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β-Catenin in the Liver: An Integrator of Proliferation and Metabolism?

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In this issue of GASTROENTEROLOGY, Tan et al¹ use the Cre/lox P system to conditionally inactivate β-catenin in hepatocytes, exploring the consequences on liver growth, regeneration, and gene expression. This system allowed them to generate mice that escaped the gastrulation defect that causes global β-catenin knockout animals to die during embryogenesis.²

Although the liver can increase its growth rate in response to injury or to altered physiologic demands, such as pregnancy or hyperphagia, hepatocyte proliferation is minimal in the adult. Unlike renewing epithelial cell populations of the gastrointestinal tract, which rely on stem and migrating progenitor cells to replace the daily cell bulk that sloughs into the alimentary lumen, the liver normally has a low rate of cell loss or death and a decreased requirement for cell replacement by proliferation.

The liver is unique among organs in how it responds to cell loss and how it increases its cell number to preserve or regain cell mass and function. When exposed to cell-damaging toxins, or if it cedes 70% of its mass to surgical resection, the liver quickly regenerates to its normal size³ through the proliferation of mature differentiated hepatocytes. However, when hepatocyte proliferation is blocked, other mechanisms for organ regeneration are recruited that rely on intraorgan stem cells.

The molecular regulators of cell proliferation after liver resection have been the object of intense study.³ Based on the cell culture models, much work has been done to establish roles in liver regeneration for growth factors, their receptors, and downstream signaling cascades. In the past, validation of the relevance of these pathways has involved defining the spatial and temporal expression patterns of genes and proteins belonging to these signaling cascades or administering growth factors to animals in different experimental models and monitoring growth response. Recently, however, many investigators have approached the problem of normal and regenerative growth in the liver by use of gene knockout mice.⁴ Initial studies used mouse embryonal stem cells to mutate and knock out in mice the potential expression of a single gene in all cell types. Because some genes are normally expressed by a single cell type (such as albumin in the hepatocyte), a global knockout can serve as a tissue-specific knockout in this specialized case.

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Unfortunately, global gene knockouts have several drawbacks, particularly for signaling proteins that regulate or act as transcription factors. β -Catenin, the object of the current paper by Tan et al, is an example of a protein that regulates signaling in the early stages of embryogenesis. Because its expression is ubiquitous, there is a potential for lethality during embryonic development. In fact, the deletion of β -catenin leads to mouse embryos that die at gastrulation,² eliminating the possibility of studying the effect of β -catenin deletion on developmental or regenerative liver growth.

Furthermore, when studying regenerative growth in a global gene knockout mouse that survives into adulthood, it may be difficult to determine if the knockout gene acts in the liver itself or at a distance. For example, the liver produces low levels of epidermal growth factor (EGF), which increases during regeneration.^{5,6} However, the submandibular glands and Brunner's glands in the intestine are also sources of EGF. Removal of the submandibular gland reportedly retards liver regeneration, presumably owing to the loss of this major source of EGF.⁷ A global EGF knockout mouse might show decreased regeneration, but it would not be possible to establish definitively whether the phenotype was due to the loss of EGF production by the liver, by the submandibular gland, by another EGF producing site, or by an effect of EGF knockout on another organ or cell type that indirectly influenced liver regeneration.

Finally, mice are generally used for gene knockout studies. There are over 500 inbred strains of mice that arise from the *Mus musculus* tree, complicating the analysis of gene deletion on function because of inherent genetic differences between strains.⁸ Different strains may employ different signaling mechanisms to compensate for gene inactivation in one organ during development, but not for another organ. For example, the use of gene targeting to create a global knockout at the epidermal growth factor receptor locus (EGFR) produces divergent strain-dependent phenotypes.⁹ An EGFR deficiency on a CF-1 strain background caused peri-implantation death owing to inner cell mass degeneration. On a 129/Sv background, homozygous mutants resulted in midgestation death secondary to placental defects. Finally, on a CD-1 background, the mutant mice survived for up to 3 weeks after birth, but showed an array of abnormalities in skin, kidney, brain, and gastrointestinal tract.

The Cre/loxP system allows the deletion of a gene in a specific cell type at a specific stage of development.¹⁰ The cre gene encodes a 341 amino acid, site-specific DNA recombinase, Cre protein. It can excise and recombine the intervening DNA it detects between pairs of P1 bacteriophage-derived 34-bp sequences known as loxP sites. With pairs of loxP sites inserted into critical coding sequences of mammalian genes of interest, the "floxed" alleles remain functional until the loxP sites are recognized by Cre recombinase, which is expressed under the positive control of a specific cell type promoter, such as the albumin promoter in the hepatocyte.

Tan et al¹ used the Cre-lox system in mice expressing albumin-cre to generate a floxed allele lacking exons 2-6. This inactivates β -catenin, resulting in a dramatic decrease in the total β -catenin protein by 1 month of age. Others have previously used a conditional gene inactivation strategy in similar Cre-lox β -catenin mice to study the loss of the β -catenin protein in the blood vessel, brain, or skeletal muscle.

β -Catenin belongs to the armadillo family of proteins, which form complexes with cadherin cell adhesion molecules. The latter are expressed on the surfaces of cells and contribute to intercellular adhesion. The majority of β -catenin in a cell is involved in structural functions at adherens junctions, so it is of some interest that neither Tam et al nor Sekine et al,¹¹ who recently reported on a comparable albumin-Cre/loxP model, detected significant aberrations in the cellular architecture of β -catenin-deficient hepatocytes. It appears likely that plakoglobin (γ -catenin, a homolog of β -catenin) can compensate for the loss of β -catenin.¹² Plakoglobin,

however, appears unable to substitute for the minority of cellular β -catenin that is not associated with the cytoskeleton and acts as the crucial downstream effector of the Wnt pathway where it can play key signaling roles in the positive regulation of cell proliferation.

Wnt is a secreted extracellular glycoprotein. When Wnt binds to its cell surface receptor Frizzled and coreceptor low-density lipoprotein-related protein, it inactivates the cytoplasmic APC-axin-GSK3 β -CK complex, which results in hypophosphorylation of β -catenin.³ Hypophosphorylated β -catenin maintains a monomeric structure that can enter the nucleus, where it binds to TCF/LEF transcription factors to alter gene expression.

Without an extracellular Wnt signal, several enzymes, including CK1 α or GSK3 β , phosphorylate β -catenin at specific serine and threonine residues, rendering phosphorylated β -catenin prone to ubiquitination and cytoplasmic destruction. The careful balance between the nuclear translocation and cytoplasmic destruction of β -catenin contributes to the homeostatic regulation of cell growth. Downstream of this pathway appear to be genes known to be involved in hepatocyte proliferation, including the EGF receptor, cyclin D1, and c-myc.¹³ Unregulated, β -catenin functions as an oncogene, and a large fraction of hepatocellular cancers and hepatoblastomas bear mutations in the genes that impair β -catenin degradation.¹⁴

β -Catenin has been previously reported to play an important role in the regulation of normal and regenerative liver growth. The overexpression of β -catenin in liver increases hepatocyte proliferation and the liver/body weight ratio.¹³ The studies of Sekine et al¹¹ in comparable hepatocyte-specific β -catenin knockout animals documented reduced liver size, as do Tan et al.¹ Within 5 minutes of a 70% hepatectomy, β -catenin translocates from the cell surfaces to the nuclei of hepatocytes in the remnant lobes,¹⁵ and the administration of an antisense oligonucleotide that blocks β -catenin expression impairs regeneration.¹⁶

In the present paper, Tan et al¹ use a conditional gene inactivation strategy to study the role of β -catenin in the hepatocyte. This strategy required the interbreeding of β -catenin loxP-loxP mice with albumin-cre transgenic mice to create mice that were cre-loxP-loxP “knockout” or loxP-loxP “wild-type” mice, allowing them to compare liver growth, regeneration, and gene expression in knockout and wild-type mice. Two important underlying assumptions of this paper are that the hepatocellular expression of the cre recombinase transgene by itself does not interfere with liver regeneration¹⁷ and that β -catenin deletion yields similar findings in other strains of mice with different genetic backgrounds.⁹

The investigators show that although normal development is slightly inhibited and characterized by increased apoptosis and altered global gene expression, liver regeneration is more significantly impaired. During regeneration, hepatocytes in β -catenin knockout livers show not only increased apoptosis, but also altered gene expression as revealed by microarray profiling. This included changes in the expression of genes for various cytochrome P450s, confirming a role for β -catenin in the regulation of xenobiotic metabolism.¹⁸⁻²⁰ A convincing case is made that β -catenin plays a role in the regulation of liver regeneration following surgical hepatectomy, but lesser roles in liver development and normal liver growth.

The mechanism by which β -catenin inactivation inhibits normal and regenerative growth in the liver requires further study. Based on studies in other cell types, it seems logical that β -catenin inactivation reduces cell proliferation by down-regulating proliferative signaling derived from activation of the Wnt/frizzled pathway.³ Of potential relevance are the findings of Sekine et al¹¹ that the hepatocyte-specific loss of β -catenin blocked the glutamine synthase pathway, suggesting that β -catenin serves as a link between hepatocellular proliferation and metabolic signals. Glutamine synthesis is a significant mechanism of ammonia metabolism in the liver, and although β -catenins-deficient mice did not have elevated ammonia levels,

knockout mice stressed by a high-protein diet did. β -Catenin deletion also presumably decreased the hepatic glutamine levels. Glutamine, which plays an important role in cell proliferation in many cell types, is critical in the synthesis of nucleic acids, glutathione, citrulline, arginine, and glucose, and is the major nitrogen source for the hexosamine pathway, whose products are necessary for the synthesis of glycolipids and glycoproteins.^{21,22} Glutamine has also been shown to be a significant modulator of apoptosis^{23,24} and promotes hepatic regeneration in rats.²⁵

In summary, the works of Tan et al¹ and Sekine et al¹¹ demonstrate the power of the Cre-lox system to inactivate a critical growth regulatory gene in a hepatocyte-specific fashion. However, we have come to learn that this approach often leads to more questions than it answers. Both reports make it clear that β -catenin expression is not critical to day-to-day liver function, but both reveal interesting deficits when the organism is metabolically or functionally stressed. It will be important to determine whether alterations in either ammonia or glutamine metabolism are the proximal modulators of hepatocyte proliferation in these animals, and what happens to β -catenin knockout livers when exposed to other stresses such as diabetes, steatosis, toxic injury, and carcinogens. Future studies combining Cre/lox with inducible transcriptional systems may allow investigators to regulate temporally the deletion of β -catenin and other genes in hepatocytes with even greater precision, and minimize the genetic compensatory changes that may occur with the long-term inactivation of important genes.

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