

Animal models of GI cancer

Animal models are employed to explore basic mechanisms in the progression from infection to malignancy in the gut and liver. The importance of animal husbandry and environment when designing and interpreting rodent models of infectious GI and liver cancer cannot be overstated. Especially in the case of the enterohepatic system, differences in study outcomes may be attributed to endogenous gut microflora. Awareness of the potential influence of endogenous gut microbiota in general, and *Helicobacter* spp. in particular, is indispensable when interpreting results of infectious models of gastric, lower bowel, and hepatic cancer in rodents.

Infectious stomach cancer

Tumors of the stomach, liver, and lower bowel are the second, third, and fourth leading causes of human cancer mortality, together accounting for more than two million deaths annually. ~10% of the world's total cancer burden and 20-30% of deaths are attributable to infections of the gastrointestinal (GI). Most stomach tumors in humans are associated with chronic *Helicobacter pylori* infection, which colonizes half of the world's population. Although only a small percentage of *H. pylori*-infected individuals develop tumors, the ubiquity of persistent colonization explains why stomach cancer follows only lung cancer in mortality prevalence. *Helicobacter* spp. are the only proven bacterial cause of gastric tumors in rodents. The two most commonly used rodent species to model *H. pylori*-associated gastritis and cancer are the mouse and the Mongolian gerbil. Mongolian gerbils chronically infected with *H. pylori* develop gastroduodenitis, ulcers, and antral cancer closely resembling the human disease. Mouse strains such as C57BL, which mount strong Th1 responses to Helicobacter infection. Although susceptible mice develop gastritis following *H. pylori* infection, WT strains (i.e., without genetically engineered mutations) have not been described that develop gastric adenocarcinoma. *H. pylori* has been shown to induce oxidative damage-induced gastric DNA mutations in C57BL Big Blue mice, which carry a lambda phage transgene that acts in vitro as a chromogenic mutation biomarker. Hypergastrinemic INS-GAS mice, which constitutively express humanized gastrin under an insulin promoter, acquire spontaneous gastric tumors within 2 yr and develop severe gastritis and carcinoma within months when infected with *H. felis* or *H. pylori*. Histological progression in *H. felis*-infected C57BL mice closely mimics the sequence of events in human *H. pylori* disease including gastritis, oxyntic gland atrophy, surface epithelial proliferation, metaplasia, dysplasia, and neoplastic transformation, making this natural murine model a valuable tool in the characterization of inflammation-associated gastric carcinogenesis. Indeed, the progression from acute to chronic inflammation, epithelial proliferation, dysplasia, and, ultimately, neoplastic transformation is repeated in all murine models of Helicobacter-induced infectious GI and liver cancer. Multiple *Helicobacter* spp. can induce lesions typical of MALT lymphoma in persistently colonized BALB/c mice.

Inflammatory bowel disease and lower bowel cancer

Although only a small percentage of human colorectal carcinomas (CRC) are attributable to IBD, patients with Crohn's disease or ulcerative colitis have significantly increased risk of developing CRC versus the general population. Although no specific infectious agent have been causally linked to IBD, it is generally acknowledged that intestinal bacteria initiate the cascade of events

leading to chronic enterocolitis in susceptible individuals. In the broadest sense, then, IBD-associated CRC may be acknowledged as an "infectious" cancer. Under "conventional housing conditions," genetically engineered mice with a proinflammatory phenotype develop typhlocolitis and lower bowel tumors in some facilities but remain free of disease in others. In contrast, the murine pathogen *H. hepaticus* reliably induces disease in susceptible mouse strains in virtually all non-germ-free environments. Availability of the recently published complete genome of *H. hepaticus* should facilitate studies on the role of putative bacterial virulence properties on disease induction. Genetically engineered mice on a 129/Sv strain background appear to be especially susceptible to IBD-like disease, suggesting that host genetic determinants, as well as the targeted gene disruption influence inflammation severity and tumor risk. Adoptive transfer of syngeneic CD4⁺ T lymphocytes or their subsets into immunodeficient (e.g., SCID and Rag^{-/-}) mice confirms that CD45RB^{hi} T effector (Teff) cells induce severe typhlocolitis and tumors, whereas CD25⁺ T regulatory (Treg) cells ameliorate or completely reverse the process. Adoptive transfer of CD25⁺ cells from IL-10-deficient mice fails to ameliorate typhlocolitis and tumorigenesis in these models, confirming the critical role of IL-10 for proper Treg function.

Infectious Liver Cancer

Nearly 10% of people worldwide are infected with either hepatitis B virus (HBV) or hepatitis C virus (HCV). More than 1 million people die from HBV-associated liver failure and cancer every year. About 70% of individuals exposed to HCV become chronically infected, and of those 5-10% will develop fatal cirrhosis or cancer. Rodents cannot be infected with HBV or HCV. However, studies using HBV- and HCV-transgenic mice clearly demonstrate that viral gene products can induce tumors a priori. The main drawback to mouse HBV and HCV models is that transgenes expressed during embryogenesis induce immunotolerance, and carriers fail to develop hepatitis. Investigators have applied a number of strategies to address this shortcoming including adoptive transfer of cytotoxic T lymphocytes (CTL) from antigen-primed syngeneic WT mice, intrahepatic viral DNA injection, and splenocyte adoptive transfer into transgenic SCID mice.

In the early 1990s, *H. hepaticus* was isolated from the livers of untreated male A/JCr mice with unusually high cancer and hepatitis prevalence in a 2-yr National Toxicology Program carcinogenesis study. *H. hepaticus* was the first member of the genus shown to persistently colonize the lower intestinal tract and migrate to the liver, making it the prototype enterohepatic *Helicobacter* species. To date, enterohepatic *Helicobacter* spp. are the only natural murine infectious pathogens known to induce HCC. Recently, our laboratory has described the pivotal role that *Helicobacter* spp play in the development of cholesterol gallstones in mice fed a lithogenic diet. Thus *H. hepaticus* infection of mice provides a uniquely valuable animal model for exploring basic mechanisms underlying infectious HCC and hepatobiliary cancer. Do *Helicobacter* spp. promote liver tumors in human beings? After reports of an intriguing association between *Helicobacter* spp. infection and gall bladder cancer in Chilean women, observational and case-control studies have documented significant associations between human hepatobiliary disease, including cancer, and detection of this group of bacteria. Very high rates of human HCC are reported in southeast Asia, where hepatobiliary *Helicobacter* spp. join other environmental agents with tumor-promoting potential.

In conclusion, rodent models of infectious gastrointestinal and liver cancer are critical in bridging the gap between the research laboratory and clinical applications. These models will

continue to play an instrumental role in elucidating new targets for the prevention and treatment of gastrointestinal and liver cancer in humans.

Table 1. Summary of key rodent models of infectious gastrointestinal and liver cancer

Rodent	Infectious Agent/Transgene	Tumor	Comment
C57BL mice	<i>H. felis</i>	Gastric adenocarcinoma	Natural gastric pathogen, but lacks <i>cag</i> and <i>vacA</i>
INS-GAS FVB mice*	<i>H. felis</i> and <i>H. pylori</i>	Gastric adenocarcinoma	Constitutive hypergastrinemia promotes tumorigenesis
Mongolian gerbil	<i>H. pylori</i>	Gastric adenocarcinoma	Closely mimics human disease, but long time course and few reagents
BALB/c mice	Several <i>Helicobacter</i> spp.	Gastric MALT lymphoma	Usually requires 18—24 mo
Genetically engineered mice: IL-10-, IL-2-, G _{αi2} -, Muc2-, etc.-deficient; especially on 129Sv background	"Endogenous microbiota" or <i>H. hepaticus</i>	Lower bowel carcinoma	Bacteria in endogenous microbiota models not well defined; <i>H. hepaticus</i> reliably induces disease
Lymphocyte-deficient mice: SCID or Rag ^{-/-} ; especially on 129Sv background	"Endogenous microbiota" or <i>H. hepaticus</i>	Lower bowel carcinoma	Often used for adoptive transfer studies; <i>H. hepaticus</i> induces tumors in untreated Rag2 ^{-/-} mice
Transgenic mice*	HBV or HCV transgene(s)	Hepatocellular carcinoma	Prove tumorigenic potential of viral gene products; adoptive transfer or inducible gene strategies required for hepatitis
A/JCr and other mice*	<i>H. hepaticus</i>	Hepatocellular carcinoma	Natural murine pathogen induces chronic active hepatitis and HCC

MALT, mucosal-associated lymphoid tissue; PAI, pathogenicity-associated islands; HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma. * Male predominant. (Rogers, A. B., and J. G. Fox. 2004. Inflammation and cancer - I. Rodent models of infectious gastrointestinal and liver cancer. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **286**:G361-G366.)