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INSIDE LAB INVEST

MAPPING DIVERSE CELL CLUSTERS: A number of recent reports in the tumor biology literature emphasize the degree of heterogeneity found in tumor cells. The studies demonstrate that, even when considering point mutations in a gene of pathogenetic importance for the tumor, diversity is invariably found. These results pose a paradox, given the widely held view of the clonality of mutations that are presumably selected for and give rise to dominant clones that take over the tumor. In this issue, **Axel Walch and collaborators** (Lab Invest 2001, 81: 1457–1459) show the power of fluorescence in situ hybridization (FISH) to delineate diverse cell groups or populations forming a tumor. By using sequential hybridizations for different loci, they can appreciate diversity in the number of copies in interphase nuclei. This allows the definition of complex patterns of increased dosage for genes such as *c-met*, *c-myc*, *Her-2/neu*, *cyclin D1*, and *20q13.2*, as well as other chromosomal loci containing candidate tumor genes. It is not yet clear what the significance of micro-heterogeneity is and how it can be explained. However, there is little doubt that, as therapies targeting specific molecular defects in tumor cells appear in the clinic, defining and understanding tumor heterogeneity will be important for predicting response and evaluating the innate resistance of tumor cells to a given therapy.

NOVEL CHROMOSOMAL LOSSES IN FAMILIAL OVARIAN CANCER: Although genetic damage is the sine qua non of all cancers, making sense of the myriad gene deletions, amplifications, and chromosomal rearrangements that characterize advanced malignancy remains a daunting challenge. One tool useful in this quest is comparative genomic hybridization (CGH). Although the resolution (≈ 10 MBase) of this method is too imprecise to identify specific genes, it does allow a global view of chromosomal integrity and the opportunity to spot regions of amplification, deletion, or rearrangement. In this issue, **Zweemer and his colleagues** (Lab Invest 2001, 81: 1363–1370) use CGH to present the most extensive survey yet of chromosomal alterations in 36 cases of familial ovarian cancer. Although historically, more than 90% of such cancers harbor mutations in either *BRCA1* or *BRCA2*, other genes must be involved since *BRCA1/2* mutations are rare in sporadic ovarian cancer, and in the familial cancers presented this month, only 15 of the 36 have identified *BRCA1/2* mutations. So what else is involved? In the 36 tumors examined, there were on average nearly 10 chromosomal losses and four amplifications per tumor. The frequency of chromosomal loss was greater than that detected in earlier studies of ovarian cancer using CGH, presumably because of the difficulty of detecting somatic deletions in specimens that have not been microdissected and are thus likely to be contaminated with nontumoral tissue. Many of the changes identified are not specific for hereditary cancer and occur with equal frequency in sporadic lesions. Some changes point to the usual gene suspects, such as *p53* on chromosome 17, or *c-myc* on chromosome 8. However, losses in five regions that do not include *BRACA1/2* were identified at high frequency in familial ovarian cancers and only rarely in sporadic cases. These sites are on chromosomes 8, 22, 15 (two regions), and 12. Several of these loci have not been previously observed in ovarian cancer, although deletions in chromosomes 8, 22, and 15 have been reported in other tumors. Interestingly, a likely candidate gene on chromosome 15 is *hRAD51*, a gene that appears to work in concert with *BRCA1* and *BRCA2* to effect double-stranded DNA repair. Finally, the loss of 12q appears to be specific for familial ovarian cancer (vs sporadic tumors). In this sense it is similar to the rarity of *BRCA1/2* mutations in sporadic tumors. Although the specific gene on chromosome 12q responsible for hereditary ovarian cancer has not yet been identified, it is likely that, when it is, a new pathway for hereditary ovarian cancer development may be revealed.

ASTHMA: CYTOKINES VS INFLAMMATION: Over the past 20 years, the predominant view of asthma has shifted from one based upon the position that asthmatics have intrinsic defects in the responses of airways smooth muscle to bronchoconstrictors (ie, hyperresponsiveness) to one based on the belief that the smooth muscle changes that lead to airways hyperreactivity (AHR) are secondary to inflammation, especially inflammation enriched in eosinophils. The inflammation hypothesis has been supported by various mouse models in which administration

of antigens into the airways of immunized animals leads to both eosinophil-rich inflammation and AHR. Although airways inflammation is a common feature of human asthma and of the various animal models of asthma, it remains formally possible that bronchial smooth muscle hyperresponsiveness and inflammation are parallel, but independent, features of the same primary process. Because it is now well appreciated that cytokines are the mediators of inflammation, an alternative interpretation of the data is that the same cytokines that act on peribronchial endothelium to cause inflammation act in parallel on airways to cause AHR. This alternative interpretation is supported by the work of **To and colleagues** (Lab Invest 2001, 81: 1385–1396). Using a mouse model of airways inflammation in response to an experimental antigen, ovalbumin, these authors have carefully compared the time course of inflammation with that of the onset of AHR (measured by the noninvasive enhanced pause [Penh] method) in immunized animals. They find that, although both AHR and eosinophilic inflammation are reduced in parallel by administration of anti–IL-4 antibody (or in IL-4 knockout mice), AHR precedes the onset of eosinophilic inflammation. These data do not rule out the idea that inflammation contributes to the persistence of AHR, but they strongly favor the notion that the onset of AHR may be a direct effect of cytokines (eg, IL-4) on the airway, independent of inflammatory infiltrates. The source of the cytokine is not determined by this study, but candidates include resident mast cells or T cells rather than infiltrating eosinophils. If this model extends to human disease, then cytokine antagonists may be a better therapeutic intervention than other anti-inflammatory agents.

MURINE ORTHOTOPIC AND ECTOPIC TUMOR MODELS: TOOLS FOR UNDERSTANDING TUMOR PATHOPHYSIOLOGY: Animal models of tumor formation, progression, and metastasis, although plentiful and used extensively, are often limited in their usefulness as models of specific tumors and their behavior in situ in their organ/tissue of origin. For example, our understanding of the biology of pancreatic adenocarcinoma would be enhanced by the development, use, and systematic analysis of its behavior at orthotopic and ectopic sites during its growth. In this issue, **Tsuzuki et al** (Lab Invest 2001, 81: 1439–1451) describe the use of abdominal wall windows in an orthotopic and ectopic model of human ductal pancreatic adenocarcinoma, which allows for intravital microscopy and biochemical analyses of the tumors. The authors found that orthotopically implanted human ductal pancreatic adenocarcinoma (PANC-1) tumors grew better within the pancreas compared with the ectopic site. Further, orthotopically implanted tumor showed clinical features similar to those observed in the human disease, including direct invasion into retroperitoneal areas and adjacent organs, peritoneal dissemination, and metastasis to mesenteric lymph nodes and the liver. In contrast, ectopically implanted tumors showed less invasion and no metastasis. In addition to these findings, the authors demonstrated higher levels of vascular endothelial growth factor (VEGF) isoforms, maintenance of vascular density and hyperpermeability, and fewer leukocyte-endothelial interactions in orthotopically implanted tumors. These findings underscore the roles that the microenvironment may play in modulating tumor development, progression, survival, and metastasis in primary and metastatic lesions. These observations underscore the potential usefulness of both orthotopic and ectopic tumor models in determining the tumor's pathophysiology in primary (orthotopic) and metastatic (ectopic) sites and may ultimately lead to the development of novel and specific therapeutic strategies.