Background We have discovered that galectin-9 (Gal-9), a carbohydrate-binding protein, is expressed in biliary tract cancer (BTC). Upon binding to the receptor Tim-3 on T cells, Gal-9 regulates immune evasion of tumors by inducing apoptosis. Defining the role of galectin-9 in the BTC microenvironment will inform how to leverage this checkpoint as a novel therapeutic target.

Methods Tissue microarrays (TMAs) were constructed from the tumors of 66 patients who underwent curative-intent resection of BTC from 2000 to 2015 at our institution. These TMAs underwent immunohistochemical staining for CD4, CD8, Gal-9 and Tim-3; the percentage of positive staining cells was quantified using QuPath software. For each biomarker, martingale residual plots and bias-adjusted log rank tests were used to identify a cut-off value for ‘low’ versus ‘high’ expression with maximal survival difference. For in vitro experiments, CD3+ T cells were isolated from human donors, activated and expanded in the presence of exogenous Gal-9 (1 μg/mL), and analyzed by flow cytometry on day 5.

Results CD8+ T cell infiltration positively correlated with high Gal-9 and Tim-3 expression on tumors ($R^2=0.538$, $R^2=0.430$: linear regression). OS and recurrence-free survival (RFS) were significantly improved in patients with high Gal-9 expression in their tumors, compared to those patients with low Gal-9-expressing tumors ($p=0.018$, $p=0.025$). A significant improvement in RFS was evident in patients with high CD8+ T cell infiltration ($p=0.047$), although there was no difference in OS. No differences in survival were observed based on CD4+ T cell infiltration or Tim-3 expression. Interestingly, when tumors from patients with high Gal-9 expression were stratified by Tim-3 expression, there was improved OS and RFS in individuals with low Tim-3 expression ($p=0.031$, $p=0.005$), implying that engagement of the Gal-9-Tim-3 pathway impairs T cell survival. Indeed, viability and proliferative capacity of human lymphocytes was impaired when cultured in vitro in the presence of Gal-9 versus control ($p=0.016$, $p=0.008$).

Conclusions This study provides unique insight into the role for Gal-9 in BTC. Although its elevated presence in resectable tumors is associated with longer RFS and OS, it may also signify immune reactivity. This idea is supported by a correlation between CD8+ T cells and Gal-9 and subgroup analysis showing that clinical outcomes are strongly influenced by expression of both Gal-9 and Tim-3. Our in vitro data shows soluble Gal-9 directly inhibits T cell survival and function. Taken together, we posit that Gal-9 may circumvent attempts at immune recognition in early-stage BTC.

REFERENCES

Ethics Approval Tumor tissue was obtained from patients under an IRB-approved protocol at Emory University Winship Cancer Institute.