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Myc, Max, and Mnt: Molecular Mechanisms of Enhancement of Cholangiocarcinogenesis by Cholestasis

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Recent studies have revealed a strong effect of cholestasis on the development of cholangiocarcinoma, and the currently preferred model of cholangiocarcinoma progression uses bile duct ligation to enhance growth and metastasis of these tumors.¹ However, the specific mechanisms by which cholestasis contributes to development of cholangiocarcinoma have not been completely elucidated. In this issue of *Gastroenterology*, Yang et al² report on the influence of cholestasis induced by left and median bile duct ligation (LMBDL) on development of diethylnitrosamine (DEN)-induced biliary preneoplastic lesions and cholangiocarcinomas in a mouse model. In accompanying functional studies, they elucidate several mechanistic events accompanying the induction of cholestasis that contribute to cholangiocarcinogenesis.

c-Myc is a member of the Myc family of helix loop helix proteins that heterodimerizes with Max to activate target gene transcription by mediating DNA binding.^{3–5} Mnt is a c-Myc antagonist that competes with c-Myc for binding to Max. The Mnt–Max complex represses transcription of target genes. Mnt seems to inhibit cell-cycle entry, because the lack of Mnt in cells accelerates the G0 to S-phase transition.⁶ Mnt levels do not fluctuate during the G0 to S-phase transition; however, strong induction of c-Myc leads to a transient decrease in Mnt–Max complexes and a change in the ratio of Mnt–Max to c-Myc–Max complexes on shared target genes in primary mouse embryo fibroblasts (MEFs), triggering cell-cycle entry.^{6,7} Correspondingly, Mnt overexpression suppresses cell-cycle entry and cell proliferation, suggesting that the relative ratio of c-Myc–Max to Mnt–Max is critical for cell-cycle entry. These results demonstrate that Mnt–Myc antagonism plays a fundamental role in regulating cell-cycle entry and proliferation. A previous study by Yang et al⁸ showed that the nuclear binding activity of Myc to the E-box element of cyclin D1 increased in mouse liver after bile duct ligation, whereas that of Mnt decreased in a time-dependent fashion. In the current report, Yang et al have assessed the contribution of the switch from Mnt to Myc to the increase in cyclin D1 during cholangiocarcinogenesis induced by the

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See “A mouse model of cholestasis-associated cholangiocarcinoma and transcription factors involved in progression,” by Yang H, Li TWH, Peng J, et al, on page 378.

Conflicts of interest

The author discloses no conflicts.

combination of 2 weekly intraperitoneal injections of the liver carcinogen DEN at a dose of 100 mg/kg body weight, followed 2 weeks later by LMBDL to induce cholestasis, and then by weekly oral gavage with 25 mg/kg DEN in corn oil, designating the entire procedure as DLD. LMBDL-induced cholestasis was relatively well tolerated by mice, but significantly accelerated DEN-induced biliary neoplasia, resulting in development of cholangiocarcinomas with associated stromal fibrosis at 28 weeks after surgery. LMBDL induced a marked increase in c-Myc expression in the first 8 weeks after surgery, whereas IP and oral treatment with DEN (DD) without bile duct ligation resulted in increased c-Myc at later time points between 12 and 28 weeks. When combined into the DLD treatment, c-Myc expression was induced early and persisted to 28 weeks.

To investigate the regulation of c-Myc and Mnt expression during cholestasis-associated cholangiocarcinogenesis, the investigators examined the effects of LMBDL and DD on known microRNA regulators of c-Myc expression, miR-34a (which suppresses c-Myc expression) and the Lin-28B/let-7 axis (in which the RNA binding protein Lin-28B is a post-transcriptional repressor of the miRNA let-7, which in turn represses c-Myc expression; hence increased Lin-28 leads to de-repression of c-Myc expression).^{9–11} They found that LMBDL alone led to decreased expression of miR-34a from weeks 1 to 8; DD led to decreased expression of miR-34a from weeks 12 to 28; and combined cholestasis and DEN treatment (DLD) led to persistent down-regulation of miR-34a from weeks 1 to 28. Similarly, LMBDL alone led to increased expression of Lin-28B from weeks 8 to 12; DD led to increased expression from weeks 12 to 28; and combined DLD led to increased expression of Lin-28B from weeks 8 to 28. Thus, both miR-34a and the Lin-28B/let-7 axis may contribute to the regulation of c-Myc during combined cholestasis and carcinogen-induced cholangiocarcinogenesis, but the early effect seems to be driven by miR-34a rather than the Lin-28B/let-7 axis. Furthermore, the authors investigated the potential role of regulation of Mnt in cholestasis-enhanced biliary carcinogenesis. Mnt is a known target of miR-210, which in turn is induced by the hypoxia-induced factors HIF-1 α and HIF-2 α , the increased expression of which is characteristic of the fibrotic liver stroma.¹² Similar to their effects on miR-34a and Lin-28B, LMBDL and DD cooperated to induce both early and late expression of HIF-1 α and HIF-2 α . miR-210 expression was also increased by combined DLD, but with a time course that correlated better with HIF-2 α than with HIF-1 α expression.

Next, the investigators determined whether the observed changes in miRNAs regulating c-Myc and Mnt were reflected in changes in c-Myc and Mnt expression and the downstream cell-cycle regulator cyclin D1. Over time after DLD, both electrophoretic mobility shift assays and chromatin immunoprecipitation assays showed a shift from Mnt to c-Myc binding activity to the E-box element of the cyclin D gene promoter. Finally, to determine the effect of the switch from Mnt to Myc on the expression of cyclin D1 and cholestasis-enhanced cholangiocarcinoma progression, the investigators transduced mice with lentiviruses expressing shRNA targeting Mnt or c-Myc using hydrodynamic injection through the tail vein. They found that shRNA knockdown of Mnt in DLD mice increased cholangiofibrosis, cholangioma, and proliferation of the ductular cells compared with mice transduced with lentiviruses expressing scrambled shRNA. By contrast, the result in mice

expressing shRNA targeting c-Myc was opposite to that in mice expressing shRNA targeting Mnt. Knockdown of c-Myc decreased the levels of c-Myc, increased Mnt, and decreased cyclin D1 expression. These results suggest that the switching between c-Myc and Mnt plays an important role in the regulation of cyclin D1 and progression of CCA.

In summary, Yang et al² have performed elegant proof-of-principle experiments in a combined LMBDL and DEN mouse model to investigate the mechanisms of cholestasis-accelerated CCA progression (Figure 1). Their work shows that a switch in binding activity from Mnt to c-Myc in the E-box element of cyclin D1 induces tumor progression of cholangiocarcinoma in mice. These findings enhance our understanding of the process by which cholestasis contributes to carcinogenesis and identify potential targets for prevention of cancer in patients with cholestatic liver diseases as well as for treatment of progressive and metastatic biliary cancer. The findings also potentially underline the importance of achieving adequate biliary drainage when feasible in patients with cholangiocarcinoma and associated biliary obstruction, as this may be important not simply for reducing cholestasis-induced injury to hepatocytes but also for abrogating pro-carcinogenic signaling pathways. It will be exciting to see whether future developments based on rational targeting of the molecules and pathways elucidated in this study will lead to improvements in the treatment of patients with cholangiocarcinoma.

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Figure 1.

A scheme for the mechanism of the effect of combined cholestasis and DEN in promoting cholangiocarcinogenesis. In this model, cholestasis is induced by an LMBDL. Neoplasia is induced by DEN treatment. Cholestasis decreases the expression of miR-34a resulting in the expression of c-Myc. Increased Lin-28 expression with consequent down-regulation of the c-Myc suppressor let-7a miRNA appears to play a lesser role in activation of c-Myc expression. Liver injury induced by partial bile duct ligation increases the expression of miR-210 via HIF-2 α resulting in decreased Mnt expression. The combination of increased c-Myc and down-regulation of Mnt shifts the balance toward c-Myc–Max activation of cyclin D1 expression, activating the cell cycle and leading to progression of cholangiocarcinogenesis.