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The origins of bladder cancer: gene clusters and the earliest genetic changes

Molecular analysis of epithelial malignancies has been the lodestar of research into the origins of human cancer for the past two decades. The progression of microscopically identifiable *in situ* precursor lesions through dysplasia to carcinoma has enabled the mapping out of the multiple cumulative molecular events that characterize cancer. While colon cancer has garnered the most attention over the years, carcinoma of the bladder also is a superb candidate for such studies. It is the fifth most common human malignancy, and accounts for 3% of all cancer-related deaths. The most common papillary form arises from superficially growing lesions that originate from urothelial hyperplasia. Early tumors can be excised in a minimally invasive fashion, and recurrent tumors usually do not behave aggressively. Bladder cancers arising as solid, nonpapillary lesions originate from *in situ* dysplasia, and tend to be aggressive with bladder wall invasion and a propensity for distant metastasis.

In the April issue of *Laboratory Investigation*, Kim *et al*¹ (p. 532) present a comprehensive analysis of gene expression patterns in the development of human bladder cancer from preneoplasia along the papillary and nonpapillary pathways. A total of 19 pairs of RNA samples from bladder tumors and adjacent urothelium were analyzed using complementary DNA microarrays. Selected genes of interest were further analyzed in a cohort of 251 bladder cancer tissues using tissue microarray and immunohistochemistry. These molecular findings were then related to clinicopathological parameters including clinical follow-up data. Both papillary and nonpapillary cancers exhibited alterations in gene clusters controlling proliferation, differentiation, and programmed cell death. Nonpapillary cancer alone exhibited changes in genes controlling cellular and stromal interactions. In particular, tumors characterized by low expression of e-cadherin and high expression of DNA α -topoisomerase had a high propensity for distant metastasis and were associated with poor survival. This study represents a tour-de-force for comprehensive molecular analysis of a human cancer, verification of gene expression in an expanded tissue set, and exploration of the clinical significance of variably expressed genes. Remarkable at first sight is the substantive simi-

larities between gene expression alterations in papillary and nonpapillary bladder cancer. This makes more conspicuous the finding that the signature expression profiles for altered genes in the more aggressive nonpapillary cancers could also be detected in the adjacent urothelium. This finding suggests that genetic changes occurring in the preneoplastic phase of bladder cancer may have a profound effect on the ultimate histologic and clinical outcome.

In the current issue, Tuziak *et al*² (p. 689) from the same research group present a comprehensive strategy for identifying the earliest genetic changes in incipient neoplasia of the bladder epithelium. The key concept in play is that neoplasia begins as *in situ* clonal expansion of phenotypically normal cells. Identification of the chromosomal regions involved and their contained target genes is likely to be critically important in establishing the incipient genetic events leading to human carcinogenesis. Including the paper by Kim *et al*,¹ this research group has previously reported the identification of putative tumor-suppressor gene loci involved in the preinvasive phases of human bladder cancer. In the paper by Tuziak *et al*,² chromosomal regions 13q14 and 17p13 containing tumor-suppressor gene loci *RB1* and *p53*, respectively, were examined for deleted segments, as a function of clonal expansion of *in situ* bladder neoplasia as identified by whole-organ histologic and genetic mapping of single nucleotide polymorphisms (SNPs). In all, 27 Mb of DNA sequence around the chromosome 13 *RB1* locus, and 5 Mb around the chromosome 17 *p53* locus, were mapped by 661 and 960 SNPs, respectively. These genotypic maps were matched to whole-organ histologic maps. The result was identification of anatomic clusters of deleted regions associated with clonal expansion of intra-urothelial neoplasia, ranging from 0.001 to 4.3 Mb, with an average of 0.67 Mb. Interestingly, the genetic maps suggest that noncoding DNA sequences characterized by a high concentration of repetitive sequences may predispose to chromosomal deletions, and hence risk for neoplasia. This highly novel approach of high-resolution mapping enables analysis of the overall genomic landscape of a preneoplastic anatomic region. In particular, opportunity is given for identification of chromosomal events that precede definitive alterations in tumor-suppressor loci.

References

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Interferon gamma: a burr collar for the gastric neck cell?

Chronic inflammation of the gastric mucosa, in the form of *Helicobacter pylori*-induced chronic gastritis, is believed to significantly increase the risk of gastric adenocarcinoma. The molecular mechanisms underlying progression of chronic gastritis through intestinal metaplasia, dysplasia, and eventual adenocarcinoma remain elusive. Major consideration is being given to the direct effects of bacterial virulence factors, and the initiating and promoting effects of chronic inflammation. The best opportunity for elucidating the mechanisms for carcinogenesis appear to be animal models of chronic gastric inflammation, and genetically deficient mice feature prominently. One such animal model is the gastrin-deficient mouse (G^{-/-}), which exhibits chronic inflammation in a hypochlorhydric stomach. The cover image of this issue highlights a study suggesting that interferon gamma is central to the pathology of this chronic gastritis. Merchant and co-workers¹ previously identified a prominent fundic mucous cell population whose expansion occurred shortly after colonization of gastrin-deficient mice (G^{-/-}) with non-*Helicobacter* bacteria. They postulated that these cells might be a precursor to the gastric cancers that are common in these mice. In the present issue, **Kang *et al***² (p. 702) from this same research group sought to identify the origin of these cells. Based on expression of trefoil factor 2 and MUC6, Griffonia Simplifolia II (GSII) lectin staining, and absence of the intestinal transcription factor Cdx2, the authors conclude that this cell population is derived from gastric mucous neck cells. These authors went on to show that interferon gamma is the most abundant proinflammatory cytokine in the stomachs of these mice. Infusion of this cytokine into mice increased the abundance of these same mucous neck cells. Based on these *in vivo* findings, they developed the hypothesis that interferon gamma might be the driving force behind the expansion of these mucous neck cells. To test this hypothesis, they treated a gastric epithelial cell line with interferon gamma *in vitro*. Remarkably, expression of trefoil factor 2, MUC6 and pepsinogen II, but not of pepsinogen I or intrinsic factor, were increased. Thus, **Kang *et al*** conclude that inflammation, and especially the proinflammatory cytokine interferon gamma, induces expansion of fundic mucous neck cells. Although the question of whether these are the cells that ultimately become neoplastic remains open, it is notable that increased numbers of morphologically similar cells have been

associated with gastritis in humans and in other mouse models.

References

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The complex origins of pancreatic neo-islets

One of the profound enigmas of diabetes has been the fact that the pancreata of animals and humans with overt type I diabetes feature destruction of the islets of Langerhans, yet islets can still be found that appear normal, without infiltrating immune cells, and express insulin. Diagnosis and early treatment of type I diabetes enables preservation of a residual insulin secretory response, and may significantly delay complete loss of insulin secretory capacity. These observations have led to the exciting hypothesis that there may be mechanisms for regeneration of islet β -cell mass, even in the midst of ongoing immune-mediated destruction. Possible pathways include *de facto* regeneration, and transdifferentiation from other cell types. In the latter instance, the formation of duct-like tubular structures in the exocrine pancreas (tubular complexes) with eventual expression of insulin recapitulates embryologic formation of the endocrine pancreas. Tubular complexes have been observed in adult conditions involving pancreatic damage, including pancreatitis, pancreatic duct obstruction, partial pancreatectomy, and experimental chronic hyperglycemia. These tantalizing findings raise the issue of whether tubular complexes may represent a regenerative pathway for islet neogenesis, or another process such as acinar-to-duct transformation of the exocrine pancreas. In this issue, **Wang *et al***¹ (p. 675) hypothesized that formation of tubular complexes may occur during the active phase of islet destruction, and that these complexes may contain endocrine progenitor cells. Accordingly, they analyzed for the presence of tubular complexes in BB rats developing type I diabetes, in comparison to appropriate control populations: wild-type rats, and BB rats that did not develop overt diabetes. Tubular complexes were observed in all rat groups early in life (analyzed at 13 days of life). Only in the diabetic BB rats did the number of tubular complexes increase over the subsequent 3 months of life. These were highly proliferative foci, with transitional duct/acinar (keratin/amylase immunoreactive) and duct/endocrine (keratin/insulin immunoreactive) features. These complexes also were focally immuno-

reactive for other endocrine markers such as chromogranin, glucagon, somatostatin, and pancreatic polypeptide, along with markers significant for commitment to the endocrine phenotype, PDX-1, neurogenin 3 and protein gene product 9.5. The stem cell marker nestin also was upregulated in the tubular complex regions. This study demonstrates that tubular complexes are upregulated in the pancreata of rats undergoing β -cell destruction, and that the complexes contain potential endocrine precursors. Although not conclusive, these data are consistent with the hypothesis that reconstitution of islet β cells may occur through a transitional complex route.

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Why do men produce prolactin?

Prolactin has a well-recognized gender-specific reproduction-related hormonal effect. In addition, there is a growing body of literature documenting that it also plays a proinflammatory role in various physiological and pathological settings. The presence of the prolactin receptor on immunocytes is consistent with a place for this hormone in immune system homeostasis. Numerous reports have linked prolactin to an enhanced immune response and have associated high levels of prolactin to various

autoimmune diseases. This is consistent with the well-established heightened immune responses and higher prevalence of autoimmune diseases in women. However, the mechanisms by which prolactin plays a proinflammatory role are still largely elusive. In this issue, **Montes de Oca *et al***¹ (p. 633) evaluated the hypothesis that prolactin may stimulate an integrin-mediated adhesion of leukocytes to endothelial cells. Through a series of carefully constructed experiments, these authors demonstrated for the first time that prolactin stimulated the adhesion of peripheral blood mononuclear cells (PBMC) to IL-1 β activated human umbilical endothelial cells (HUVEC) through the integrins LFA-1 (leukocyte functional antigen-1) and VLA-4 (very late antigen-4). Their work also strongly suggests that the integrin-mediated adhesion involves G-protein chemokine receptors, such as CXCR3, and the tyrosine phosphorylation of JAK-2, STAT-3 and STAT-5. Further elucidation of this mechanism may help to clarify some aspects of gender differences in the immune system. Moreover, it shows that this hormone, which has evolved a function beyond milk production, is not secreted in vain by men.

References

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