Yale SCHOOL OF MEDICINE

YALE LIVER CENTER

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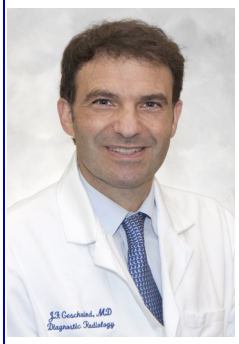
New Member Spotlight

Jeff Geschwind, MD

Professor of Radiology and Biomedical Imaging
Chairman, Department of Radiology and Biomedical Imaging



Dr. Geschwind's clinical expertise focuses on treating hepatic cancer and other malignancies. He is considered a key opinion leader in the field of liver cancer.

A native of France, Dr. Geschwind received his medical degree from Boston University School of Medicine in 1991. He performed his residency training as a research scholar (sponsored by the NIH) in diagnostic radiology at the University of California at San Francisco (UCSF) in 1996, where his research focused on cardiac magnetic resonance imaging. Dr. Geschwind completed his training in vascular and interventional radiology at Johns Hopkins and joined the faculty in 1998 as an Assistant Professor. Dr. Geschwind was recruited to the Chairmanship position in Radiology and Biomedical Imaging at Yale in April of 2015.

Dr. Mario Strazzabosco (M) sat down with Dr. Geschwind (J) to discuss why he was interested in joining the Liver Center and his views on the future of HCC:

M: *Tell us about your career*
J: I started my research career at UCSF using MR imaging to assess residual myocardial viability after an ischemic insult, and did the exact opposite after I came to Johns Hopkins when I used functional MR imaging to characterize tumor cell death (instead of viability) after loco-regional therapy for liver cancer. This research on liver cancer really launched my career in this field. The era of loco-regional treatment for liver cancer had just begun in the US and I was amazed by the fact that there was very little science to support its use. I was fascinated by the fact patients did very well after these procedures and clearly benefitted from their impact in terms of survival, but I wanted to dive more deeply into the mechanism of tumor cell death and obtain a better understanding at the tissue level. This is where functional MR imaging played a key role. Soon after generating pilot data, I received my first grant from the American Cancer society and liver cancer became the focus of my career. I obtained further funding from the NIH and then focused on developing new therapeutics for liver cancer, as well as drug delivery systems to improve our ability to maximize drug concentration within tumors and finally exploit our knowledge of imaging so that it could be use to visualize tumors, guide us during the procedures and assess the response to therapy.

M: *You are an expert in the treatment of Liver Cancer. Where do you think the field of Liver Cancer is going now that we have several treatments but not many new ones?*
J: Liver cancer is truly unique and by definition unlike any other cancer because it is really two diseases in one; the underlying liver dysfunction or cirrhosis and the cancer itself. This is why treating patients with liver cancer is so challenging and also why the traditional therapies against cancer specifically chemotherapy have mostly failed in the case of liver cancer. In addition, because patients with liver cancer usually die of liver failure or local progression but not from extrahepatic metastases, local control of the cancer within the liver is of critical importance. This is where loco-regional therapies for liver cancer play a major role. New treatments are being developed all the time that improve our ability to target the tumors better and kill them more effectively. Here, we are talking about micro- or nanospheres loaded with new drugs or radiation that can be delivered directly to the tumor using the unique blood supply of liver tumors. We are looking into combining drugs given systemically but able to target very specific pathways in cancer cells with loco-regional treatments. I truly believe this is the way the field is going. For example, we have been at the forefront of this when we started developing a new approach by delivering a drug that targets the metabolism of cancer cells locally to liver tumors. The results have been incredible and we are hoping to initiate clinical trials soon.

M: *How do you see your research blending into the Liver Center as a whole? What would you like to build here?*
J: It is very clear to me that the liver center at Yale is an incredible place. So much research is being performed here. So to be an integral part of this liver center—by the way it was one of the main reasons why I left Johns Hopkins to come to Yale- was tremendously important to me and my team. We have a huge potential in the field of liver cancer but we are not quite on the map yet and I think we absolutely must be. We have much more name recognition here at Yale than many of the other centers in the country and It is therefore very exciting to be at stage where we can create a liver cancer center of excellence. The goals is to create a center where basic and pre-clinical research can be performed, translated and used in the clinic. The incidence of liver cancer is on the rise and liver cancer is here to stay. We need to provide the best possible care to these patients with few therapeutic options. Yale should be at the forefront of that fight against liver cancer.

For a full list of Dr. Geschwind's publications please click here.

Inside this issue:

Director's Corner

The Yale Liver Center (YLC) is one of 18 Digestive Diseases Research Core Centers (DDRCC) supported by NIH/NIDDK. The YLC has been funded continuously for over 30 years and is one of only four that focus on the liver.
Full story >

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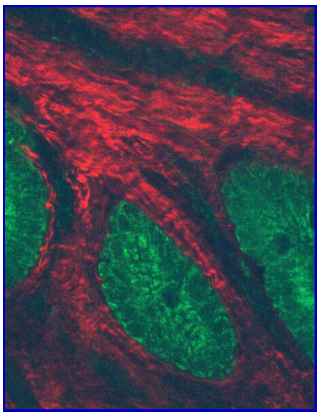
PSC PARTNERS SEEKING A CURE

Primary Sclerosing Cholangitis (PSC) is a rare, chronic cholestatic liver disease that eventually progresses to liver failure requiring liver transplantation and also greatly increases the risk of bile duct and colon cancers. The cause of this autoimmune disease is not known although interestingly 75% of cases are associated with inflammatory bowel disease. There currently is no proven medical therapy for PSC. Thus there is considerable interest in its pathogenesis and the development of novel therapeutic agents. Several promising drugs are currently being studied including 24-norursodeoxycholic acid, a homologue of ursodeoxycholic acid, which significantly reduced serum alkaline phosphatase compared to placebo in a multi-national Phase-2 study that was just reported at the European Association for the Study of Liver Disease (April 2016). The safety profile was excellent. Obeticholic acid (OCA, INT-747), a 6-ethyl derivative of chenodoxycholic acid, a naturally occurring bile acid, with 100 fold affinity for the bile acid nuclear receptor FXR that regulates the enterohepatic circulation of bile acids, is currently in a Phase-2 clinical trial in PSC patients. The FDA is anticipated to give preliminary approval within the next few months for its use in primary biliary cholangitis, another cholestatic liver disorder. Taking a different approach, drugs have been designed to inhibit the uptake of bile acids by the apical sodium dependent bile acid transporter (ASBT) on the apical membrane of the terminal ileum. Positive results from animal models of cholestasis have led to a Phase-2 trial with an ASBT inhibitor (LUM001) in PSC. Animal studies suggest that there may be a role for the gut microbiome in the metabolism of bile acids and the pathogenesis of PSC. Several studies have shown that various antibiotics including oral vancomycin, metronidazole and minocycline have improved the level of serum alkaline phosphatase, the surrogate marker for a clinically significant effect in patients with PSC. Vedolizumab and Simstuzumab, two monoclonal antibodies directed against a4/b7, a cell surface glycoprotein expressed on B and T cells and lysyl oxidase-like protein 2 (LOXL2) that cross links collagen fibers, respectively, are also being investigated in Phase-2 clinical trials in PSC. Thus there is much activity and anticipated excitement for therapeutic breakthroughs in this disease. There is also a need to develop in vitro models for high throughput screening of small molecules that might be beneficial in this disease. To this end the Yale Liver Center has equipped a new facility for the development of induced pluripotent stem cells and organoids from patients with PSC due to the generosity of an anonymous donor. We anticipate that this new development should help advance drug therapy for this orphan disease.

MORPHOLOGY CORE

Michael H. Nathanson, MD, PhD
Director

Carol Soroka, PhD — Technical Director
Al Mennone, MS — Research Director



The Morphology Core Facility provides instrumentation and technical expertise for the preparation, acquisition and analysis of images of cells and tissues at both the light and electron microscopic level. Given the cost of such instrumentation and the high level of technical expertise required to perform these investigational techniques, this Core was established to ensure the availability of these techniques for Center members. In recognition of the broad usefulness of this Core facility, the School of Medicine has partnered with the Liver Center by making ongoing, major investments to ensure that the facility remains state-of-the-art.

The Morphology Core offers the following specific activities and services, plus associated training and technical support:

1. confocal microscopy
2. epifluorescence microscopy, including quantitative and ratio imaging
3. multiphoton microscopy
4. time lapse microscopy and image processing and analysis.
5. Leica gated STED super-resolution microscope*

*The Liver Center recently purchased a new Leica Gated STED super-resolution microscope, the first of its kind at Yale and one of the first in the country. This microscope permits collection of images with spatial resolution approximately five-fold below the diffraction limit. For training and access to this microscope, please contact Al Mennone.

The Yale Liver Center is built on a tradition established by the late Gerald Klatskin, one of the country's founders of the discipline of Hepatology and a member of Yale's faculty for over 50 years.

MEMBERS RECENT PUBLICATIONS

Self-Expanding Metal Stents for Acute Refractory Esophageal Variceal Bleeding: A Systematic Review and Meta-analysis. McCarty TR, **Njei B**. Dig Endosc. 2016 PMID: 26845490

Variceal Hemorrhage in a Patient With Hepatitis C Virus Cirrhosis in Whom Liver Synthetic Function had Normalized After Viral Elimination. Sack J, **Garcia-Tsao G.** Hepatology. 2016; (5):1733-5. PMID: 26806550

Malignancies in Primary Sclerosing Cholangitis--A Continuing Threat. Bonato G, Cristoferi L, **Strazzabosco M**, Fabris L. Dig Dis. 2015; 2:140-8. PMID: 26641079

Statins are Associated with a Decreased Risk of Decompensation and Death in Veterans with Hepatitis C-related Compensated Cirrhosis. Mohanty A, Tate J, **Garcia-Tsao G**. Gastroenterology, 2015, (15)01476-6, PMID: 26484707.

Pigment Epithelium-Derived Factor (PEDF) Inhibits Wnt/β-catenin Signaling in the Liver. **Protiva P,** Gong J, Sreekumar B, Torres R, Zhang X, Belinsky GS, Cornwell M, Crawford SE, **Iwakiri Y**, **Chung C.** Cell Mol Gastroenterol Hepatol. 2015;1(5):535-549. PMID:26473164

Canalicular membrane MRP2/ABCC2 internalization is determined by Ezrin Thr567 phosphorylation in human obstructive cholestasis. Chai J, **Cai SY**, Liu X, Lian W, Chen S, Zhang L, Feng X, **Cheng Y**, He X, He Y, Chen L, Wang R, Wang H, **Boyer JL**, Chen W. J Hepatol. 2015; 63(6):1440-8. PMID: 26212029

Stimulation of nuclear receptor peroxisome proliferator-activated receptor-γ limits NF-κB-dependent inflammation in mouse cystic fibrosis biliary epithelium. Scirpo R, **Fiorotto R**, Villani A, Amenduni M, **Spirli C, Strazzabosco M.** Hepatology. 2015; 62(5):1551-62. PMID: 26199136

Na(+) /H(+) exchanger regulatory factor 1 knockout mice have an attenuated hepatic inflammatory response and are protected from cholestatic liver injury. Li M, **Mennone A**, **Soroka CJ,** Hagey LR, **Ouyang X,** Weinman EJ, **Boyer JL.** Hepatology. 2015;62(4):1227-36. PMID: 26108984

Calcium signaling and secretion in cholangiocytes. Guerra MT, **Nathanson MH**. Pancreatology. 2015;15(4 Suppl):S44-8. PMID: 26100660

Nuclear Factor, Erythroid 2-Like 2 Regulates Expression of Type 3 Inositol 1,4,5-Trisphosphate Receptor and Calcium Signaling in Cholangiocytes. Werachayaphorn J, Amaya MJ, **Spirli C**, Chansela P, **Mitchell-Richards KA**, **Ananthanarayanan M, Nathanson MH**. Gastroenterology. 2015; 149(1):211-222. PMID: 25796361

Hepatic arteriolosclerosis: a small-vessel complication of diabetes and hypertension. Balakrishnan M, **Garcia-Tsao G**, Deng Y, **Ciarleglio M**, **Jain D.** Am J Surg Pathol. 2015; 39(7):1000-9. PMID: 25786083

Liver X receptor regulates hepatic nuclear O-GlcNAc signaling and carbohydrate responsive element-binding protein activity. Bindesbøll C, Fan Q, Nørgaard RC, MacPherson L, **Ruan HB**, Wu J, Pedersen TÅ, Steffensen KR, **Yang X**, Matthews J, Mandrup S, Nebb HI, Grønning-Wang LM. J Lipid Res. 2015; 56(4):771-85.

Controlled-release mitochondrial protonophore reverses diabetes and steatohepatitis in rats. Perry RJ, Zhang D, Zhang XM, **Boyer JL**, **Shulman GI**. Science. 2015, (6227):1253-6. PMID:25721504

Fibrates and cholestasis. Ghonem NS, **Assis DN**, **Boyer JL**. Hepatology. 2015; 62(2):635-43. PMID: 25678132

The origins of hepatobiliary and gastrointestinal physiology. **Boyer JL**. Hepatology. 2015; 61(5):1452-4. PMID: 25677183

Hepatic acetyl CoA links adipose tissue inflammation to hepatic insulin resistance and type 2 diabetes. Perry RJ, Camporez JP, Kursawe R, Titchenell PM, Zhang D, Perry CJ, Jurczak MJ, Abudukadier A, Han MS, Zhang XM, **Ruan HB**, **Yang X**, **Caprio S**, Kaech SM, Sul HS, Birnbaum MJ, Davis RJ, Cline GW, **Petersen KF**, **Shulman GI**. Cell. 2015; 160(4):745-58. PMID: 25662011

TAM receptor-dependent regulation of SOCS3 and MAPKs contributes to pro-inflammatory cytokine downregulation following chronic NOD2 stimulation of human macrophages. Zheng S, Hedl M, **Abraham C**. J Immunol. 2015;194 (4):1928-37. PMID: 25567680

Immunohistochemical pitfalls in the diagnosis of hepatocellular adenomas and focal nodular hyperplasia: accurate understanding of diverse staining patterns is essential for diagnosis and risk assessment. Kakar S, Torbenson M, **Jain D**, Wu TT, Yeh M, Ferrell LD. Mod Pathol. 2015; 28(1):159-60. PMID: 25560413

Post-translational regulation of the type III inositol 1,4,5-trisphosphate receptor by miRNA-506. **Ananthanarayanan M,** Banales JM, Guerra MT, **Spirli C**, Munoz -Garrido P, **Mitchell-Richards K**, Tafur D, Saez E, **Nathanson MH**. J Biol Chem. 2015; 290(1):184-96. PMID: 25378392

LIVER CENTER ANNOUNCEMENTS

Yale Liver Center Will Host Two Key PSC (Primary Sclerosing Cholangitis) Meetings in June 2016

The PSC Partners Seeking a Cure group will bring its annual national patient and caregiver meeting to New Haven, from 6/24 - 6/26. More than 300 patients and caregivers will be in attendance. The meeting will include several educational sessions in large and small group settings, given by a multidisciplinary group of Yale providers in addition to an international group of leading PSC experts. It is considered the premier patient meeting for this disease, and represents an unparalleled opportunity for Connecticut patients and caregivers to come together with clinicians and researchers to seek support and promote new approaches to PSC management. The second gathering is the 4th workshop of the International PSC Study Group (IPSCSG), a biennial meeting of the world's leading experts in clinical and basic science research in the field of PSC. This is the first time in which this state-of-the art meeting will be held outside Europe, thus providing a unique opportunity for the Yale Liver Center to assume a leadership role in promoting greater participation and collaboration among North American centers. The meeting will take place on 6/26-6/27, and will include a joint session with the PSC Partners meeting on the morning of the 26h in which researchers, clinicians, and patients will extensively interact and exchange ideas for how to best move the field forward. For more information about these meetings, please contact Jennifer Horn.

2015-2016 New Liver Center Members

Choukri Ben Mamoun, PhD
Ying Chen, MD, PhD
Jeff Geschwind, MD
Martin Kriegel, MD, PhD
Zhaoxia Sun, PhD
Vasilis Vasiliou, PhD
Silvia Vilarinho, MD
Narendra Wajapeyee, PhD
MingDe Lin, PhD
Carla Rothlin, PhD

If you are interested in becoming a member of the Yale Liver Center, please contact Christine Abu-Hanna for an application.

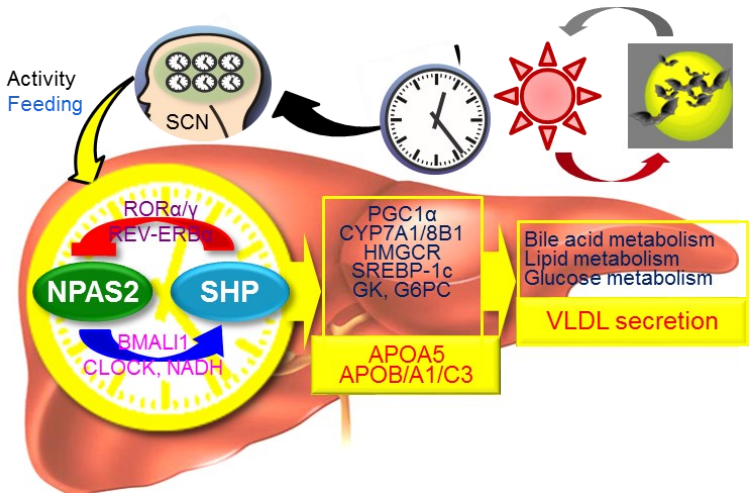
Membership Criteria

Featured Publications

Small Heterodimer Partner/Neuronal PAS Domain Protein 2 Axis Regulates the Oscillation of Liver Lipid Metabolism

Lee, SM., Zhang Y., Tsuchiya H., Smalling R., Jetten AM., **Wang, LI**. Hepatology. 2015

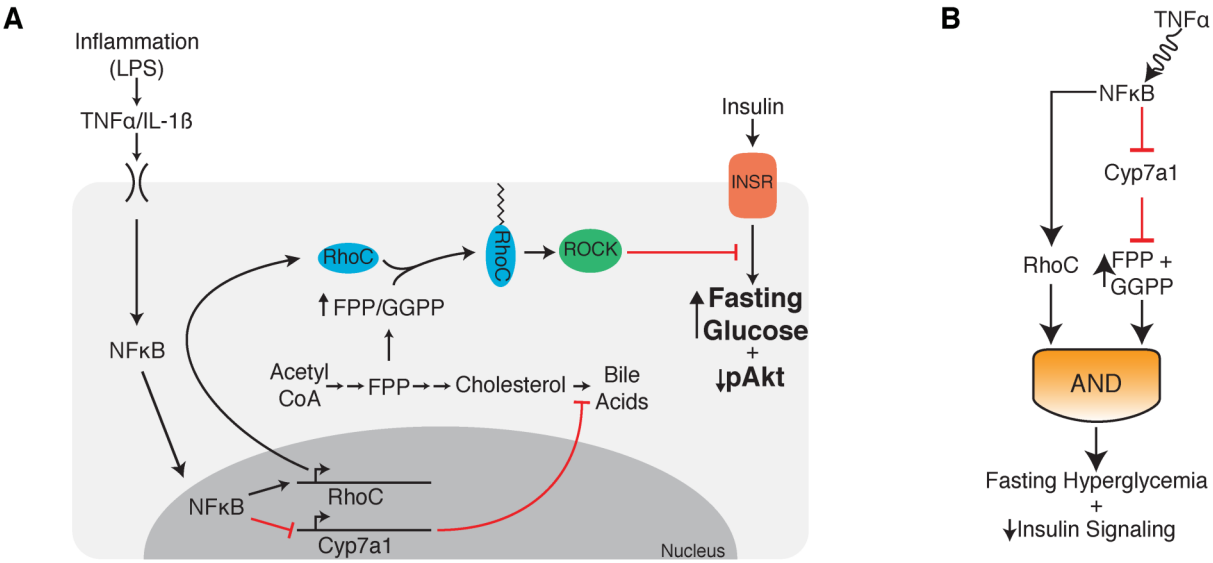
Hepatic steatosis, the accumulation of triglyceride droplets in the hepatocytes, is a common hepatic pathology seen in subjects with obesity/metabolic syndrome. Wang lab showed that the liver circadian machinery plays a key role in regulating the oscillation of liver lipid metabolism. The study revealed a feedback regulation between core clock gene neuronal PAS domain protein 2 (NPAS2) and nuclear receptor small heterodimer partner (SHP). NPAS2 binds to Shp promoter to activate it, whereas SHP inhibits Npas2 promoter activity through two mechanisms: by interacting with ROR gamma to repress ROR gamma transactivation or by interacting with REV-ERB alpha to enhance its inhibition of ROR alpha activity. Npas2-deficiency induces steatosis in Shp-/- mice by diminishing VLDL secretion.



Two-signal requirement for growth-promoting function of Yap in hepatocytes

Su, T., Bondar T., Zhou X., Zhang C., He, H., **Medzhitov, R.** eLife. 2015

Many diseases that occur as individuals age are associated with sustained inflammation, however we currently do not understand the reasons behind this association. Inflammation is an adaptive response to noxious stimuli that occurs at the expense of normal tissue function. Specifically, inflammation suppresses normal tissue function as a component of removing the inciting stress. We became interested in the role of inflammation in the regulation of systemic glucose levels, with an eye toward understanding the role inflammation plays in causing diabetes. We found that inflammation induces fasting hyperglycemia and hyperinsulinemia. Further investigation tied this to inflammatory suppression of CYP7A1, the rate limiting enzyme of bile acid biosynthesis, leading to accumulation of key intermediate metabolites in the mevalonate pathway. Accumulated metabolites result in activation of Rho-associated protein kinase (ROCK) which is important for the development of fasting hyperglycemia and hyperinsulinemia. Furthermore, treating obese mice with a ROCK inhibitor results in improved glucose control. This research has demonstrated that inflammatory suppression of tissue function can have unpredictable systemic consequences that resemble known human diseases. Our identification of the bile acid synthesis pathway as key to development of fasting hyperglycemia suggests a mechanism by which treatment of patients with bile acid sequestrants reduces hemoglobin A1c levels. Additionally, we have identified a potential new target for treating humans with diabetes with the goal of improving their glucose control. Finally, this paper provides evidence for how sustained signaling is translated into a meaningful change in homeostatic parameters.



Featured Publications (cont.)

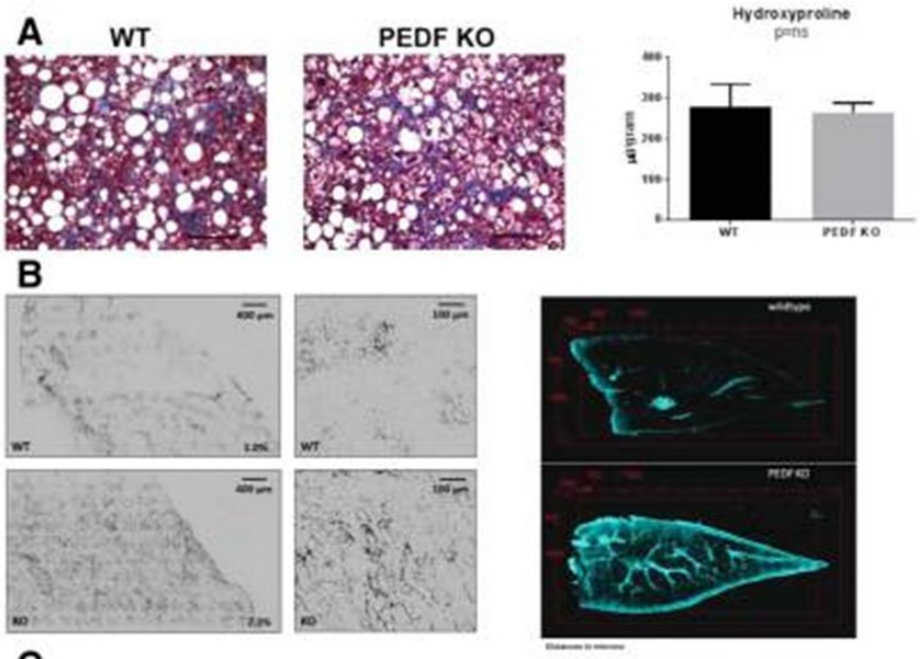
Pigment Epithelium-Derived Factor (PEDF) Inhibits Wnt/β-catenin Signaling in the Liver

Protiva, P., Gong, J., Sreekumar, B., Torres, R., Xuchen, Z., Belinsky , GS., Cornwell, M., Crawford, SE., **Iwakiri, Y., Chung, C.**

Hepatocellular carcinoma (HCC) is a major cause of cancer-related deaths worldwide. Gene expression "signatures" of the HCC and/or adjacent liver tissue was shown to correlate with clinical outcomes and extracellular matrix plays a key role in determining tumor behavior. Therefore, modulators of the extracellular matrix can activate signaling pathways that drive hepatocarcinogenesis.

Pigment epithelium-derived factor (PEDF) is a circulating 50-kDa protein with extracellular matrix binding domains and broad tumor suppressive and anti-angiogenic properties. The absence of this protein in animal models is permissive to stromal abnormalities and PEDF delivery ameliorates liver fibrosis. In humans PEDF expression declines in liver cirrhosis and HCC. The data suggest that PEDF regulates tissue matrix quiescence and its absence is permissive for malignant transformation. In the eye, PEDF inhibits the Wnt coreceptor, low-density lipoprotein receptor-related protein 6 (LRP6) hinting a possible link between a signaling pathway that drives hepatocarcinogenesis.

We aimed to examine the interplay between PEDF, fibrosis, hepatocarcinogenesis, and Wnt/β-catenin signaling. To investigate the impact of the PEDF loss in vivo we performed the whole genome expression analysis on PEDF knockout (KO) and control mice livers. We have identified that KO liver expression signatures resemble those of well established experimental mouse HCC models, and there was also a striking resemblance to gene signatures of human HCC tissues characterized by overactive Wnt/β-catenin signaling. The PEDF loss was also associated with increased fibrosis and enhanced cellular proliferation (see accompanying figure). We then interrogated components of the Wnt/β-catenin signaling pathway in PEDF KO livers before and after PEDF reconstitution. PEDF KO livers showed enhanced phospho-LRP6 levels and active β-catenin compared with controls and restoration of PEDF in KO mice resulted in decreased LRP6 phosphorylation indicating that PEDF functions as an antagonist of hepatic LRP6 activation and that exogenous PEDF can inhibit LRP6 activation in vivo. In human HCC cells a siRNA-mediated PEDF knockdown also led to increased phospho-LRP6 and active β-catenin levels extending the relevance of these findings to humans. Finally, we showed that PEDF deficiency combined with a chronic Western diet led to formation of sporadic HCC in mouse model (see accompanying figure).



(A) Six months of Western diet feeding induced liver fibrosis in wild-type (WT) and PEDF KO mice as demonstrated by trichrome staining (magnification 20×; size bars: 100 μM) and measured by hydroxyproline content. (*B*) Second harmonic generation (SHG) imaging shows increased fibrillar type I/III collagen deposition in PEDF KO mice livers (bottom panels) compared with WT (top panels) mice fed a Western diet. Magnification: left 4×; right 20×. Three-dimensional reconstruction of serial SHG images reveals prominence of fibrillar collagen around blood vessels in PEDF KO livers. (*C*) PEDF KO mice showing macroscopic tumor in mice fed the Western diet versus control diet. Bottom panel shows histology of a well-differentiated HCC arising in KO mouse fed a Western diet. L, liver; T, tumor; magnification 10×, arrow at demarcation between liver and HCC; 20×, arrows highlighting unpaired blood vessels in HCC.

We conclude that the absence of pigment epithelium-derived factor (PEDF) in mice and human hepatocellular carcinoma cells results in enhanced Wnt/β-catenin signaling. Genomic profiling of PEDF knockout livers correlates with gene expression signatures of human HCC associated with aberrant Wnt/β-catenin signaling. PEDF is an endogenous inhibitor of Wnt/β-catenin signaling.