethyl nitrous urea on, and only on, day 15 of gestation, approximately 15 percent of the offspring develop neuroblastomas. High molecular weight DNA isolated from these malignant cells transforms NIH 3T3 cells, and antibodies to the oncogene encoded product define a surface protein on both the original neuroblastoma cells and on cells transformed by the oncogene-containing DNA.

The amino acid structure of this protein (p185), as deduced from cDNA clones, has all the earmarks of a receptor—an extracellular domain recognized by the antibodies, a membrane-spanning region, and an intracellular portion with tyrosine kinase activity. When Greene used panels of monoclonal antibodies to monitor the appearance of the *neu* receptor during normal rodent embryogenesis, he found that the expression of the proto-oncogene was developmentally regulated in both temporal and tissue-specific manners. Only on day 15 of development, he told the audience, does the cellular *neu* gene become active, and then only in a particular subset of neuronal cells. By day 18, *neu* protein is substantially gone from their surface, but appears briefly on cells destined to form intestinal and kidney tissue. Not coincidentally, Greene said, exposure to ethyl nitrous urea on day 18 leads to tumors of the kidney and intestines.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

The photograph shows nude mice inoculated 14 days earlier with *neu*-transformed B104-1-1 tumor cells. The lower animal was simultaneously injected with non-specific immunoglobulins and shows a characteristically large tumor at the site of injection. The upper animal received a mixture of monoclonal antibodies—directed against two non-overlapping domains of the *neu* receptor—and remained tumor free.

These same antibodies, when directed against *neu* protein on the surface of transformed cells, have dramatic effects on the oncogenic protein. They cause aggregation and subsequent removal of mutant receptor, and enhance the intracellular degradation of mature p185. Simultaneously, the cells lose their transformed phenotype. And most dramatically, when appropriate combinations of the antibodies are administered to mice implanted with *neu*-transformed cells, tumor formation is completely and stably inhibited in 60 to 80 percent of the animals (see illustration).

A number of human tumors, including adenocarcinomas of the salivary and prostate glands, and carcinoma of the breast, have been characterized by amplification of the *neu* gene and over-production of cell-surface *neu* receptor. Greene is beginning to investigate the therapeutic potential of anti-*neu* antibodies in these tumors. An early sign that this approach might be productive is that the antibodies can retard further growth of already established tumors in nude mice.

The product of another proto-oncogene, the epidermal growth factor (EGF) receptor, is also aberrantly expressed on the cell surface in certain human tumors. Ira Pastan and his collaborators at the National Cancer Institute are attempting to create specific reagents against such tumors by joining the genes for the EGF receptor and a carefully redesigned *Pseudomonas* exotoxin.

Pastan chose to work with the *Pseudomonas* toxin for a number of reasons: It is a single-chain protein of 60 kD molecular weight and is non-glycosylated, thus making it relatively easy to clone, modify and express. And few people have established immunity to the protein—unlike another candidate, the diphtheria toxin, which kills cells by an identical mechanism.

However, toxin-receptor conjugates posed two biological problems: At doses which kill tumor cells, enough toxin binds to normal cells, through its own receptor, that sufficient selectivity is not achieved. Second, the ligand-complexed EGF receptor is rapidly internalized and maintained within membrane-limited compartments. For the toxin to kill cells, it must pass into the cytosol.

The breakthrough, Pastan said, came through the recently published solution of the exotoxin's crystal structure. This allowed researchers to make predictions of the functional domains of the toxin in relation to the sequence of the toxin gene. A series of elegant subcloning and expression experiments resulted in a chimeric protein with EGF receptor-binding activity, enough hydrophobicity to cross intracellular membranes, no binding affinity for cells not expressing the EGF receptor, and significant killing of transformed cell lines in which the receptor is over-expressed. A number of protocols utilizing this conjugate are presently being developed.

—Harvey Bialy