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INSIDE LAB INVEST

ARE TUMORS DERIVED FROM TISSUE STEM CELLS?: The maintenance of tissue function throughout the life of an organism requires replenishment of terminally differentiated cells by cells derived from a proliferative compartment. The proliferative population is in turn maintained by tissue stem cells. These undergo asymmetrical division, a mitosis that results in the generation of a daughter stem cell and a cell that will enter a number of symmetrical divisions, thus effectively amplifying the cell population that will eventually differentiate. The length of time required for the genesis of naturally occurring sporadic tumors, together with the fact that malignant tumors result from the accumulation of transmissible (genetic or epigenetic) alterations of the somatic cells, suggests that tissue stem cells may be the substrate for carcinogenesis. Inside this issue, **Böcker et al** (Lab Invest 2002, 82: 737–745) present evidence suggesting that tissue stem cells responsible for the maintenance of breast function can be identified in the mammary ductules. Restricted expression of cytokeratin 5 distinguishes cells that are committed stem cells for both myoepithelial and glandular mammary cell types. In turn, these two cell types can be identified by their differential expression pattern of intermediate filaments. These findings should provide a conceptual and practical way for the classification of benign and malignant proliferative breast lesions. It is reassuring to see that comprehensive analysis of breast cancers using expression microarrays (Perou et al) indicates the existence of cell types and tumor types (basal and luminal) that are a good fit to the concepts proposed by Böcker et al.

CANCER PROGNOSIS—A BETTER MEANS TO A (CHROMOSOME) END?: One of the cardinal features of carcinogenesis is up-regulation of telomerase activity. Telomerase stabilizes chromosome ends by extending telomere sequences that would otherwise be shortened at each cell division because of the end-replication problem. Recent studies have indicated potential for telomerase measurements in prognosis of breast, lung, prostate, and hepatocellular carcinoma. The utility of telomerase assays for prognosis depends upon the use of uniform and sensitive methods for quantification. Telomerase is typically measured using a "TRAP" assay, which detects enzymatic activity extracted from specimens, or by an assay for expression of the telomerase reverse transcriptase hTERT mRNA. The former assay has the advantage of providing direct measurement of telomerase, but may be subject to problems with stability of the enzyme and with other technical factors. mRNA levels are readily quantified, but are less directly linked to functional activity. Both assays can be confounded by the heterogeneity of specimens. In this issue, **Marchetti and associates** evaluate the relative utility and independence of these two approaches for telomerase measurements in a series of nonsmall cell lung carcinoma specimens (Lab Invest 2002, 82: 729–736). All samples expressed hTERT mRNA as quantified by real-time RT-PCR, but high hTERT expression was associated with reduction of disease-free and overall survival. The TRAP assay identified a subset of patients with poor prognosis. Significantly, combination of TRAP and real-time RT-PCR provided the best combination of sensitivity and specificity, suggesting that neither test on its own is ideal, or that, in a subset of tumors, telomerase up-regulation is dissociated from mRNA alteration. Hence, until further technical development, combining TRAP and mRNA expression information may be the best strategy for evaluating telomerase.

CONNECTIVE TISSUE GROWTH FACTOR—A MASTER SWITCH IN THE INDUCTION OF HEPATIC FIBROSIS?: Cirrhosis is listed among the top ten causes of death in the Western world. Its causes include alcoholism, hepatitis, biliary disease, and iron overload, and it is characterized by bridging fibrous septa, leading to effacement of the lobular hepatic architecture and reorganization of the hepatic vasculature. Hepatic fibrosis is thought to be a cytokine/growth factor-mediated process affecting the hepatic stellate cell population. Recent studies have implicated TGFβ1 and PDGF-BB as inducers of hepatic stellate cell extracellular matrix expression, leading to hepatic fibrosis. In this issue, **Paradis et al** investigate the regulation and effects of connective tissue growth factor (CTGF) (a 38 kDa protein known to be involved in a variety of human fibrotic disorders) on hepatic stellate cells (Lab Invest 2002, 82: 767–773). Their studies illustrate the stimulatory effects of

TGFβ1 and PDGF-BB on stellate cell CTGF expression. Specifically, TGFβ1 was observed to stimulate CTGF expression directly, while PDGF-BB was thought to act indirectly, via a TGFβ1-mediated pathway. In addition to its inductive effects on stellate cell connective tissue synthesis, CTGF was also noted to stimulate the proliferation and migration of stellate cells. Thus, CTGF seems to be a critical central component in the cytokine/growth factor-mediated pathway leading to hepatic stellate activation and subsequent hepatic fibrosis. These findings suggest that modulation of CTGF levels, its expression, and/or receptor activation will be likely therapeutic targets in the near future. This may have importance in controlling the fibrotic responses associated with a variety of genetic, metabolic, infectious, and neoplastic disease processes.

CORNEAL AMYLOIDOSIS—TEARS OF TROUBLE?: Disorders of protein folding have emerged as a major pathway of pathologic injury. Perhaps the clearest and best known examples collect under the rubric of amyloidosis, protean disorders in which extracellular deposits of protein fold into congophilic beta-sheet–like secondary structures that form insoluble aggregates, clog tissues, and destroy cells. Alzheimer's disease is such a disorder; so also are many otherwise dissimilar conditions including cardiac amyloid, hereditary amyloidosis, amyloid of immune origin, etc. Amyloid deposition may be localized or systemic. In each case, unanswered questions relate to the nature of the proteins involved and the conditions that lead to their abnormal accumulation. Ocular tissues are one type of tissue involved in both systemic amyloidosis syndromes and in more localized forms. Conditions associated with localized corneal amyloid include inherited mutations in gelsolin (also a cause of systemic disease). Gelsolin mutations lead to lattice corneal dystrophy. In a second inherited condition called gelatinous drop-like corneal dystrophy, the precursor protein remains unknown; lactoferrin has been found in these deposits but has not been thought to be causal. A number of secondary corneal amyloidosis disorders arising with infection or other processes are also recognized. One of these is trichiasis. In this issue**, Ando and colleagues** explore the genesis of a novel type of corneal amyloidosis that follows trichiasis (Lab Invest 2002, 82: 757–765) and find that like gelatinous drop-like corneal dystrophy, it is associated with the deposition of lactoferrin, a component normally found in tears but not other ocular tissues. Of the three patients they studied, they also found that all had a Glu561Asp mutation in their lactoferrin gene, and one was a compound heterozygote with a second Ala11Thr mutation. These mutations (polymorphisms?) cannot be causal by themselves, because the Glu561Asp replacement is present in over 40% of healthy Japanese volunteers. However, it is demonstrated in vitro that modified lactoferrin does indeed form amyloid fibrils, and that the deposits found in the patient's corneal samples have undergone several modifications including deglycosylation and N-terminal truncation. Given the abundance of lactoferrin in these deposits, its level of modification, the presence of some suspicious mutations that may signal a more amyloidogenic form of the protein, and the in vitro demonstration of the amyloidogenic potential of lactoferrin, the authors conclude that lactoferrin is the causative factor in this form of amyloid. Their case is solid, if not conclusive. What remains, of course, is to understand the covariables that lead to corneal amyloidosis under some conditions, but not others. Clearly, this disorder is much more rare than are the observed mutations in lactoferrin. Serum amyloid precursor protein (SAP) is also present in the deposits; this is a common accompaniment of many other forms of amyloid and is presumably one cofactor. A second and important factor may simply be the level and extent of lactoferrin exposure that the cornea experiences and how other pathologies (such as infection with trichiasis) may alter lactoferrin levels or its processing. As noted above, this protein is not normally present in the cornea but is abundant in tears and inflammatory cells. Since infiltration by leukocytes is an unusual feature of these corneal diseases, the most likely lactoferrin source is tears. It will thus be interesting in future work to explore this hypothesis. If true, novel therapeutic interventions should be possible, given the easy accessibility of the cornea and lachrymal secretions. If true, it should thus be nothing to cry about!