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Permeability properties of tumor surrogate blood vessels induced by VEGF-A

Angiogenesis is a biologic process present in tissues in numerous pathological conditions. It is intimately associated with inflammatory responses to agents that cause tissue injury and disease. It is an essential feature of tumors associated with tumor growth and metastasis. Angiogenesis is also an important process in tissue repair and the generation of granulation tissue, and is induced in ischemic conditions. The theory put forth in the literature is that angiogenesis is so intimately involved in the conditions noted above that alterations in angiogenesis will have profound effect on the viability and development of these conditions. There are general features of angiogenesis, which appear to be universal in all conditions and others that are more specific to a given condition. Thus, study of angiogenesis continues to be very important in order to identify biologic targets that play regulatory roles, and thus can be utilized to control human disease conditions by interfering with steps in the angiogenic process. Major efforts have been directed at the identification of both pro- and antiangiogenic factors that regulate either the initiation and progression or the inhibition of angiogenesis, respectively. Vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) is a well-studied cytokine that not only promotes angiogenesis but is also a potent hyperpermeability factor, rendering it to be an agent that regulates two important processes—the initiation and growth of new blood vessel in tumor tissue and the hyperpermeability of the tumor blood vessel bed to plasma proteins. The latter condition is thought to be important, at least in part, in the regulation of stromal tissue growth and remodeling in tumors.

Progress in understanding the pathogenesis of angiogenesis has been very good; however, numerous questions remain unanswered. In this issue, the study by **Nagy *et al***[1] (p. 767) has advanced our understanding of tumor hyperpermeability. The authors choose to utilize a nude mouse model to investigate the finer details of hyperpermeability in tumors. The model utilizes the study of one angiogenic/permeability factor VEGF-A^{164} in the induction and development of surrogate blood vessels, which are free of association with tumor tissue, inflammatory processes and tissue necrosis. The model is useful since it is simpler than actual solid tumors, it results in large numbers of new blood vessels that are similar to the full range of vessels developed in *in vivo* tumor tissue, with reproducible kinetics of development, and it is amenable to evaluations of morphology and permeability. The authors found that different types of blood vessels, similar to those identified by this group in tumors, show different hyperpermeability responses to VEGF-A^{164} with mother vessels and glomeruloid microvascular proliferations being leaky. They also showed that the amount of VEGF-A^{164} in tissue does not always correlate with the extent of measured hyperpermeability, and that increased permeability is associated with changes in vesiculovascuolar organelles and fenestrae. An intriguing finding that hyperpermeability is not seen very early on despite high VEGF-A^{164} levels is puzzling and the suggestion that this is related to downregulation of VEGF receptors needs to be demonstrated. Other interesting possibilities exist such as the presence of inhibitors that are constitutive and need to be overcome to affect permeability changes. Future investigative challenges will be to continue to use this current model to understand the complex molecular interactions that control permeability after initiation of angiogenesis, during transformations into the several distinct vascular entities, and during remodeling as the vasculature returns to normal.

There is a very valuable lesson in the Nagy *et al* study for all who study disease processes, that is, that evaluations that look at the sum total of an outcome may indeed miss important information that is masked by studying the whole and is only revealed by focusing on the components of the whole. Thus, assessment of permeability of an entire tumor tissue makes the assumption that all vessels may be affected and does not provide important information on which blood vessels are involved. Once these hyperpermeable vessels are identified, then morphological-molecular studies can be initiated with confidence and investigations on hemodynamic forces can be appropriately designed as well. As biomedical investigators pursue the molecular pathobiology of disease, it is becoming clear that the precise identification of the structure and/or the cell involved is essential. This is best reflected by the development of laser capture microscopy and its emergence as a transformative tool in the new age of molecular pathobiology.[2] This current permeability study also utilizes high-quality microscopy and imaging in combination with molecular investigations to further understand the complexities of tumor angiogenesis. These types of studies will need to be extended to more complex *in vivo* experimental tumor models to better understand

tumor angiogenesis and hyperpermeability, and especially the role of mechanotransduction of hemodynamic forces on regulating the structure and function of the new blood vessels as has been done in large vessel[3] and in microvascular endothelial cells.[2]

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References

1 Nagy JA, Feng D, Vasile E, *et al*. Permeability properties of tumor surrogate blood vessels induced by VEGF-A. Lab Invest 2006;86:767–780.
2 Bonner RF, Emmert-Buck M, Cole K, *et al*. Laser capture microdissection: molecular analysis of tissue. Science 1997;278:1481–1483.
3 Cunningham K, Gotlieb AI. The role of shear stress in atherogenesis. Lab Invest 2005;85:9–23.
4 Chatterjee S, Al-Mehdi AB, Levitan I, *et al*. Shear stress increases expression of a KATP channel in rat and bovine pulmonary vascular endothelial cells. Am J Physiol Cell Physiol 2003;285:C959–C967.

Mice on ventilators: modeling the adverse effects of mechanical ventilation

Mechanical ventilation is a common life-saving procedure that is frequently complicated by ventilator-associated pneumonia (VAP). VAP results from the colonization of the oropharynx by bacteria, most prominently *Staphylococcus aureus*, and subsequent aspiration. This puts the patients at a high risk for the development of acute lung injury, acute respiratory distress syndrome and multiple organ dysfunction syndrome (MODS), a major factor of morbidity and mortality. The mechanisms responsible for MODS pathogenesis in this context are not clear. Experimental studies have previously linked mechanical ventilation to acute lung injury, but the tidal volumes utilized (15–21 ml/kg) were significantly higher than the ones commonly used in critically ill patients (10–12 ml/kg), and the ventilation was applied only for a short period of time (3–6 h), diminishing the clinical revelance of these findings. In a landmark study published in this issue, **Dhanireddy *et al***[1] (p. 790) have successfully developed for the first time a clinically relevant model by ventilating mice for 12 h using tidal volumes of 10–12 ml/kg and exposing them to either *S. aureus* or *E. coli*. The major finding of the study was the demonstration of the synergistic effect of mechanical ventilation and bacterial infection in the development of acute lung injury. When compared to either normal respiration plus bacterial infection or mechanical ventilation without introduction of bacteria, the combination of mechanical ventilation and bacterial instillation resulted in a significantly higher loss of pulmonary alveolar integrity, along with higher levels of pulmonary neutrophil infiltration, systemic inflammatory cytokine expression. MODS also developed. Interestingly, elevations in pro-inflammatory cytokines were not associated with bacterial clearance, arguing that the inflammatory process occurred as the normal host response to the bacteria. The significance of this study is that with this model in hand, investigators now have the opportunity to tap the rich catalog of genetically altered mice to initiate extensive mechanistic studies of the role played by mechanical ventilation in acute lung injury and multiple organ failure.

Reference

1 Dhanireddy S, Altemeier WA, Matute-Bello G, *et al*. Mechanical ventilation induces inflammation, lung injury, and extra-pulmonary organ dysfunction in experimental pneumonia. Lab Invest 2006;86:790–799.

Generating liver-derived insulin-producing cells: timing the switch

Previously in this journal, Yang *et al*[1] demonstrated that hepatic stem cells could be reprogrammed *in vitro* to become pancreatic endocrine precursor cell phenotype. This was accomplished by lentivirus transduction of the rat hepatic stem-like WB cell system with pancreatic-duodenum homeobox protein-1 (Pdx1) or Pdx1-VP16, a fusion protein of Pdx1 with the activating domain of the viral VP16 transcription factor, followed by *in vitro* culture in a high glucose media. The cultured transduced cells were then capable of becoming completely functional β-like insulin-producing cells following *in vivo* transplantation into the murine renal subcapsular space; experimental diabetes could be ameliorated by this maneuver. This strategy is attractive for the goal of 'cell therapy' as a potential cure for diabetes, since it takes advantage of the 'master control gene' Pdx1 for pancreatic differentiation. Unfortunately, Pdx1 initiates phenotypic programming for both the exocrine and endocrine portions of the pancreas, so that the high-glucose *ex vivo* treatment is required for generation of a predominant endocrine β-cell-like phenotype.

In an attempt to be more specific, in the current issue, this same group has traveled further down the endocrine differentiation pathway to explore the phenotypic role of 'late' transcription factors (**Tang *et al***,[2] p. 829). Specific attention was given to the *Pax4* gene, which normally is expressed in the early pancreas, but is later restricted to β-cells. Early expression of *Pax4* in a subset of endocrine pancreas progenitors is thought to be essential for the differentiation of the beta and delta cell lineages. Moreover, inactivation of *Pax4* by homologous recombination results in the absence of mature insulin- and somatostatin-producing cells (β and δ, respectively) in the pancreas of Pax4 homozygous

mutant mice, but glucagon-producing alpha cells are present in considerably higher numbers. Thus, the hypothesis was tested whether lentivirus-mediated *Pax4* expression in *Pdx1-VP16*-expressing WB cells could promote a more completely differentiated pancreatic phenotype with β-cell properties. At face value, the experiment was a success. Activation of Pax4 in WB cells that had already been transduced with Pdx1-VP16 resulted in the expression of the late-stage pancreatic transcription factors *Pax6*, *Isl-1*, and *MafA*, and generated a gene expression profile for the doubly transduced WB cells similar to that of functional rat insulinoma INS-1 cells. The doubly transduced cells exhibited glucose-responsive insulin release *in vitro*, and caused a rapid reversal of hyperglycemia following cell transplantation into streptozotocin-induced diabetic mice. Intraperitoneal glucose tolerance tests showed a normal glucose response, and removal of the transplanted WB cells resulted in a return of hyperglycemia, confirming that they were responsible for the observed normoglycemia. Notably, the doubly transduced cells produced high-insulin content in the explanted tissue with similar intensity on insulin immunostaining to the native pancreatic islet beta cells. These studies indicate that activation of Pax4 in Pdx1-VP16-expressing WB cells reprograms these pancreatic precursor cells into glucose-responsive, more mature insulin-producing cells. There was, however, one problem. If the doubly transduced cells were left in recipient mice for an extended period, the mice became progressively hypoglycemic and ultimately died. Hence, the cell product is not fully regulated, and does not therefore qualify as a fully functional insulin-producing cell system. This study nevertheless provides further important insights into the differentiation programming of pancreatic endocrine precursor cells, both *ex vivo* and in the *in vivo* setting of diabetic hyperglycemia. Given that pancreatic endocrine cell regeneration is itself a potentially major host response to the diabetic state,[3] the molecular insights gained in the current study will likely be of substantial value for the ultimate goal of curing diabetes.

References

1 Tang DQ, Lu S, Sun YP, *et al*. Reprogramming liver-stem WB cells into functional insulin-producing cells by persistent expression of Pdx1- and Pdx1-VP16 mediated by lentiviral vectors. Lab Invest 2006;86:83–93.
2 Tang DQ, Cao LZ, Chou W, *et al*. Role of Pax4 in Pdx1-VP16-mediated liver-to-endocrine pancreas transdifferentiation. Lab Invest 2006;86: 829–841.
3 Nishio J, Gaglio JA, Turvey SE, *et al*. Islet recovery and reversal of murine type 1 diabetes in the absence of any infused spleen cell contribution. Science 2006;311:1775–1778.