INSIDE LI

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Creating accurate *in vitro* ovarian carcinoma models

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Ovarian carcinoma is the leading cause of death from gynecological cancers in Western societies. Although most ovarian carcinomas initially respond to chemotherapy, the median progression-free survival is only 18 months. Therefore, more effective therapies are sorely needed to treat these common gynecological malignancies. Drug screening has traditionally been performed in two dimensional (2D) cultures. However, most therapeutic compounds that appear to have significant activity in 2D culture models fail in clinical trials. The success rate is estimated to be 5%. Because of the high failure rate, attention has turned to 3D culture systems, which are thought to more accurately reflect tumor biology and susceptibility to chemotherapy.

With the aim of developing more accurate ovarian carcinoma models, Lee *et al* cultured 31 ovarian carcinoma cell lines as 3D cultures. By growing the cell lines on polyhydroxyethylmethacrylate (polyHEMA)-coated plates that prevented attachment and 2D growth, most of the cell lines grew as 3D aggregates. When histology was performed on these cell lines, several displayed obvious histological differentiation. One line resembled a well-differentiated serous ovarian carcinoma with

psammoma bodies; others were moderately or poorly differentiated. Two cell lines grown as xenografts were similar in appearance when grown as 3D cultures. It was also found that, in general, 3D cultures proliferated more slowly and were more resistant to chemotherapy, as has been seen in other studies. Overall, this study highlights the importance of 3D cultures in predicting the biology of ovarian cancer and supplies much-needed data about relevant models for drug screening and evaluation.

Hic-5 plays key role in glomerulosclerosis

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Glomerulosclerosis, which is characterized by the accumulation of abnormal extracellular matrix (ECM) and mesangial transdifferentiation to a myofibroblast-like phenotype, can result from numerous different insults. The condition is responsible for up to 50% of cases of chronic renal failure requiring dialysis. The insults that initiate glomerulosclerosis vary, but the associated molecular pathways are thought to be the same. Previous work has shown

that Hic-5, a protein that contains four zinc-finger LIM domains, is expressed more strongly in mesangial cells after attachment to collagen I. Collagen I is expressed in trace amounts in normal glomeruli but at higher levels in glomerulosclerosis. Transglutaminase-2 (TG-2) catalyzes the formation and deposition of ECM proteins, including collagen I. NTU281 is a pharmacological inhibitor of TG-2 that results in decreased levels of ECM, including collagen I, in experimental glomerular disease leading to glomerulosclerosis.

To understand the relationship among collagen I, Hic-5, and glomerulosclerosis, Hornigold *et al* used a rat model of diabetic nephropathy and mesangial cell cultures. They found that treatment with NTU281 decreased glomerulosclerosis, which was associated with decreases in collagen I, Hic-5, and smooth-muscle actin—all of which correlated with increased renal function as compared with control animals that did not receive NTU281. When mesangial cells attached to collagen I in cell culture, Hic-5 was upregulated along with collagen I precursors and smooth-muscle actin, leading to further accumulation of collagen I, which in turn was blocked by depletion of Hic-5 by small interfering RNA. These results demonstrate that Hic-5 is a critical element of a pathway that leads to glomerulosclerosis.

Treating ischemic heart tissue with endothelial progenitor cells

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Steady advances are being made to treat a litany of diseases with stem cell-based therapies. In particular, there has been

substantial progress in stem cell–based therapies for ischemic myocardium. Bone marrow–derived endothelial progenitor cells (EPCs) are known to home naturally to damaged myocardium and participate in repair but the process is inefficient. Strategies to modify stem cells genetically to improve their fitness to participate in myocardial repair have had some success. The serine protease tissue kallikrein (TK) processes low-molecular-weight kininogen to produce bradykinin and Lys-bradykinin (kallidin), which are potent vasoactive peptides. TK is known to enhance angiogenesis and activate cell-survival pathways and therefore has great potential to enhance the performance of EPCs.

Yao *et al* hypothesized that EPCs modified to express TK would be superior to unmodified EPCs in the treatment of acute ischemic cardiac injury. Using a nude-mouse cardiac ischemia model and human umbilical vein–derived EPCs, they demonstrated that EPCs expressing TK via an adenovirus vector were therapeutically superior to EPCs harboring vector alone. Mice treated with TK-expressing EPCs had less cardiac injury and better cardiac function. This was attributed to less EPC apoptosis due to activation of Akt as well as increased angiogenesis. EPCs were also shown to secrete factors that acted in a paracrine fashion to protect cardiomyocytes *in vitro* from oxidative damage. Thus, TK expression not only enhanced the establishment of EPCs in the myocardium but also had paracrine effects on blood vessel formation and provided protection from oxidative damage.

MI/Ad.Null-hEPC

MI/Ad.TK-hEPC

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New mutations in adrenal adenomas Primary aldosteronism, which results in drug-resistant and often life-threatening hypertension, can be caused by unilateral aldosterone-producing adenomas (APAs). To identify somatic mutations that lead to APAs, Beuschlein *et al*, as described in a recent letter in *Nature Genetics*, performed exome sequencing of APAs. They identified novel somatic mutations in *ATP1A1* and *ATP2B3*, which encode an Na^+/K^+ ATPase α subunit and a Ca^{2+} ATPase, respectively. These P-type ATPases control sodium, potassium, and calcium ion homeostasis in adrenal cells. The mutations identified in *ATP1A1* and *ATP2B3* are found in 5.2% and 1.6% of APAs, respectively. They inactivate pump function and are predicted to increase intracellular calcium concentrations, which activates calcium-dependent signaling and aldosterone output. These findings indicate that these P-type ATPases are potential therapeutic targets in hypertension caused by APAs.

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NSAIDs mobilize hematopoietic stem cells for transplant

Stem cell transplantation has become a mainstay of modern cancer therapy. In a recent letter in *Nature*, Hoggatt *et al* explain how inhibition of prostaglandin E_2 (PGE_2) signaling by nonsteroidal anti-inflammatory drugs (NSAIDs) results in increased egress of hematopoietic stem cells (HSCs) from the bone marrow to the peripheral blood. Interestingly, they demonstrate that PGE_2 signals via the E-prostanoid 4 (EP4) receptor. Further molecular dissection revealed that EP4 increases egress in hematopoietic progenitor cells (HPCs) and HSCs through different mechanisms. Whereas EP4 expression on hematopoietic cells is required for NSAID-mediated egress of HPCs, EP4 on stromal cells within the bone marrow niche is required for HSC egress. Importantly, these results highlight a strategy for increasing the efficacy of stem cell transplants using NSAIDs in addition to other compounds that mobilize HSCs for transplant.

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Hepatotoxicity induced by rifampicin and isoniazid

Rifampicin (RIF) and isoniazid (INH) are antituberculosis drugs that are effective in treating tuberculosis but often result in drug-induced liver injury. As recently reported in a Brief Communication in *Nature Medicine*, Li *et al* developed a humanized mouse model expressing the human pregnane X receptor (PXR) to prove that PXR was responsible for RIF- and INH-induced hepatotoxicity. PXR is a ligand-dependent transcription factor that regulates a transcriptional program involved in the metabolism of xenobiotics and endobiotics. It was necessary to generate a PXR-humanized (hPXR) mouse model because the ability of chemicals to activate PXR is species-dependent and RIF and INH do not induce liver damage in mice. The hPXR mouse model recapitulated the liver damage seen in humans after exposure to INH and RIF. These results suggest a mechanism for generation of the hepatotoxicity.

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Prevention and treatment of radiation-induced oral mucositis

Oral mucositis is a dreaded side effect of radiation therapy used before bone marrow transplantation as well as of craniofacial radiotherapy for cancer. Smad7 has been characterized as a signaling antagonist of the transforming growth factor- β (TGF- β) superfamily, which blocks TGF- β -induced growth inhibition and apoptosis in keratinocytes. This prompted Han *et al*, as recently described in *Nature Medicine*, to study Smad7 as both a preventive measure and treatment for a mouse model of radiation-induced oral mucositis. They found that topical administration of Smad7 protein, conjugated with a cell-permeable Tat tag, was effective in both prevention and treatment of radiation-induced oral mucositis. The mechanism of action appeared to be related more to keratinocyte migration and prevention of apoptosis than to proliferation. The finding that palifermin, another therapeutic compound, primarily promotes keratinocyte proliferation suggests that Smad7 and palifermin can work together to prevent and treat radiation-induced oral mucositis.

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