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## Inflammation and Cancer

### I. Rodent models of infectious gastrointestinal and liver cancer

**Arlin B. Rogers and James G. Fox**

*Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139*

**Rogers, Arlin B., and James G. Fox.** Inflammation and Cancer. I. Rodent models of infectious gastrointestinal and liver cancer. *Am J Physiol Gastrointest Liver Physiol* 286: G361–G366, 2004; 10.1152/ajpgi.00499.2003.—Chronic gastrointestinal and liver infections account for a significant percentage of human cancer deaths. Rodent models help elucidate how infection can lead to malignancy. *Helicobacter pylori*, the leading cause of human gastric tumors, produces similar disease in Mongolian gerbils. *H. pylori*, *H. felis*, and *H. hepaticus* induce stomach, lower bowel, or liver tumors in susceptible wild-type and genetically engineered mice. Immune dysregulated mice recapitulate features of inflammatory bowel disease including colon carcinoma. Hepatitis B and C virus transgenic mice provide insights into viral hepatitis and hepatocellular carcinoma. Rodent models enhance our understanding of infectious cancer pathogenesis and suggest novel targets for intervention.

digestive system neoplasms; *Helicobacter*; viral hepatitis

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 (18). Most tumors of the stomach and liver are associated with infectious agents. Inflammatory bowel disease (IBD), which predisposes to colorectal carcinoma, likely results from aberrant host responses to commensal or pathogenic bacteria. All told, ~10% of the world's total cancer burden and 20–30% of deaths are attributable to infections of the gastrointestinal (GI) and hepatic systems (18).

Despite knowledge that viral, bacterial, and metazoal infections are underlying causes of cancers of the enterohepatic system, there is limited understanding of the specific host-pathogen interactions leading to chronic inflammation and tumors in humans. Animal models are employed to explore basic mechanisms in the progression from infection to malignancy in the gut and liver. Although valuable models of infectious GI and liver tumorigenesis include ferrets, nonhuman primates, gnotobiotic pigs, woodchucks, and other species, most investigators prefer to use rodents due to relatively low costs, quick breeding times, detailed characterization, range of commercial reagents, and availability of genetically engineered animals. Some commonly used rodent models of infectious GI and liver cancer are summarized in Table 1.

#### RODENT MODEL CONSIDERATIONS

No meaningful conclusions can be drawn from studies in rodents without knowledge of how the species, strain, and gender of the animal may affect experimental outcomes. Results may be highly divergent when the same protocol is applied to different mouse populations, and care must be taken

when extrapolating findings from a single inbred mouse to human disease. Gender greatly influences disease risk in both humans and rodents exposed to tumorigenic infectious agents, with males being much more susceptible to gastric and hepatic cancers. For this reason, rodent studies of infectious GI or liver cancer that do not include males likely miss significant outcomes. Information about rodent inbred strains can be found in texts of laboratory animal medicine and from most commercial suppliers. For pragmatic reasons, genetic manipulations in mice are routinely performed with strain 129 or FVB embryos, and offspring are backcrossed for 10 or more generations onto the desired genetic background, most often C57BL. Because mouse strain knowledge is indispensable to the interpretation of published studies, inclusion of parental or wild-type (WT) strain in all abstracts describing rodent models of infectious GI and liver cancer would be a welcome step forward.

The importance of animal husbandry and environment when designing and interpreting rodent models of infectious GI and liver cancer cannot be overstated. Unfortunately, little attention is often given to these critical issues, and still less is reported. Significant confusion, and sometimes outright confrontation, emerges when investigators performing similar experiments in different settings report highly divergent outcomes. Animal stress associated with overcrowding, inadequate sanitation, and variations in temperature, humidity, and light cycles may predispose otherwise resistant animals to adverse disease outcomes. Especially in the case of the enterohepatic system, differences in study outcomes may be attributed to endogenous gut microflora. Microbial population differences may be pronounced even between different rooms and cages within the same facility. 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.

Rodents and humans share many anatomic and physiological features of the enterohepatic system, but a few comparative features are worthy of mention. The proximal third of the rodent stomach, including the fundic pouch, is lined by squamous rather than glandular epithelium. Oxyntic glands, comprised of chief and parietal cells, are limited to the gastric corpus, although some authors use the phrase “fundic glands” as a generic term for oxyntic glands in rodents. The cardiac portion of the stomach (at the esophageogastric junction in humans) is only a few glands in length in rodents, and some authors (21) refer to this transitional zone as the squamocolumnar or forestomach/zymogenic junction. Gastric *Helicobacter* spp. in mice colonize glands in the border zone between oxyntic and nonoxyntic regions; thus organisms following

Address for reprint requests and other correspondence: A. B. Rogers, Comparative Pathology Laboratory, Division of Comparative Medicine, Massachusetts Institute of Technology, 77 Massachusetts Ave., Bldg. 16-849, Cambridge, MA 02139 (E-mail: abr@mit.edu).

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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-, $\alpha_{12}$ -, 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 <sup>-/-</sup> ; 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 <sup>-/-</sup> 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.

initial infection are most readily demonstrated in the cardia and in the proximal antrum (21). Interestingly, in mice, severe proliferative and dysplastic lesions usually arise in the cardia and progress aborally in conjunction with oxyntic gland atrophy, although bacteria can be identified in antral glands throughout the course of infection (8, 10). The unfortunate term “atrophic gastritis,” referring to the loss of chief and parietal cells in a setting of inflammation, is ensconced in the medical lexicon and has been transferred to rodent models, even though subjects with atrophic gastritis often have a markedly thickened mucosa due to reactive epithelial proliferation. When compared with humans, rodents have a much larger cecum to facilitate bacterial digestion of their herbivorous diet. The anatomy of the rodent liver parallels that of humans except that rats lack a gallbladder. However, there are key functional differences between species including high susceptibility to tumors induced by peroxisome proliferators in rodents. Species differences do not invalidate animal models of infectious GI and liver cancer, but comparative pathophysiology should be understood before extrapolating findings in rodents to equivalent human diseases.

### INFECTIOUS STOMACH CANCER

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. In addition to adenocarcinoma, chronic *H. pylori* infection induces tumorlike proliferations of mucosal-associated lymphoid tissue (MALT) lymphomas, which may regress with bacterial eradication. As with humans, *Helicobacter* spp. are the only proven bacterial cause of gastric tumors in rodents. Mouse strains such as C57BL, which mount strong T<sub>h</sub>1 responses to *Helicobacter* infection, demonstrate lower colonization levels but increased gastritis and hyperplasia/dysplasia versus BALB/c and other strains with T<sub>h</sub>2-predominant responses that maintain higher bacterial burdens but suffer less epithelial damage.

The murine pathogen *H. felis* induces severe gastritis that can lead to adenocarcinoma in C57BL mice (9). In mice with

hemizygous deletion of p53, a paradoxical protective effect is observed against the progression of *H. felis*-induced gastric tumorigenesis, attributable in part to depressed T<sub>h</sub>1 immune responses (9). As with humans, chemotherapeutic immune suppression decreases *Helicobacter*-induced gastric lymphoepithelial lesions in mice. Coinfection of a T<sub>h</sub>2-provoking helminth parasite with *H. felis* ameliorates gastric disease in C57BL mice and has been offered as a potential explanation for the “African enigma,” wherein gastric adenocarcinoma is underrepresented in countries with concurrently high *H. pylori* and gastrointestinal parasite prevalence (4). The main differences between *H. felis* in mice and *H. pylori* in humans are that neutrophils are a less prominent feature of the murine gastritis and that *H. felis* lacks key pathogenicity-associated islands (PAI) found in *H. pylori* including *cag*. Nevertheless, 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 (Fig. 1).

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 (17). Mice have been successfully infected with several strains of *H. pylori*, including the mouse-adapted Sydney strain-1 (SS1), human isolate B128, and others (10). 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, probably a reflection of poor host adaptation. Nevertheless, *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 (23). Hypergastrinemic INS-GAS mice, which constitutively ex-

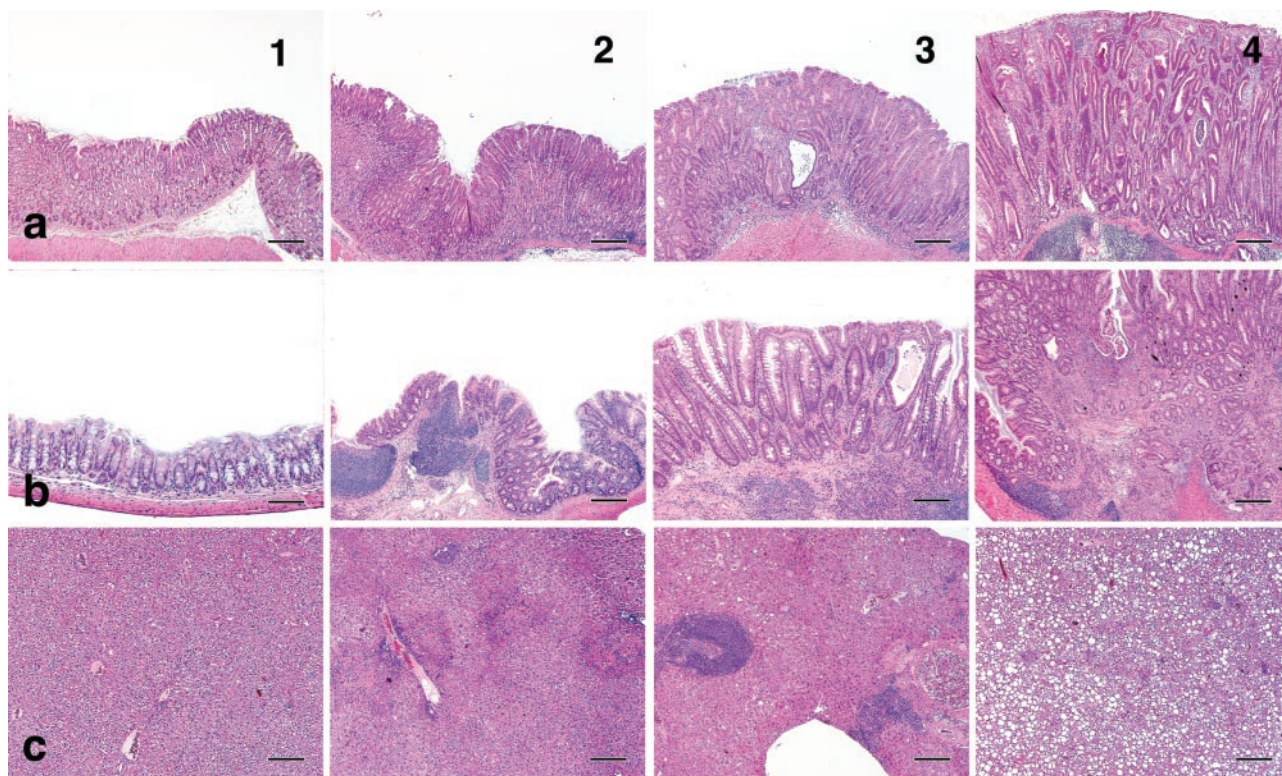


Fig. 1. Sequential histopathology of *Helicobacter*-induced infectious murine gastrointestinal and liver cancers: *H. felis* in C57BL stomach (a), *H. hepaticus* in IL-10<sup>-/-</sup> lower bowel (b), and *H. hepaticus* in A/JCr × C57BL F1 liver (c). Left to right each row: normal tissue (1), acute inflammation (2), chronic inflammation with hyperplasia and dysplasia (3), and carcinoma (4). Hematoxylin and eosin; bar = ~300  $\mu$ m.

press 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* (10, 24). As is the case with humans, males are at increased risk (8). Rodent models provide opportunities for in vivo analysis of putative bacterial virulence factors. For example, deletion of *H. pylori cagE* (*picB*) dampens immune responses and retards but does not prevent tumorigenesis in INS-GAS mice (10).

Multiple *Helicobacter* spp. can induce lesions typical of MALT lymphoma in persistently colonized BALB/c mice. Microarray analysis of laser capture microdissected gastric lesions from BALB/c mice chronically infected with *H. heilmannii* demonstrates transcriptional alteration of many genes associated with immune maturation and mucosal epithelial responses, and the gene calgranulin A/Mrp-8 appears strongly associated with development of MALT lymphoma (15).

#### 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 has yet 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. As our knowl-

edge of the pathogenesis of these human diseases increases, it seems plausible that some bacteria will be identified as inducers of disease in susceptible individuals, whereas other microbes may exert a protective effect.

Although no animal model faithfully recapitulates all features of IBD and its associated cancers, rodent systems that reproduce important hallmarks of the human disease are used to investigate basic pathogenetic components of Crohn's disease and ulcerative colitis. Studies of the roles of endogenous microflora in IBD-like disease in mice are complicated by the fact that results are dependent on the setting. 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 PAI on disease induction (20). Other bacteria of rodents, including *Citrobacter rodentium* and *Lawsonia intracellularis*, induce proliferative enterocolitis and can act as tumor promoters when combined with chemical mutagens or predisposing genetic mutations such as APC<sup>min</sup> (16).

With the exception of known tumor-associated genes such as APC<sup>min</sup> and Cdx2, microbial-induced inflammation appears to be a prerequisite for development of lower bowel tumors in most genetically engineered mice (GEM) with targeted mutation of Smad3, IL-2, IL-10, G $\alpha_{i2}$ , Muc2, and other genes

involved in immune signaling (1). GEM on a 129/Sv strain background appear to be especially susceptible to IBD-like disease, suggesting that host genetic determinants besides the targeted gene disruption influence inflammation severity and tumor risk. Interestingly, GEM on a C57BL background are sometimes resistant to IBD-like tumorigenesis compared with equivalent “knockout mice” on other genetic backgrounds. Although NF- $\kappa$ B is traditionally considered a proinflammatory signaling molecule, mice with targeted disruption of p50/p63 heterodimers develop more severe cecal and colonic inflammation (typhlocolitis) than corresponding NF- $\kappa$ B-viable mice, highlighting the complex regulatory functions of this key signal-transduction molecule. Results from a study using p50<sup>-/-</sup>p63<sup>+/-</sup> (“3X”) Rag2<sup>-/-</sup> mice demonstrate that NF- $\kappa$ B exerts influence on innate immune responses in part through modulation of IL-12 p40 expression (22).

TGF- $\beta$  signaling pathways lie at the nexus of inflammation and neoplastic transformation in ulcerative colitis-associated colorectal carcinoma (1). TGF- $\beta$ 1-deficient mice die in utero in part due to uncontrolled lymphocyte proliferation. TGF- $\beta$ 1-deficient GEM rescued from embryonic lethality by coexpression of a lymphocyte-deficient phenotype, including SCID and Rag2<sup>-/-</sup> mice, develop typhlocolitis and tumors under “conventional” but not germ-free housing conditions (1). *H. hepaticus* infection has been identified as a likely causative factor for tumor development in TGF- $\beta$ 1-deficient mice (1). However, TGF- $\beta$ 1 deficiency is not required for tumorigenesis, because 129/SvEv Rag2<sup>-/-</sup> mice with normal TGF- $\beta$  expression develop severe typhlocolitis and tumors as early as 2–3 mo after inoculation with *H. hepaticus* (2).

Adoptive transfer of syngeneic CD4<sup>+</sup> T lymphocytes or their subsets into immunodeficient (e.g., SCID and Rag<sup>-/-</sup>) mice confirms that CD45RB<sup>hi</sup> T effector (T<sub>eff</sub>) cells induce severe typhlocolitis and tumors, whereas CD25<sup>+</sup> T regulatory (T<sub>reg</sub>) cells ameliorate or completely reverse the process (2). CD25, the  $\alpha$ -subunit of the IL-2 receptor, is not exclusively expressed by T<sub>reg</sub> cells, but selection for this marker results in T<sub>reg</sub> enrichment. Absence of CD25 ligand helps explain why IL-2-deficient mice also exhibit a proinflammatory phenotype in the lower bowel. Adoptive transfer of CD25<sup>+</sup> cells from IL-10-deficient mice fails to ameliorate typhlocolitis and tumorigenesis in these models, confirming the critical role of IL-10 for proper T<sub>reg</sub> function (3).

### INFECTIOUS LIVER CANCER

Nearly 10% of people worldwide are infected with either hepatitis B virus (HBV) or hepatitis C virus (HCV). The estimated prevalence of HCV infection is 170 million individuals, or 3% of the global population, whereas the number of carriers of HBV exceeds 350 million or 6% of all people (14). More than 1 million people die from HBV-associated liver failure and cancer every year (14). About 70% of individuals exposed to HCV become chronically infected, and of those 5–10% will develop fatal cirrhosis or cancer (14). Because most HCV carriers are unaware of their infection status, the true prevalence of HCV-associated disease may exceed current estimates. Chronic viral hepatitis of either type B or C greatly increases the risk of hepatocellular carcinoma (HCC). Indeed, the vast majority of HCC diagnoses worldwide are made in people seropositive for HBV, HCV, or both. As with other

causes of HCC, males are at 3–10 times greater risk for cancer than females. Importantly, this male-predominant tumor risk is recapitulated in rodents, making these animal models a valuable resource to investigate the role of sex hormones in hepatocellular carcinogenesis.

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 (12). Although results have been variable, several mouse models transgenic for one or more HBV or HCV genes develop HCC, usually after 1 yr (reviewed in Refs. 12 and 19). Some HCV transgenic mice also develop the steatosis characteristic of human hepatitis C (12). Viral transgenes are often placed under the control of the albumin promoter to limit expression to the liver or under the broadly transactivating HBV<sub>X</sub> promoter in the case of some HCV models (12). Although HBV<sub>X</sub> is a known tumor promoter and tumorigenic incompetence of avian hepadnaviruses is attributed to the absence of a corresponding X gene, mice that produce only surface and large envelope proteins in high quantity also develop HCC (19). As is the case in humans, transgenic mouse models of HBV and HCV demonstrate higher quantities of virus production and greater risk of HCC in males as well as accelerated tumor development when exposed to mutagenic chemicals.

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 (19). These strategies have recapitulated hepatocellular damage accrued during acute or fulminant hepatitis, highlighted the importance of interferon-mediated hepatocyte-sparing virus clearance orchestrated by CTL, and produced a low-grade chronic inflammatory state, which, in some instances, has contributed to tumorigenesis (19). A second strategy to overcome immune tolerance in transgenic mice is placement of the viral transgenes under an inducible promoter such as the *Cre/lox* system in which *lox P* inhibits transgene expression until *Cre* recombinase is separately administered; however, mice thus far generated using this strategy have only developed mild hepatitis.

To date, the only proven nonviral infectious causes of human liver tumors are biliary trematodes, including *Opisthorchis viverrini* and *Clonorchis sinensis*, which increase the risk of cholangiocarcinoma (11). Although rats harboring *Cysticercus fasciolaris* (the larval form of the tapeworm *Taenia taeniaeformis*) are susceptible to hepatic tumors, these sarcomas are presumed to arise from chronic granulomatous inflammation directed against the parasite capsule, likely representing a disease process significantly different from human fluke-associated biliary carcinomas. A better model is infection of Syrian hamsters with *O. viverrini*. Infected hamsters develop cholangitis, biliary dysplasia, and oxidative DNA damage; however, an additional mutagen such as dimethylnitrosamine is required for cholangiocarcinoma development (11). Hepatic schistosomiasis appears to increase the risk of cholangiocarcinoma in humans, although the link is less clear than for the

flukes described above. Mice and/or hamsters infected with *Schistosoma japonicum* exhibit oxidative cholangiohepatic DNA damage but require additional mutagens for tumor development (11).

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 (25). *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 (6). Such organisms are now known to commonly infect humans and a wide array of animals (13). An association has been proven between *H. hepaticus* infection and hepatocellular tumors in susceptible strains of mice (7). To date, enterohepatic *Helicobacter* spp. are the only natural murine infectious pathogens known to induce HCC. Thus *H. hepaticus* infection of mice provides a uniquely valuable animal model for exploring basic mechanisms underlying infectious liver cancer. A/JCr mice are classically used for *H. hepaticus* studies, but other strains are also susceptible to hepatitis  $\pm$  HCC including BALB/cCr, SJL/NCr, SCID/NCr, C<sub>3</sub>H/HeNCr, B<sub>6</sub>C<sub>3</sub>F<sub>1</sub>, and AxB recombinant inbred strains. Studies in this system demonstrate intrahepatic tertiary lymphoid tissue, upregulated expression of oncogenes, DNA adducts, and a male-predominant tumor susceptibility, as is the case for HCC in humans.

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 (5, 13). Recent evidence implicates hepatobiliary *Helicobacter* spp. in severe HCV infection outcomes, including HCC (13). Very high rates of human HCC are reported in southeast Asia, where hepatobiliary *Helicobacter* spp. join other environmental agents with tumor-promoting potential (13). *H. pylori* DNA has been found within the livers of patients with HCC; however, culture results have been negative, and a causative association remains unproven. Clearly, more work is needed to determine the potential role of *Helicobacter* spp. in human hepatobiliary carcinogenesis.

In conclusion, following the discovery by Peyton Rous over 100 years ago that a virus could induce tumors in chickens, the list of identified infectious agents associated with human and animal cancers has continued to grow. The gastrointestinal and hepatic systems are especially vulnerable to neoplastic transformation following infection by viruses, bacteria, and helminths. Because of the difficulty in studying specific and chronological disease events in human beings, animal models are used to characterize infectious cancer pathogenesis at the molecular, cellular, tissue, and organismal level. Rodent models of infectious gastrointestinal and liver cancer help bridge the gap between bench science and clinical applications and will continue to play an instrumental role in elucidating new targets for the prevention and treatment of gastrointestinal and liver cancer in humans.

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