Background  Liver cancer remains the third leading cause of worldwide cancer-related deaths. Despite clinical advances, established immunotherapies largely fail patients due to poor immune responses. Peripheral nerves influence tumors, but brain-liver interactions remain largely unstudied. A recent study reported a decreased risk of liver cancers in patients that underwent truncal vagotomy (vagus snip) compared to simple suture procedure. Here, we identify a vagal-immune arc regulating hepatic cancer. Methods  C57BL/6 or BALB/c mice (9–12 weeks old) underwent a surgical hepatic vagotomy (HV) or sham procedure (SV). Following established protocols, HV and SV mice received orthotopic tumors via intrahepatic injection (2.5×10⁵ RIL-175 or B16-F10 cells) or tail vein/flank injection (1.0×10⁶ A20, RIL-175 cells) to model primary and metastatic liver cancer. Growth of luciferase-labeled cells was measured via in vivo imaging and immune profiling was conducted via flow cytometry and scRNASeq. Open field test, Y maze, and phenotyper cages assessed mouse behavior. Highly-multiplexed immunofluorescence (CODEX platform) revealed peripheral nerves in clinical resection samples. Results Precise liver denervation reduced tumor growth in three models of primary (RIL-175) and metastatic (B16-F10, A20) tumors (figure 1A). Outcomes remained organ specific as HV mice exhibited reduced tumor burden of intrahepatic, but not subcutaneous, models. HV livers exhibited decreased levels of vagal neurotransmitter acetylcholine (ACh). As immunofluorescent analyses revealed colocalization of peripheral nerves and lymphocytes in clinical liver cancer, we profiled HV immunity. HV livers exhibited broad anti-tumor immunity, notably increased CD8+ T cells and higher expression of intracellular cytokines (IFNγ, TNFα) (figure 1B). We then examined whether immune alterations were a cause or consequence of HV tumor burden. ACh exposure reduced intracellular cytokine levels in ex vivo CD8+ T cells following anti-CD3/CD8 activation. Treatment with bethanechol (ACh receptor agonist, 400 µg/mL drinking water) increased tumor burden and reduced CD8+TNFα+ subsets. Bethanechol failed to promote tumor growth in Rag1KO mice lacking mature B and T cells, and targeted depletion of CD8+ T cells abrogated the effects of vagotomy (figure 1C). Finally, as the vagus nerve is largely comprised of afferent fibers, we assessed murine behavior and ambulation. Tumor-bearing HV mice displayed decreased anxiety-life features and fatigue compared to sham controls. Conclusions Our findings highlight a vagal-CD8+ T cell axis modulating hepatic tumor burden and behavior. This work furthers the emerging field of cancer neuroscience and identifies ACh signaling targets to alter hepatic immunosuppression and cancer outcomes.

REFERENCES

Ethics Approval  This research was approved by the NCI Division of Intramural Research Animal Care and Use Committee, proposal numbers: MOB-028 and TGOB-015. Patients provided informed consent for clinical tissue acquisition: Institutional Review Board protocol #2017-0365.

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Abstract 988 Figure 1  (A) RIL-175 tumor weight 21 days after intrahepatic injection (2.5×10⁵ cells/mouse). (B) Median fluorescence intensity (MFI) of ex vivo CD4+ and CD8+ T cells following PMA/ionomycin stimulation, proinflammatory IFNγ and TNFα displayed. (C) CD8+ T cell depletion RIL-175 model at 21 days (200 µg anti CD8 or IgG control/mouse 1X per week). Bar graphs indicate mean and SEM, data assessed by unpaired t-test, significance determined as p<0.05.