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INSIDE LAB INVEST

PROSTATE STEM CELLS: A fundamental principle, oft taught to medical students, is that cancer is a caricature of normal tissue development. What is meant, of course, is that although tumors are pathologic, and sometimes confusing; they are not new biologic phenomena. Like the tissues from which they arise, tumors have the capacity to both regenerate and differentiate. This property is most apparent in the case of epithelial tumors, but probably holds for most, if not all, tumors. Indeed, it is this feature upon which the entire morphologic nosology of diagnostic oncologic pathology is based. Thus, one speaks of a tumor as well differentiated, poorly differentiated, or undifferentiated, but nevertheless still seeks to classify it as a carcinoma, sarcoma, lymphoma, or germ-line tumor on the basis of subtle or not-so-subtle differentiated features. To move beyond the limitations of this descriptive approach and to seek a deeper understanding of the cellular origins of cancer, one must understand the normal pathways of cellular differentiation and tissue renewal. Central to this understanding is an appreciation of the nature of the elusive stem-cell, the progenitor cell that must be present in each renewable tissue compartment, the cell that with each division, replenishes itself while committing other of its progeny to terminally differentiate into one or more of the mature cell types that define each tissue.

However, the search for stem-cells in normal or even hyperplastic tissues has proven to be a daunting task. Typically, they comprise only a tiny fraction of the cells in a tissue and by definition lack many or all differentiated features that might allow them to be sorted out into a pure state. Making matters worse, in a sort of biologist's equivalent of the uncertainty principal, they are by nature labile cells, programmed to metamorphose into more mature elements at seemingly the slightest provocation or with every attempt to measure or purify them. Thus, it is with some excitement that in this issue of *Laboratory Investigation*, two papers offer real progress toward our understanding of the nature of the prostatic epithelial stem-cell vs committed but still proliferating intermediate (transiently proliferating/amplifying) cells. While the techniques employed in these carefully done studies are neither esoteric or difficult, clear insights have been gained. Using instrumentation that makes possible the simultaneous monitoring of three cytokeratin markers, **van Leenders and his colleagues** (Lab Invest 2000, 80: 1251–1258) identify for the first time the clear presence of transition cells in the prostate, cells that have cytokeratin markers typical of basal cells (K5/14), but with an additional up-regulation of cytokeratin K18 relative to K5/14, suggesting that these cells are well along their differentiation pathway to luminal cells (which predominantly express cytokeratin 18. Also identified are basal cells with a K5/18 profile, which are presumably also intermediate-type cells. What is important here is that these changes in cytokeratin profiles are demonstrated to be occurring within individual cells, refining our understanding of how the putative stem cells of the basal layer mature gradually into the exocrine and neuroendocrine luminal cells.

In a second study published in this issue, **Hudson and his colleagues** (Lab Invest 2000, 80:1243–1250) take a complimentary approach to analyzing prostatic cell populations. Reasoning that the sine qua non of a stem cell is its ability to replenish the mature cell compartments of a tissue, these authors evaluate by clonal analysis the ability of individual cells derived from human prostate biopsies to fully recapitulate epithelial differentiation in collagen matrices. Two cell populations are identified. The first and most abundant type (type I) are those that grow in culture (ie, they still have proliferative capacity), but fail to develop structures reminiscent of prostatic epithelium. Presumably, these cells are the intermediate cells, those that transiently proliferate and would be cytokeratin The second type (type II) are those that grow, but also are able to regenerate all prostate epithelial layers in vitro. These cells, forming type II colonies, constitute less than 0.5% of the total proliferating cell population and are the best bet yet to represent the true prostate stem cell. While these findings offer no immediate insights into proliferative prostatic disease and prostatic cancer, two major health problems, they do open the door to future studies that will seek to understand in detail the factors that control epithelial cell growth and renewal in the prostate.

THE CELL BIOLOGY OF TNF SIGNALING: The cellular response to TNF is initiated by binding of this heterotrimeric cytokine to cell surface receptors, causing clustering of individual receptor proteins. In vascular endothelial cells, a principal target of TNF action, the biological responses are largely mediated through TNF receptor type I (TNFRI). It has previously been shown that TNFRI is predominantly localized to the Golgi apparatus rather than the cell surface. The biological significance of this observation is uncertain. Two possibilities are either that the Golgi receptor pool represents a reservoir of receptors that can be rapidly mobilized or that the Golgi contains functional receptors that respond to endogenously synthesized TNF molecules that must pass through the Golgi on their way to the cell surface. Since endothelial cells do not synthesize TNF and since TNFRI shedding has been shown to rapidly down-regulate sensitivity of endothelial cells, the existence of a receptor replacement pool is an attractive idea. The key limitation of this interpretation is that there is no evidence as yet that the Golgi-associated pool of TNFRI molecules can be mobilized to the cell surface. In the present issue of this journal, **Gaeta and colleagues** (Lab Invest 2000, 80: 1185–1194) investigate the structural basis of receptor sequestration within the Golgi. The approach used is based on the observation that transfected receptors recapitulate the behavior of the endogenous receptor, which incidentally rules out the possibility that the Golgi receptor pool arises from an alternatively spliced form of TNFR1 mRNA. Deletion analysis of the transfected receptor identifies the carboxy-terminal intracellular death domain as being necessary for Golgi retention, but studies with chimeric receptors show that this region is not sufficient to prevent another protein (TNFRII) from reading the cell surface. These results contribute to a growing body of evidence that a full explanation of TNF signaling needs to be approached as a problem in cell biology as well as one in biochemistry.

RHEUMATOID ARTHRITIS: VEGF AND ANGIOGENESIS, ANOTHER THERAPEUTIC TARGET? Rheumatic arthritis is a disabling chronic inflammatory disease of unknown etiology whose incidence approaches 1% among adults aged 18 years and older, and it is characterized by the manner in which it involves the joints. In rheumatoid arthritis a nonsuppurative proliferative synovitis (pannus) causes the destruction of articular cartilage and subsequent ankylosis of the joints. The synovial microvasculature is thought to be injured, resulting in edema, complement activation, synovial lining cell proliferation, polymorphonuclear leukocyte infiltration, and angiogenesis. Several cytokines, chemokines, and growth factors have been found to be present in synovial fluids obtained from affected joints. These findings have given rise to a number of newer treatments directed at abrogating the effects of activated complement proteins and particular cytokines in this disease process. These treatments include the use of inhibitors of complement activation and antibodies directed against specific cytokines (anti-TNFα). As changes in synovial microvascular permeability and angiogenesis have been observed in the synovia in rheumatic arthritis, the notion of influencing vascular behavior in this disease has received attention, and several approaches have been taken. Inhibition of complement activation appears to reduce endothelial cell activation and subsequent polymorphonuclear leukocyte infiltration in murine models. Since vascular permeability changes and angiogenesis would amplify and prolong the inflammatory response and VEGF (a potent vascular permeability and angiogenic factor) is known to be present in the synovial fluid of affected joints, **Miotla et al,** in this issue of the journal (Lab Invest 2000, 80: 1195–1205), tested the ability of a soluble VEGF receptor (sFlt-1), delivered interperitoneally, to reduce the severity of a murine collagen-induced model of rheumatoid arthritis. Treatment with sFlt-1 reduced clinical score, paw swelling, joint inflammation, and bone and cartilage destruction compared with untreated controls. These results suggest that, in addition to the current conventional and newer therapeutic approaches used and being developed to treat rheumatic arthritis, modulation of vascular permeability and angiogenesis may prove to be beneficial as an additional therapy in treating this debilitating disease.

INTERLEUKIN-4 IS MORE THAN A TH2 CYTOKINE: Inflammatory reactions have long been divided into antigen-independent responses, typically initiated by infection or injury (innate inflammation) and responses initiated by specific recognition of antigen by lymphocytes or antibodies (immune inflammation). More recently, immune inflammation has been subdivided into responses dominated by IFN-gamma-producing T helper 1 (TH1) lymphocytes, and those dominated by IL-4, IL-5, and IL-13-producing TH2 lymphocytes. Innate inflammatory infiltrates are typically dominated by neutrophils and, at later times, by macrophages. In contrast, TH1 infiltrates are composed mainly of macrophages and T cells, whereas TH2 infiltrates are enriched in eosinophils. TH2 cells,

through secretion of IL-4, are also thought to be mediators of chronic fibrosis. In the current issue of this journal, **Salmon-Ehr and colleagues** (Lab Invest 2000, 80:1337–1343) upset this neat classification by showing significant local production of IL-4 at early times (ie, at one day) in a nonimmune injury caused by a sterile excisional skin wound. Even more surprisingly, reduction of IL-4 production by antisense treatment delays wound healing, an effect that could be reversed by providing exogenous IL-4. Several questions arise from this study. What cell types produce IL-4 in response to wounding? Mast cells are obvious candidates, but circulating leukocytes could also contribute. How does IL-4 work to accelerate healing? Does it act directly on keratinocytes to promote their growth? Finally, is IL-4 a potential therapy to promote wound healing and is IL-4 antagonism a potential therapy for controlling too exuberant would healing (eg, in keloids)? Regardless of the answers to these questions, IL-4 must now be seen as a cytokine of innate, as well as of antigen-dependent, immunity.

ERRATUM

In the July issue the legend for the cover figure was incorrect. The correct legend for the July cover figure appears below. We regret the error.

Cover: Representative karyotypes of NSCLC cell lines (a) Colo-699 and (b) A427. Numbers adjacent to a derivative chromosome indicate the origin of the translocated or inserted material. “OL” in (b) indicates a color change caused by overlapping chromosomes. See article by Speicher et al.

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