CANCER MICROBIOME

Cancer immunity thwarted by the microbiome

Microbial bile acid metabolites promote liver metastasis

By **Nadine Hartmann**[1] *and* **Mitchell Kronenberg**[1,2]

Liver cancer rates have tripled since 1980, making it one of the leading causes of cancer deaths worldwide (*1*). The efficacy of a variety of cancer treatments, as well as carcinogenesis itself, can be influenced by the microbiome (*2*). Microbiome-cancer interactions are complex, as the microbiome can promote chronic inflammation or directly affect cancer cells. Furthermore, by influencing the immune system, the microbiome may either promote or inhibit antitumor immune responses (*3*). On page 876 of this issue, Ma *et al.* (*4*) show that a type of commensal bacteria, *Clostridium* species, prevents an effective immune response to both primary liver tumors and liver metastases by a previously unknown mechanism: not by means of its own metabolites, but by modifying a host product—the bile acids that liver cells are exposed to.

About 75% of the blood that passes through the liver is supplied from the intestine by the portal vein, thus constantly exposing the liver to components and metabolites from the intestinal microbiota. To avoid excessive immune stimulation, at steady state, hepatic tissue promotes immune tolerance rather than immune activation (*5*). The liver has a mostly noncirculating immune system consisting of T lymphocytes, B lymphocytes, and macrophages, among others (*6*). In the absence of infection or injury, these immune cells are predominantly in the sinusoidal blood vessels rather than the tissue parenchyma. The liver-resident lymphocyte populations have different properties from their circulating counterparts. For example, natural killer T (NKT) cells constitute up to 40% of the total intrahepatic T lymphocytes in mice, but they are much less frequent in other sites.

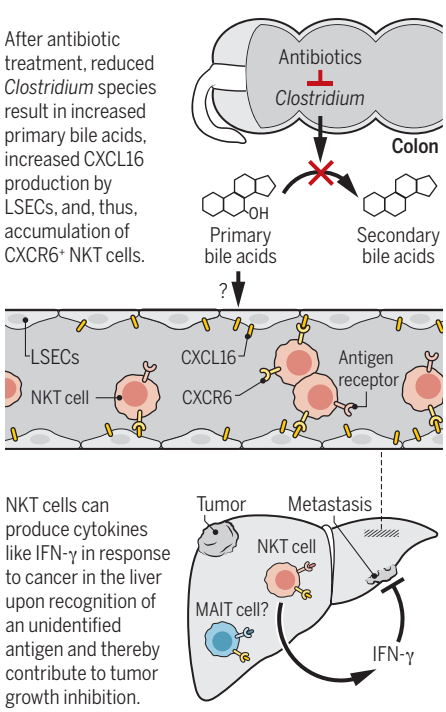
Although most T lymphocytes recognize peptides bound to or presented by antigen-presenting molecules encoded by the highly polymorphic major histocompatibility complex (MHC) genes, NKT cells recognize lipids presented by the monomorphic MHC class I–like protein CD1d (*7*). NKT cells are distinguished by their rapid and intense cytokine responses: Intrahepatic NKT cells produce copious amounts of interferon-γ (IFN-γ) when activated, which has been associated with their ability to respond to tumors (*8*).

To understand the role of the microbiome in hepatic cancers, Ma *et al.* analyzed primary liver tumors in transgenic mice as well as liver metastases from lymphoid or melanoma tumor cell lines engrafted outside the liver. All mice received a cocktail of three antibiotics to minimize the microbiome. Antibiotic treatment reduced tumor growth or liver metastases, but not metastases to other sites, such as the lungs. This tissue-specific effect correlated with an increase in activated liver NKT cells. Furthermore, tumor-bearing CD1d-deficient mice were unaffected by antibiotic treatment. These mice lack not only NKT cells but also other CD1d-reactive cells in the liver. The accumulation of NKT cells in the liver requires expression of the chemokine receptor CXCR6 (C-X-C chemokine receptor type 6), which binds the ligand CXCL16 expressed on the surface of liver sinusoidal endothelial cells (LSECs) (see the figure). Antibiotic treatment increased LSEC expression of CXCL16 by an unknown mechanism. In CXCR6-deficient mice, antibiotic treatment did not augment tumor growth, suggesting that the increased antitumor response following antibiotics was due to enhanced NKT cell accumulation in the liver mediated by CXCL16-CXCR6 interactions.

How does the gut microbiome signal to LSECs? Bile acids—which are synthesized in the liver, stored in the gall bladder, and secreted into the intestine—are involved in dietary fat absorption. Recently, bile acids have been found to shape the intestinal microbiome composition, but microbiome metabolism also affects bile acid profiles (*9*). Primary bile acids that are synthesized in the liver stimulate hepatocytes to produce proinflammatory cytokines and chemokines like CXCL16, thus contributing to the recruitment of CXCR6$^+$ lymphocytes to the liver (*10*). However, primary bile acids are metabolized to secondary bile acids by intestinal bacteria and are returned to the liver by the enterohepatic circulation (*9*). Ma *et al.* showed that LSECs up-regulate *Cxcl16* messenger RNA (mRNA) production when exposed to primary bile acids, whereas *Cxcl16* mRNA production was decreased upon secondary bile acid exposure. Also, treatment with antibiotics depleted the main producers of secondary bile acids and increased CXCL16 expression in LSECs. Importantly, increasing primary bile acids (for example, through feeding) increased intrahepatic NKT cells and reduced tumor metastases. Because the selective use of antibiotics implicated Gram-positive bacteria, and because *Clostridium* species carry out a major enzymatic step in secondary bile acid formation, Ma *et al.* determined that colonization with a commensal *Clostridium* species led not only to decreased intrahepatic NKT cells but also increased liver tumor metastases. Interestingly, it has been shown that a high-fat diet-induced increase of intestinal *Clostridium* species increases levels of secondary bile acids in the liver, which in turn promotes liver cancer (*11*).

Primary bile acids promote liver tumor immunity

Clostridium species modify bile acids, which prevents antitumor immune responses in the liver.



GRAPHIC: V. ALTOUNIAN/*SCIENCE*

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Why is a specialized T cell subset critical for immune protection from liver metastases? Although NKT cells recognize lipids containing fatty acids, bile acids have an entirely different structure and are not known to be antigens. Therefore, the role of primary bile acids in NKT cell activation is indirect, by promoting NKT cell accumulation but not their activation. Ma *et al.* analyzed CD1d-expressing tumors, and they suggest that CD1d-presented lipid antigens from the tumors could be directly recognized by NKT cells. It is also possible that NKT cells could be activated by lipid antigens from $CD1d^-$ tumors presented by $CD1d^+$ macrophages or dendritic cells or even by inflammatory cytokines such as interleukin-12 (IL-12) (*12*). Regardless, the prevalence of NKT cells in mouse liver and their strong effector function allow them to be a dominant player in the anticancer response of this organ. The situation is different in humans, as intrahepatic NKT cells are much less frequent. However, mucosal-associated invariant T (MAIT) cells, which recognize microbial vitamin B metabolites presented by MHC-related protein 1 (MR1), are prevalent in human liver, and they also exert rapid effector functions, such as IFN-γ secretion (*7*). Interestingly, MAIT cells also express CXCR6, and, although their antitumor potential is uncertain, they do infiltrate some metastatic lesions in the liver of colorectal carcinoma patients (*13*).

Although there are bile acid profiles that are associated with cancer, several studies advocate modifying bile acid production to improve cancer therapy, for example, through agonists of bile acid receptors or simply by changes in diet (*14*). Thus, as for most biological systems, bile function has different facets. It is designed to emulsify fats and help absorb dietary lipids, but after modification by the microbiome, secondary bile acids alter immune function to promote liver cancer and liver metastases. ■

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CELL BIOLOGY

The RNA face of phase separation

RNA regulates the formation, identity, and localization of phase-separated granules

By **Magdalini Polymenidou**

Phase separation or liquid unmixing—a phenomenon resembling the formation of oil droplets in vinegar—has emerged as a major driver of functional compartmentalization within cells, allowing the rapid and dynamic isolation of specific activities from the surrounding cellular environment, without the need of a membrane (*1*). A flurry of exciting recent studies demonstrated the importance of RNA binding proteins (RBPs) with low complexity regions (LCRs) in this process [for example, (*2*, *3*)], yet the role of incorporated RNAs is less well understood. On pages 918 and 922 of this issue, Maharana *et al.* (*4*) and Langdon *et al.* (*5*) propose that cellular RNAs regulate the formation, subcellular localization, and identity of these granules, which has implications for stress responses and pathologic protein aggregation in neurodegenerative diseases.

Cellular stressors induce the formation of perhaps the best studied of these so-called membraneless organelles, stress granules (SGs), which consist of multiple RBPs in complex with cytoplasmic RNAs. SGs stall the translation of incorporated RNAs and contain RBPs with LCRs—also called prion-like domains—which can phase-separate, both in vitro and within cells (*1*–*3*). Several of these SG-incorporating prion-like RBPs form pathologic protein aggregates in affected neurons of patients with neurodegenerative diseases. The same proteins form phase-separated liquid droplets in vitro, which are dynamic in a similar manner to SGs in cells. These liquid droplets transform over time into solid-like structures (*1*–*3*), somewhat resembling the pathologic protein aggregates in patients with neurodegeneration. These observations have reinforced the hypothesis that SGs act as precursors of pathologic aggregates (*6*, *7*). How this transition occurs in the degenerating brain remains unclear, and a direct in vivo demonstration is still missing.

A main driver of protein phase separation is concentration, so Maharana *et al.* asked whether the intrinsic cellular concentrations of RBPs known to form membraneless organelles could explain this behavior. Surprisingly, they found that in the absence of any other components, several RBPs implicated in neurodegeneration phase-separate in vitro in concentrations similar to those physiologically found in the nucleus. Yet inside the cell, the same proteins remain diffused in the nucleus and are typically excluded from phase-separated compartments, even under cellular stress conditions, which drive their condensation into cytoplasmic SGs. Searching for which nuclear component may prevent the phase separation of RBPs, they found that high RNA:protein ratios—like those typically found in the nucleus—prevent phase separation, whereas low ratios, similar to those in the cytoplasm, promote it (see the figure). This effect seems to be independent of sequence specificity for RBP binding. How these RNAs keep RBPs from phase-separating in the absence of specific binding and what the role of LCRs is in this process remain unresolved. Interestingly, Maharana *et al.* show that in vitro high amounts of short, nonspecific RNAs keep prion-like RBPs soluble. Conversely, longer RNAs that can form higher-order secondary structures and which specifically bind RBPs can nucleate phase separation. This is potentially the reason that *Neat1* (nuclear paraspeckle assembly transcript 1), a long noncoding RNA with specific FUS (fused in sarcoma) binding sites (*8*), can capture FUS out of the diffused nuclear pool to form paraspeckles (phase-separated nuclear compartments that regulate gene expression).

> ***"...high RNA:protein ratios... prevent phase separation... low ratios...promote it..."***

Maharana *et al.* propose that these observations may explain the somewhat preferential phase separation of nuclear proteins into cytoplasmic SGs, which in turn may transform into aggregates in the degenerating brain. Cytoplasmic aggregates are toxic

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