SUMMARY

Helicobacter pylori colonizes the gastric epithelium by interacting with carcinoembryonic antigen-related cell adhesion molecules (CEACAMs), notably CEACAM1, CEACAM5 and CEACAM6, expressed on the gastric epithelial cells. Colonization may give rise to gastritis, occasionally advancing to gastric cancer (GC). This work explored alterations in CEACAM expression triggered by *H. pylori* infection. Results demonstrated a significant upregulation of CEACAM6 after infection which promoted gastric cancer cell (GCC) proliferation, migration and invasion. The tumor microenvironment (TME) promotes tumorigenesis through released mediators. Therefore, the paracrine effects of the *CEACAM6*-expressing GCCs were also studied on surrounding non-aggressive GCCs. Supernatants from *H. pylori*-infected CEACAM6-transfected GCCs further enhanced tumorigenic traits in neighboring cells. Within the TME, macrophages are key drivers of tumor progression. Co-culture experiments revealed that epithelial-CEACAM6 facilitated *H. pylori*-driven M2 macrophage polarization as confirmed by flow cytometry and immunofluorescence microscopy. Cytokine signatures linked to macrophage polarization were identified through antibody array analysis of the *CEACAM6*-expressing cell supernatants.

Extracellular vesicles (EVs) are key secretory modulators that contribute substantially to tumor development and progression. Significantly elevated levels of CEACAM6 were found to be present in the EVs secreted by *H. pylori*-infected gastric cancer cells (GCCs). To study the impact of CEACAM6-containing EVs, on tumor inducing capabilities, EVs were isolated from *CEACAM6*-expressing cells. Those were thoroughly characterised. Functional assays revealed that CEACAM6-positive EVs enhanced proliferation, clonogenicity, migration and invasion of recipient GCCs, with infection further amplifying these effects. EV sonication and fractionation confirmed that CEACAM6 was largely confined to the vesicle membrane. Consistent with tissue findings, sera from GC patients exhibited significantly higher

CEACAM6 in EVs compared to those in healthy controls. Collectively, these findings suggest that CEACAM6-containing EVs act as potent mediators of tumorigenesis and potential biomarkers in *H. pylori*-associated GC.