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Building a better mouse prostate

Current techniques for generation of transgenic mouse models, while considerably cheaper than in the past, still require a significant financial investment. Furthermore, it is difficult to predict if a specific genetic manipulation will result in a desirable phenotype. Tissue recombination is a relatively rapid and inexpensive method that has been used to study the developmental biology of a number of organ systems including the gastrointestinal tract, male and female genitourinary tracts and the mammary gland. In this issue, the paper by **Ishii *et al*¹** (p. 1086) examines the hypothesis that tissue recombinants can recapitulate the pathologic features of the neoplastic prostate seen in transgenic mice, and would thus make suitable predictive models for new mouse design.

The well-characterized prostate-specific probasin family of promoters was used in this study, along with the SV40 large T antigen (Tag) as the transgene. The authors employed bladder epithelial cells from 12T-7f and 12T-10 LPB-Tag transgenic mouse lines. These two mouse lines have different sites of transgene integration and exhibit different rates of neoplastic prostate growth paralleled by differences in histopathology. The induction of prostatic mesenchyme in fetal rats also differs. The authors demonstrated that the histologic and temporal development of prostatic lesions in tissue recombinants from each mouse line closely parallels that seen in the native prostates of the respective intact mice. The authors also observed that the recombinants using 12T-7f epithelium lacked the hypercellular stroma commonly seen in that model. Further tissue recombination experiments revealed that the hypercellular stroma is the result of direct expression of the transgene in the prostatic stroma, rather than being due to epithelial-to-mesenchymal transformation. This finding of prostatic stromal transgene expression in 12T-7f mice but not in 12T-10 animals was further explored, with a careful examination of transgene expression in other tissues and other mouse models. The study showed that each of the models has its own characteristic pattern of expression of Tag.

These observations underline the critical relationship between the site of transgene integration and the phenotype of gene expression patterns. These findings also reinforce the importance of understanding the biology of promoters used in transgenic models, many or all of which are likely to

suffer the same problem of plasticity of expression. Furthermore, pathologists characterizing such transgenic mouse models need to establish histopathologic links between primary tumors and putative metastatic lesions to ensure that the distant site is not simply an aberrant or unexpected primary lesion.

Reference

- 1 Ishii K, Shappell SB, Matusik RJ, *et al*. Use of tissue recombination to predict phenotypes of transgenic mouse models of prostate carcinoma. *Lab Invest* 2005;85:1086–1103.

A breakthrough in barrier dysfunction

Ulcerative colitis and Crohn's disease, collectively known as inflammatory bowel disease (IBD), are characterized by chronic inflammatory damage. This is accompanied by dysfunction of the epithelial permeability barrier, an observation that may have both prognostic and pathogenic significance. Since, in the absence of gross erosion and ulceration, this barrier is defined by the epithelial intercellular tight junction, recent studies have focused on mechanisms of tight junction disruption in inflammatory bowel disease.¹ In the article of this issue, **Prasad *et al*²** (p. 1139) have investigated claudin isoform expression in tissues from patients with inflammatory bowel disease. Claudins are a large family of proteins in which the expression of specific family members defines tight junction permeability to specific ions. For example, overexpression of claudin-2 in cell line models causes marked increases in tight junction permeability to Na⁺ ions.³ Prasad *et al*² show that expression of claudin 2, which is very low in nondiseased colonic crypts, is markedly upregulated in IBD. Thus, claudin-2 overexpression may explain the increased tight junction permeability that occurs in IBD. These authors continue by defining mechanisms of claudin-2 upregulation using well-established *in vitro* models. They show that activation of the IL-13 can induce increased claudin-2 expression and corresponding permeability changes that mirror those seen in human IBD tissues. This IL-13-induced claudin-2 upregulation was then shown to involve the phosphatidylinositol-3-kinase-Akt pathway, although it is not clear whether the link between Akt and claudin 2 upregulation is direct. Thus, this remarkable study provides the first data to suggest that tight junction protein composition is altered in IBD. This is likely just the beginning; as one would expect this

observation to generate future studies that may lead to major breakthroughs in our understanding, and even treatment, of IBD.

References

- 1 Clayburgh DR, Shen L, Turner JR. A porous defense: the leaky epithelial barrier in intestinal disease. *Lab Invest* 2004;84:282–291.
- 2 Prasad S, Mingrino R, Kaukinen K, *et al.* Inflammatory processes have differential effects on claudins 2, 3 and 4 in colonic epithelial cells. *Lab Invest* 2005;85:1139–1162.
- 3 Amasheh S, Meiri N, Gitter AH, *et al.* Claudin-2 expression induces cation-selective channels in tight junctions of epithelial cells. *J Cell Sci* 2002;115:4969–4976.

Genome-wide searching in metastatic colorectal carcinoma

The opening editorial of this issue describes the important role that pathology research can play in the elucidation of human disease pathogenesis, through molecular and morphological analysis of human tissues. One valuable paradigm is genome-wide analysis of diseased human tissues to enable hypothesis testing and validation. The article by **Tanami *et al.***¹ (p. 1118) in this same issue is called out as an excellent example of such upper-tier research. The question is whether genetic differences exist between primary colorectal cancers and their metastatic tumor deposits. While this question is not new, the ability to simultaneously examine all chromosomes in primary and metastatic tumor foci has only recently been possible. Chromosomal hybridization with DNA probes has been possible for over 30 years. Comparative genomic hybridization (CGH) was introduced over 10 years ago and is based on a competitive *in situ* hybridization of differentially labeled DNA samples to a normal human metaphase chromosomal spread.² This technique has been widely applied, with extensive attention given to the differences between cancer tissue and noncancerous tissue. CGH has been used to compare primary and metastatic tumor foci of colorectal carcinoma, with demonstration that primary tumors with a variety of chromosomal deletions (eg, 2q, 5q, 8p, 9p, 10q, 21q21) or gains (eg, 1q, 11, 12qter, 17q12–q21, 22q) are more likely to metastasize.^{3,4} What has not previously been assessed is whether these genetic alterations substantively affect clinical outcomes, over-and-above the obvious presence of metastases at a distant site (which does not require molecular analysis to document).

Tanami *et al.*¹ performed conventional and subtractive CGH on paired samples from 20 colorectal carcinoma patients with primary tumors and synchronous or metachronous liver metastases. The use of paired samples maximized the probability of

identifying genetic regions relevant to metastasis development. Among a variety of chromosomal alterations identified, frequent gains in DNA copy-number were detected at 6p in metastatic foci. This is consistent with the premise that amplification of chromosomal DNA is a mechanism for activating genes that promote the development and progression of cancer, and specifically one or more metastasis-related genes in the region. Eleven colorectal carcinoma cell lines were then analyzed using array-based CGH, with specific attention given to the 19 genes/loci within 6p. The CGH-array technique allows high throughput and quantitative analysis of copy-number changes at high resolution throughout the cancer genome, allowing precise and rapid identification of tumor suppressor genes and oncogenes. One amplified 6p candidate gene only was identified in the 11 cell lines, CCND3 at 6p21.1, which codes for cyclin D3. Returning to primary colorectal tissue specimens, all three cyclin D genes (CCND1, CCND2, CCND3) were shown to be upregulated. However, quantitative reverse transcriptase-PCR experiments showed that only CCND3 was significantly upregulated further in liver metastases. This finding was further supported by immunohistochemical analysis for cyclin D3, using tissue microarrays of 120 primary colorectal cancer specimens (depth of invasion pT3) with accompanying clinical follow-up data. In particular, immunohistochemical expression of cyclin D3 at the leading edge of the primary colorectal carcinoma was significantly associated with recurrence and metastasis of the cancer. There was a significant decrement in patient survival for cyclin D3-positive primary cancers over 10 years, when compared to cyclin D3-negative primary cancers.

This study illustrates the remarkable power of a comprehensive and systematic approach to cancer genomic analysis. Survey of the entire cancer genome by CGH can be followed with molecular validation studies *in vitro* and then brought back to clinical tissue specimens to establish the prognostic value of specific molecular alterations in cancer. In this particular instance, overexpression of cyclin D3 at the leading edge of primary colorectal carcinoma was shown to be a risk factor for recurrence of tumor resulting from (presumably antecedent) nonsynchronous hematogenous spread to the liver, and adverse patient survival outcomes.

References

- 1 Tanami H, Tsuda H, Okabe S, *et al.* Involvement of Cyclin D3 in liver metastasis of colorectal cancer, revealed by genome-wide copy-number analysis. *Lab Invest* 2005;85:1118–1129.
- 2 Kallioniemi OP, Kallioniemi A, Sudar D, *et al.* Comparative genomic hybridization: a rapid new method for detecting and mapping DNA

- amplification in tumors. *Semin Cancer Biol* 1993; 4:41–46.
- 3 Knosel T, Schluns K, Stein U, *et al*. Chromosomal alterations during lymphatic and liver metastasis formation of colorectal cancer. *Neoplasia* 2004; 6:23–28.
- 4 Jiang JK, Chen YJ, Lin CH, *et al*. Genetic changes and clonality relationship between primary colorectal cancers and their pulmonary metastases—an analysis by comparative genomic hybridization. *Genes Chromosomes Cancer* 2005; 43:25–36.