This Month in AJP

Molecular Profiling of Human Neoplasms: Isolation of RNA from Tissues Microdissected by Laser Capture

The analysis of large scale changes in gene expression using nucleic acid array techniques has been applied to the study of human cancers and their molecular classification. A major limitation for the application of this new technology is the amount of tissue required to obtain a sufficient amount of RNA to perform the microarray analysis. Inevitably, the tissue samples are heterogeneous and may contain normal cells, inflammatory infiltrates, etc, in addition to tumor cells. Luzzi et al (Am J Pathol 2001, 158: 2005–2010) describe the application of laser capture microdissection to obtain high quality RNA, in nanogram amounts, from breast tissue. The procedure is reproducible and has a sensitivity comparable to that of methods that usually require 20 to 1000x greater input RNA. The technique retrieved 15,000 to 20,000 cells from either nonmalignant epithelial cells or adjacent ductal carcinoma in situ, yielding 40 to 80 ng of RNA. Although expression profiles were obtained from three patients, it is still too early to reach conclusions about specific gene expression profiles for ductular carcinoma in situ. Nevertheless, future application of the technique should prove to be very useful in the molecular classification of neoplasms and the detailed analysis of patterns of gene expression in human cancers.

More Plasticity from Stem Cells: Liver to Myocytes

Recent findings demonstrate that stem, or precursor, cells derived from adult tissues have broad differentiation capacity and great plasticity. Examples include the differentiation of hemopoietic stem cells into liver cells, differentiation of stromal bone marrow cells into muscle, cartilage, and other tissues, and the generation of neural cells from bone marrow. Malouf et al (Am J Pathol 2001, 158: 1929–1935) show that a clonal rat liver stem cell line (WB-F344) inoculated into the heart of nude mice differentiated into myocytes six weeks after transplantation. The WBF344 cells were from male rats and carried a β-galactosidase gene. They were transplanted into the heart of female animals and could be recognized by the presence of the Y chromosome as well as β-galactosidase staining. Long myocytes derived from WB-F344 cells contained well-organized sarcomeres and myofibrils and formed intercalated disks as well as gap junctions with host myocytes. The results demonstrate that liver cells with stem cell properties can respond to the adult heart microenvironment and differentiate into myocytes. The donor-derived myocytes apparently integrate well in the cardiac tissue, although proof of their functionality is still lacking.

Imprinted Genes and Mutations in Patients with Congenital Hyperinsulinism, Pancreatic Adenomas, and Increased Insulin Secretion

Congenital hyperinsulinism (persistent hyperinsulinemic hypoglycemia of infancy) is a disease characterized by severe hypoglycemia caused by excess insulin secretion. In the focal form of the disease (FoCHI), there is widespread focal adenomatous hyperplasia of islets, a condition that can be treated by partial pancreatectomy. Patients with this disease have a loss of maternal alleles of the 11p15 region during pancreatic development. They also have a paternally-inherited mutation of the sulfonylurea receptor gene ABCC8, although other genetic mutations have also been identified. In a study of 31 patients with FoCHI, Fournet et al (Am J Pathol 2001, 158: 2177–2184) show that 61% of these patients had an imbalance in the expression of imprinted genes in the 11p15.5 region and a somatic reduction to homozygosity (LOH) of recessive mutations in the ABCC8 gene. In addition, there were mutations in KCNJ11, a gene that encodes a K-ATP channel. It is suggested that the alterations in these genes lead to unregulated insulin secretion while the genetic imprinting may be responsible for adenomatous growth.

Pathways to Bladder Carcinogenesis: High Frequency of Fibroblast Growth Factor Receptor 3 (FGFR3) Gene Mutations in Non-Invasive Papillary Tumors

More than 90% of bladder tumors are urothelial carcinomas and the most frequent form are papillary tumors (pTa). These tumors, confined to the epithelium, have a high rate of recurrence but a low probability of invasion to lamina propria or muscle. The other type of urothelial carcinoma confined to the epithelium is carcinoma in situ (CIS). CIS is probably the most common precursor of invasive cancer to lamina propria (pT1) or muscle (pT2 - pT4). In a study of 132 bladder tumors...
at various stages, Billerey et al (Am J Pathol 2001, 158: 1955–1959) found a 74% frequency of FGFR3 point mutations in non-invasive papillary tumors (pTa) and absence of these mutations in CIS. Invasive tumors (pT1 and pT2 - pT4) had FGFR3 mutation frequency of ~20%. These results support the notion that papillary tumors and CIS may represent two different pathways in bladder cancer development. Moreover, the high frequency of FGFR3 mutations in papillary tumors opens the possibility of detecting bladder tumor recurrence by urine sampling.

Reversal of Hypoxic Damage in Isolated Kidney Proximal Tubules

Most of the tubule cell damage in human and animal acute renal failure is sublethal and reversible. Cellular injury results from ATP depletion and protein dephosphorylation resulting in multiple subcellular defects including loss of brush border microvilli, disruption of tight and adherens junctions, abnormalities in integrin distribution and disruption of normal polarity of major membrane associated proteins. Weinberg et al (Am J Pathol 2001, 158: 2153–2164) show that in hypoxia/reoxygenation of isolated rabbit kidney tubules, ATP-generating substrates given during hypoxia promote mitochondrial recovery and reverse tyrosine dephosphorylation caused by hypoxia. In particular, the addition of these substrates induced extensive phosphorylation of focal adhesion proteins as well as recovery of fodrin and microvillar actin even after 60 minutes of severe hypoxia. These results emphasize the potential value of strategies aimed at conserving or generating energy to prevent injury in hypoxic/ischemic cells.

Presenilin-1 Mutations and Amyloid Angiopathy in Familial Alzheimer's Disease

Mutations of the presenilin-1 (PS-1) gene are present in ~50% of patients with early onset familial Alzheimer’s disease (AD). The PS-1 gene contains 11 exons encoding a primary transcript translated into a single protein. The protein is in a precursor form, which is cleaved into three fragments, causing the activation of γ-secretase activity. Although the gene has been extensively studied and >70 disease-associated mutations of PS-1 have been identified, little is known about the relationships between these mutations and AD pathogenesis. Mann et al (Am J Pathol 2001, 158: 2165–2175) studied in detail the extent and morphological features of amyloid β protein (Aβ) in 54 cases of PS-1-associated AD involving 25 different PS-1 mutations through the length of the gene. Two distinct histological profiles were identified in the study: type 1 histology, with cortex and cerebellar lesions typical of sporadic AD, and type 2 phenotype, characterized by excessive amyloid angiopathy in these tissues. Patients with type 1 histology had earlier onset age and shorter disease duration. In broad terms, the type 1 profile was associated with mutations extending up to codon 200 of PS-1 while type 2 histology was associated with mutations beyond codon 200. Furthermore, some mutations were associated with more aggressive disease, an observation that should be of great help in designing transgenic mouse models of AD. The authors speculate that the PS-1 mutations downstream of codon 200 associated with amyloid angiopathy may involve defective signaling from the Notch gene.

A New Pediatric Renal Neoplasm

Renal cell carcinoma and angiomyolipomas are the main epithelioid renal neoplasm in children. Argani et al (Am J Pathol 2001, 158: 2089–2096) describe a distinctive type of pediatric renal neoplasm which has features of an epithelial neoplasm but lacks immunoreactivity for epithelial markers. The tumors are stained with melanocytic markers, have ultrastructural features that are distinct from other tumors and carry a 6;11 chromosome translocation. The histogenesis of these tumors is unknown. Their cells do not correspond to normal cell types in the kidney.