Volume 80 • Number 3 • March 2000

INSIDE LAB INVEST

A PAPILLOMA OF A DIFFERENT STRIPE: ZEBRAFISH GENETICS: Central concepts of initiation, promotion, and progression of cancer have been developed using the mouse skin carcinogenesis model. However, despite the numerous advantages of this experimental system, it remains difficult to perform large-scale genetic screens in mouse. A new model organism of choice for vertebrate genetics is the zebrafish, *Danio rerio.* With their compact size, terrific fecundity, and short generation time, zebrafish are ideally suited for genetic screening. During the past few years the zebrafish system has yielded many advances in vertebrate development. The feedstock for many of these screens is a population of zebrafish that has been mutagenized with heavy doses of the alkylating agent ethylnitrosourea (ENU). In this issue of the journal, **Beckwith and colleagues** report that ENU efficiently induces papillomas in male zebrafish (Lab Invest 2000;80:379–386). This means that the power of zebrafish genetics can now be harnessed to investigate this early phase of carcinogenesis. This may identify crucial gene targets for papillomagenesis, and secondary screens for enhancers and suppressors of papillomagenesis may identify additional genes in the same pathways, or modifier loci. Although molecular identification of mutated genes in zebrafish still requires laborious positional cloning, insertional mutagenesis through retrovirus infection may eventually make it possible to rapidly identify target genes. These genes and their homologs can then be further studied in zebrafish or mammals. There are significant differences between zebrafish and murine skin carcinogenesis systems, because the histology of the papillomas differs. More importantly, papilloma formation is just a beginning, and it must be hoped that further development of this experimental model will make it possible to recapitulate the full range of progression to metastatic carcinoma. Nonetheless, a long swim begins with the first stroke. . .

COMPLEMENT VERSUS RENAL CELL CA: Activation of anti-tumor immunity is a long sought goal for the treatment of cancer. The complement system is a major effector arm of the immune system and is likely to play an important role in the efficacy of treatments involving anti-tumor cell antibodies. However, mammalian cells, including cancer cells, are generally resistant to lysis by complement because they express several different membrane regulatory proteins that antagonize the activation and function of complement. These include membrane cofactor protein (CD46) and decay accelerating factor (CD55), which inhibit C3 convertase, and homologous restriction factor (CD59), which blocks the assembly of the membrane attack complex. The effectiveness of these regulatory proteins in vitro is often directly related to their level of expression. Although expression levels have been characterized on normal cells and on tumor cell lines, little is currently known about expression levels on human tumor cells in situ. In this issue of the journal, **Blok and colleagues** use semi-quantitative immunostaining to evaluate the levels of these proteins on 31 renal cell carcinomas as well as normal renal tubular cells in the same specimens (Lab Invest 2000;80:335–344). They find that CD46 levels on tumor cells are inversely correlated with deposition of C3d, a stable breakdown product of activated C3b. This observation has two implications: (1) that complement is activated in vivo versus renal cell carcinomas, generating C3b; and (2) that CD46 is not only expressed, but is functional in vivo, preventing C3b generation. Moreover, the authors also find that CD46 levels are decreased on advanced metastatic tumors compared with tumors at early stages, implying that the complement system may be holding tumor growth in check. An additional implication of this study, which will need prospective confirmation, is that CD46 is an independent indicator of tumor stage and possibly prognosis in renal cell carcinoma. Finally, these data are consistent with but do not directly establish the authors' original contention, namely that CD46 levels will predict responsiveness of a tumor to passive antibody therapy.

CELL-SUBSTRATUM CONTACT DISRUPTS CELL-CELL CONTACT IN HEPATOCELLULAR CARCINOMA CELLS: A POTENTIAL PLAYER IN INTRAHEPATIC METASTASIS: Normal cellular and tissue development, differentiation, and stabilization are dependent upon complex interactions among soluble and solid

phase factors with their cognate receptors and specific spatiotemporally regulated cell-cell and cell-substratum interactions. Specifically, cadherin-based cell-cell adhesion and integrin-mediated cell-substratum interactions have been found to be major players in these interactions, mediating not only adhesion, but as initiators and modulators of several signal transduction pathways regulating cellular migration, proliferation, polarization. and communication. In the development of neoplasia, dysregulation/dysfunction of both cadherin- and integrin-based adhesion/signaling systems has been documented and correlated with tumor cells' abilities to migrate, invade, arrest, and survive at metastatic sites. In this issue of *Laboratory Investigation*, **Genda et al** demonstrate a cross-talk between specific integrin-based cell-substratum adhesion and cadherin-based cell-cell adhesion mediated, in part, via the tyrosine phosphorylation of c-src in highly metastatic hepatocellular carcinoma cells (Lab Invest 2000;80:387–394). In their in vitro studies they found that engagement of β1 and β5 integrins mediated a dissociation of the E-cadherin-catenin complex, resulting in a dissociation of the carcinoma cells. Interestingly, dissociation of cadherin complexes has recently been shown to elicit up-regulation of matrix metalloproteinases, which, in turn, have the potential of further enhancing a migratory/invasive phenotype. Integrin engagement can be associated with either the development of a mature, stable, static, differentiated phenotype or a highly motile, invasive phenotype, depending upon the cell and the context in which it is studied. Cultured hepatocytes have been shown to differentiate when in contact with several ECM components, mimicking their in vivo mitotically quiescent and static but metabolically active phenotype. The hepatocellular carcinoma cell line used in this study appears to exhibit a dysfunction in its integrin-cadherin signaling interactions manifested by a dissociation of cell-cell contact following integrin engagement, leading increased cell dissociation and a high intrahepatic migratory/invasive potential. These studies suggest novel approaches based on modulation of adhesion receptors in the treatment and control of metastasis and recurrence of hepatocellular carcinoma.

SCALPEL TO RESTRICTION ENZYMES: Recognition of peptides derived from tumors by cytotoxic T cells can ultimately result in the lysis of the tumor cell. The target peptides are generated from endogenous peptides by a multi-partitecatalytic process and presented to T lymphocytes by the highly polymorphic HLA proteins. Because proteins encoded by genes mutatated during tumorigenesis can provide tumor neo-antigens, it is not surprising that cells deficient in the processing and presentation of antigenic peptides emerge as the dominant population in advanced tumors. Only cells capable of evading the host-immuneresponse will survive and thrive. Down-regulation or absence of HLA Class I molecules is found in many human tumors. **Feenstra and colleagues**, in this issue of Laboratory Investigation (Lab Invest 2000;80:405–414) have been studying the alterations found in HLA Class I molecules and the transporters associated with antigen processing in head and neck cancers. One mechanism causing loss of HLA expression is loss of one allele encoding for the complex. In this issue of the journal Feenstra et al use a whole spectrum of dissecting techniques to further refine the information of LOH at loci encoding for HLA Class I. By dissection of the tumor cells using laser surgery on histological sections, they render dissection at the chromosomal level informative. The ability to isolate samples rich in tumor cells by microdissection enables the authors to demonstrate allelic loss of HLA loci. Thus in head and neck cancers, tumor cells may evade immunerejection interdicting the presentation of immunogenic peptides at the cell surface. The study also illustrates the role of laser microdissection methodologies in bridging between the scalpel and restriction enzymes!