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INFLAMMATION

The gut takes a toll on liver cancer

> activation of PAMP–TLR4 signalling in chronically damaged livers confers survival to pre-malignant cells

Approximately 80% of hepatocellular carcinomas (HCCs) are preceded by chronic liver disease, hepatic fibrosis and cirrhosis. Chronic liver disease is also accompanied by the translocation of intestinal bacteria and pathogen-associated molecular patterns (PAMPs) —molecular motifs only found in microbes, including intestinal bacteria — which induce inflammatory immune responses. So, it is clear that the microenvironment is important for the development of HCC, but the mechanisms and the involvement of PAMPs are unclear.

Dapito, Mencin, Gwak, Pradere and colleagues focused their attention on the contribution of intestinal bacteria and PAMPs to hepatocarcinogenesis. PAMPs are recognized by Toll-like receptors (TLRs), so the authors investigated whether TLRs contribute to HCC initiation. They treated *Tlr4*-mutant mice (which have an inactivating mutation in *Tlr4*) with the genotoxin DEN and the hepatotoxin CCl_4 to recapitulate liver injury and inflammation. Tumours developed with HCC characteristics, but the number and size of tumours that developed in the *Tlr4*-mutant mice was significantly reduced compared with controls. Next, the authors investigated HCC induction in gut-sterile mice (which do not have intestinal bacteria) and again found that tumours still formed, but that the number and size of tumours was significantly reduced. The gene expression profile of these tumours was similar to that observed in the tumours from *Tlr4*-mutant mice, indicating that gut sterilization and TLR4 inactivation have similar effects on suppressing HCC progression.

Furthermore, *Tlr4*-mutant or gut-sterilized mice that were induced to undergo HCC formation also had reduced levels of hepatocyte growth factor and epiregulin (EREG). Focusing on EREG, they found that secretion of this mitogen was increased in fibrotic livers, and that $Ereg^{-/-}$ mice that were treated with the DEN–CCl_4 regimen developed fewer and smaller tumours, although this decrease was to a lesser extent than in *Tlr4*-mutant mice, indicating that additional TLR4-regulated pathways are involved.

The authors then sought to identify the time at which PAMPs and TLR4 exert their effects on hepatocarcinogenesis. They found that gut sterilization restricted to the last 4 months of the HCC induction protocol, when tumours were not visible, significantly reduced the number and size of the tumours that formed. Tumour number and size was not affected by gut sterilization when tumours were already visible. To ascertain the effects of PAMP–TLR4 signalling, the authors found that caspase 3 cleavage — which is indicative of apoptosis — was increased in the tumours from *Tlr4*-mutant mice and gut-sterilized mice compared with tumours in $Tlr4^{+/+}$ mice. Moreover, the proportion of cells within tumours exhibiting caspase 3 cleavage inversely correlated with tumour size and number.

Together, these data indicate that activation of PAMP–TLR4 signalling in chronically damaged livers confers survival to pre-malignant cells in late stages of hepatocarcinogenesis.

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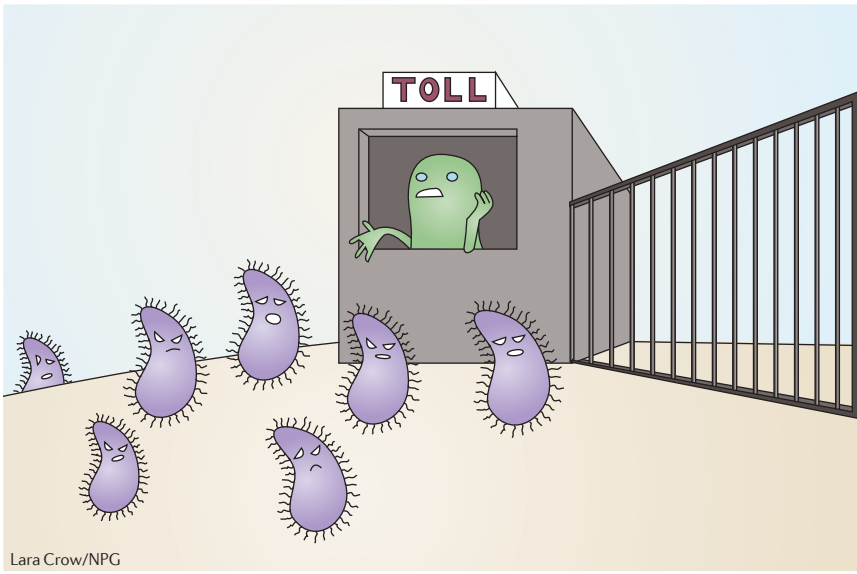
ORIGINAL RESEARCH PAPER Dapito, D. H. *et al.* Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. *Cancer Cell* **21**, 504–516 (2012)



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