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# INSIDE LAB INVEST

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## Early expression signatures provide cues to Sjögren's syndrome pathogenesis

Gene expression microarrays have been a part of experimental pathology for over a decade, and the dense heat-maps that they generate have become a familiar sight. Although this type of analysis is by nature not hypothesis-driven, it can be a powerful hypothesis-generating tool when properly used. Congenic mouse strains that share most of their genome can provide such an experimental system. In this issue, **Killedar *et al***[1] (p. 1243) have used a congenic strain carrying two Sjögren's syndrome (SS) susceptibility loci from the non-obese diabetic (NOD) mouse on a normal C57BL/6 (B6) and compared gene expression in its sub-mandibular glands to that of B6 controls. The two congenic strains are over 90% identical and differ only by the two regions that are necessary and sufficient for SS induction in this model. These authors now demonstrate that the pattern of sub-mandibular gland gene expression in the NOD congenic mice at the pre-clinical and early clinical stages of the disease was strikingly different from that of age-matched controls. In particular, a strong B-cell activation signature through members of the TNF-superfamily receptors confirms recent findings on SS pathophysiology obtained both in patients and animal models. More remarkable is a signature involving the TLR3/TLR7 and interferon pathways, which is highly reminiscent of results obtained through multiple approaches in lupus patients and murine models. These autoimmune diseases share many features, such as the production of pathogenic autoantibodies. These new results suggest now that SS and lupus may share early pro-inflammatory events in which an RNA virus may play a triggering role.

### Reference

1 Killedar S, Eckenrode S, McIndoe R, *et al*. Early pathogenic events associated with Sjögren's Syndrome (Sjs)-like disease of the nod mouse using microarray analysis. Lab Invest 2006;86:1243–1260.

## R2D2 to assess dysplasia?

Specialized epithelial cell metaplasia, that is, the presence of goblet cells, in the distal esophagus often occurs in response to chronic injury and is known as Barrett's esophagus. Challenges in this diagnosis are generally related to uncertainties regarding the location of the biopsy which can, in turn, lead to confusion as to whether identified goblet cells represent Barrett's esophagus or intestinal metaplasia of the gastric cardia. The clinical significance of the Barrett's esophagus diagnosis is that it identifies patients at risk for the development of dysplasia and adenocarcinoma. Thus, many patients with Barrett's esophagus undergo regular endoscopic surveillance and biopsy. Although this would seem to be a reasonable approach to management, it is complicated by difficulties in histopathological evaluation of Barrett's esophagus biopsies. In general, inter-observer agreement in detecting the presence of low-grade dysplasia is poor, even among experienced gastrointestinal pathologists. Thus, there is a tremendous clinical need for new ancillary techniques to improve diagnostic evaluation of these biopsies. Unfortunately, available approaches, including a variety of immunostains, have been unsatisfactory.

In this issue of *Lab Invest*, **Sabo *et al***[1] (p. 1261) have used a new approach to develop a more objective assessment of epithelial dysplasia in Barrett's esophagus. Although morphometry is not new, these authors are the first to establish a purely morphometric tool to assess dysplasia using both nuclear cytology and tissue architecture. The latter, which is often ignored in automated morphometry due to the difficulty in analyzing such variable structures, employed an ingenious method using fast Fourier transforms to study variations in spatial periodicity. After developing an analytical algorithm for analysis of Barrett's esophagus, Sabo *et al* used a test set of cases already diagnosed by experienced gastrointestinal pathologists to test the 'skill' of the computer-based diagnostic algorithm. When this approach was used to evaluate test cases, the morphometric diagnosis agreed with the consensus diagnosis 86% of the time, better than typical inter-observer agreement.

It seems possible that such an algorithm could develop into an ancillary technique, perhaps in a similar manner to immunohistochemical stains to assist in diagnosis. Perhaps even more interesting is the idea that this morphometric approach was able to identify the specific features that gastrointestinal pathologists use to evaluate dysplasia and the extent to which they weight each feature in determining an overall diagnosis. Thus, the morphometric data could be of use as a means to train and maintain consistency between pathologists and, possibly, to fine-tune the criteria and grading system for dysplasia in Barrett esophagus. Finally, and perhaps most

remarkably, the data suggest that this morphometric tool may even be able to identify cases with high grade dysplasia that are at greatest risk of progressing to invasive adenocarcinoma. While this computer-based algorithm will not eliminate the need for expert gastrointestinal pathologists, it may not be long until we have a computer, akin to Luke Skywalker's R2D2, helping us to more accurately 'fly' our microscopes.

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**Reference**

1 Sabo E, Beck AH, Montgomery EA, *et al*. Computerized Morphometry for Determining the Grade of Dysplasia and Progression to Adenocarcinoma in Barrett's Esophagus. Lab Invest 2006; 86:1261–1271.

## Are progenitor cells the 'egg'? Glypican-3 expression in hepatocarcinogenesis

A fundamental question in the field of hepatocellular carcinogenesis is whether mature hepatocytes undergo malignant transformation, or a progenitor cell population. While the occurrence of dual-phenotype hepatocellular:cholangiocellular carcinomas would argue for the latter, there is abundant evidence that oncogenic events occur in adult hepatocytes. One arbiter might be the cellular propensity for deposition of extracellular matrix. In both experimental animal models and in humans, the progenitor cell population resides in intimate relationship with an extracellular matrix that is distinctly different from the matrix of the lobular parenchyma. One striking component of the progenitor cell matrix in the developing liver is glypican-3 (Gpc3), a cell surface-linked heparan sulfate proteoglycan that is highly expressed during embryogenesis and organogenesis. Specifically, Gpc3 is highly expressed in fetal hepatoblasts (the fetal progenitors of both hepatocytes and bile duct epithelial cells), and expression decreases towards birth. Gpc3 also is expressed in hepatocellular carcinomas, and a truncated form of the protein is released into the blood in patients with such tumors. In this issue, **Grozdanov *et al***[1] (p. 1272) examined Gpc3 expression in an adult rat model of liver progenitor cell activation and in a related adult rat model of hepatocarcinogenesis, the Solt-Farber protocol. Gpc3 expression was highly induced in the progenitor cells (so-called 'oval cells') of the first model, with co-expression of a second oncofetal protein, alpha-fetoprotein (AFP). In the carcinogenic model, Gpc3 expression persisted throughout the formation of atypical duct-like structures and atypical nodules, and ultimately in the neoplastic liver lesions characteristic of the Solt-Farber protocol. Hence, this work documents for the first time that the oncofetal protein Gpc3 is a marker of hepatic progenitor cells and of early neoplastic liver lesions. Furthermore, these findings show that hepatic progenitor ('oval') cells of the rat are the target for malignant transformation in the Solt-Farber model of hepatic carcinogenesis. This experimental study is highly germaine to a recent report by Wang *et al*,[2] in which the expression profile of Gpc3 was examined in human hepatocellular carcinomas (HCCs) and preneoplastic lesions. Specifically, tissue microarrays containing HCCs, adjacent liver tissue (cirrhotic or non-cirrhotic), and cirrhotic macronodules were examined for Gpc3 immunohistochemistry. Membranous and cytoplasmic staining was identified in 90% of the HCCs and 64% of the tissue samples from adjacent non-cirrhotic liver. In the macronodules, Gpc3 immunostaining was present in 48% of high-grade dysplastic/early HCCs, but only 3% of the benign/low-grade dysplastic nodules. The unique features of the periductular/progenitor cell matrix also have been used to advantage in last month's cover article, in which Tátrai *et al*[3] used agrin immunostaining to identify foci of hepatocellular carcinoma in cirrhotic livers. Hence, Gpc3 may be considered as an early marker of hepatic carcinogenesis in humans as well. Both studies provide evidence that hepatocellular carcinogenesis may arise from progenitor cell populations, rather than mature hepatocytes undergoing oncogenic transformation.

**References**

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2 Wang XY, Degos F, Dubois S, *et al*. Glypican-3 expression in hepatocellular tumors: Diagnostic value for preneoplastic lesions and hepatocellular carcinomas. Human Pathol 2006 (e-publication ahead of print).
3 Tátrai P, Judás A, Batmunkh E, *et al*. Agrin, a novel basement membrane component in human and rat liver, accumulates in cirrhosis and hepatocellular carcinoma. Lab Invest 2006;86: 1149–1160.