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The American Journal of
PATHOLOGY

ajp.amjpathol.org

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COMMENTARY

Therapeutic Role of Sphingosine-1–Phosphate Receptor 2 in the Progression of Esophageal Adenocarcinoma

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Lindsey Kennedy*[†] and Gianfranco Alpini*[†‡]

From the Research Service,* Central Texas Veterans Health Care System, Temple; the Department of Medical Physiology,[†] Texas A&M Health Science Center, College of Medicine, Temple; and Baylor Scott & White Health Digestive Disease Research Center, Baylor Scott & White Health,[‡] Temple, Texas

Esophageal adenocarcinoma (EAC) is the most prevalent form of esophageal cancer in the United States, and its incidence has increased sixfold during the past decade.[1,2] Specifically, the overall 5-year survival rate of esophageal cancers is only approximately 17%, with an overall survival median time of less than 1 year because of late diagnosis, chemoresistance, and high incidence of metastasis.[3] Therefore, identification of EAC pathogenesis and potential therapeutic targets is critical. High-grade dysplasia Barrett esophagus (BE) is a precursor to aggressive EAC. Persistent and severe gastroesophageal reflux disease (GERD) has been established as a primary risk factor for the development of both BE and EAC.[4] As major components of GERD refluxate, gastric acids and bile acids have drawn great attention for their role in the development of EAC. Gastric acid induces excessive epithelial cell inflammation, which contributes to tumorigenesis-related mutations in *TP53* and *CDKN2A*, both common in advanced BE and EAC.[2] However, the effects of bile acids on EAC progression are poorly understood.

Conjugated Bile Acids Promote EAC Progression via S1PR2 Signaling

In this issue of the *American Journal of Pathology*, Liu et al[5] report that the conjugated bile acid taurocholic acid (TCA) promotes human EAC invasive growth and cancer stem cell (CSC) expansion through activation of the sphingosine-1-phosphate receptor 2 (S1PR2) pathway and downstream yes-associated protein (YAP) activation.[5] Previous studies have found that unconjugated bile acids, including deoxycholic acid and chenodeoxycholic acid,

induce cyclooxygenase (COX)-2 expression and chronic inflammation, which promote BE cell proliferation and EAC progression through prostaglandin E2 and inflammation-induced signaling pathways both *in vitro* and *in vivo*.[6,7] Oxidative stress and DNA damage, induced by deoxycholic acid and chenodeoxycholic acid, are also involved in EAC carcinogenesis.[6] However, several early clinical studies demonstrated that conjugated bile acids, especially TCA and glycocholic acid, but not unconjugated bile acids, are the predominant bile acid signalers in GERD refluxate.[8,9] This finding is expected because >99% of bile acids secreted in bile are conjugated. Conjugated bile acid levels are significantly increased in the refluxate from patients with advanced BE or EAC when compared with patients with benign BE.[10] A recent study in a rat esophagojejunostomy-induced reflux BE/EAC model showed a significantly higher portion of TCA in refluxate, which was strongly associated with increased incidence of advanced BE and EAC.[11] Although these results highlighted a critical role for conjugated bile acids in the pathogenesis of EAC, they did

not illustrate the downstream signaling pathways that modulate BE and EAC progression. The study by Liu et al[5] provides novel insights in the cancer-promoting role of conjugated bile acids in EAC progression and identifies the role of S1PR2-mediated YAP activation in EAC.

Two human EAC cell lines, OE-19 and OE-33, were used for this study. OE-33, which expresses higher levels of S1PR2 compared with OE-19 cells, has higher motility and invasiveness and is much more sensitive to conjugated bile acid stimulation. Although TCA, glycocholic acid, and S1P promote proliferation of OE-19 and OE-33 cells, TCA and S1P promote the migration, invasion, and growth of OE-33 cells but not OE-19 cells. Interestingly, over-expression of S1PR2 sensitized OE-19 cells to TCA and S1P stimulation, whereas pharmacologic inhibition or genetic knockdown of S1PR2 ameliorated TCA-induced damage. Overexpression of S1PR2 in OE-19 cells increased the invasive capacity of these cells; however, knockdown of S1PR2 in OE-33 cells suppressed inva-siveness. These results indicate that S1PR2 is a critical regulator of EAC cell growth independent of conjugated bile acid stimulation, possibly via S1P secretion.

S1PR2 Activation Promotes EAC Invasion and CSC Growth

S1P is a well-established signaling molecule that modulates numerous biological processes, including carcinogenesis and cancer development.[12] The role of S1PR1 and S1PR3 signaling on cancer proliferation, survival, and metastasis has been extensively studied and characterized; however, the role of S1PR2 is controversial. Several studies found that S1PR2 signaling suppresses cancer by inhibition of migration, angiogenesis, and immune cell infiltration.[13] However, recent reports suggest that S1PR2 activation promotes proliferation, migration, and metastasis through modulation of tumor suppressor genes and formation of filopodia and lamellipodia in cancer cells.[13] Previously, Liu et al[12] and Nagahashi et al[14] demonstrated that conjugated bile acid activation of S1PR2 promotes invasive growth of cholangiocarcinoma via ERK1/2 activation, COX-2 induc-tion, and inflammation. In addition, S1P promotes transforming growth factor-β signaling and migration and invasion in EAC cells; however, pharmacologic inhibition of S1PR2 by JTE-013 treatment reduces these effects.[15] These observations suggest a potential tumor-promoting role of S1PR2 in EAC, laying the background for the discussed work. Within S1P signaling, sphingosine kinase 1 (SphK1) and SphK2 synthesize S1P from sphingosine. Both SphK1 and SphK2 promote tumorigenesis, especially in gastrointestinal tract cancers.[16] SphK1 expression correlates with chemoresistance and poorer prognosis of gastro-esophageal cancer.[17] Recent work found that SphK1 is significantly up-regulated in highly invasive esophageal cancer cells and is significantly correlated with invasive

capacity.[18] More studies that focus on the role of SphK1 and SphK2 on EAC progression are required.

The current study found that OE-33 cells (high S1PR2 expression) are poorly differentiated and exhibit mesen-chymal cell−like morphologic features, decreased E-cad-herin (epithelial marker) expression, and increased motility and invasiveness when compared with OE-19 cells.[5] Furthermore, TCA and S1P enhanced transforming growth factor-β−induced epithelial-to-mesenchymal transition and promoted CSC expansion in OE-33 cells. These changes were minimal in OE-19 cells (low S1PR2 expression). In addition, OE-33−derived CSC mainly expressed CD44, a mesenchymal marker, whereas OE-19 cells had high expression of LGR5, an intestinal stem cell marker. These findings suggest that TCA and S1P significantly enhance the expansion of OE-19 and OE-33 cells. This work provides convincing evidence of the role of S1PR2 signaling in the development and maintenance of CSCs. This finding is significant because the CSC population contributes to the high incidence of metastasis, chemoresistance, and recur-rence of EAC.

S1PR2 Promotes EAC Progression via YAP and β-Catenin Activation

Liu et al[5] found that the YAP pathway is significantly activated by TCA and S1P through a S1PR2-dependent signaling mechanism. Hippo-YAP signaling is critical for organ size control during development and is a vital regu-lator of tumorigenesis. As recently published, YAP is a major regulator of the EAC CSC population, and disruption of YAP signaling in EAC cells leads to loss of CSC prop-erties through inhibition of SOX-9 expression.[19] The path-ways upstream of YAP are missing in the current study, but previous work suggests that S1PR2 triggers YAP activation independent of its upstream kinases.[20] The current study found that administration of TCA was able to induce β-catenin activation in OE-33 cells, which is consistent with a previously published study.[21] However, this study did not evaluate the mechanism by which YAP and β-catenin activation induces EAC invasion; this should be a future area of research. Further studies are necessary to bridge the gap between activated YAP signaling and CD44 expression, with subsequent CSC expansion, in EAC cells.

Gastric acid signaling exacerbates unconjugated bile acid−induced inflammation and EAC growth via acidifica-tion of bile acids.[22] However, the effect of low pH on conjugated bile acid−induced EAC invasion was not evaluated in the current study and should be analyzed in the future. The role of COX-2 activation by gastric acids, inflammatory cytokines, and bile acids during EAC devel-opment has been characterized,[23] and previous work by Liu et al[24] found that conjugated bile acids induce COX-2 expression through S1PR2-mediated signaling in human cholangiocarcinoma cells. The effect of conjugated bile
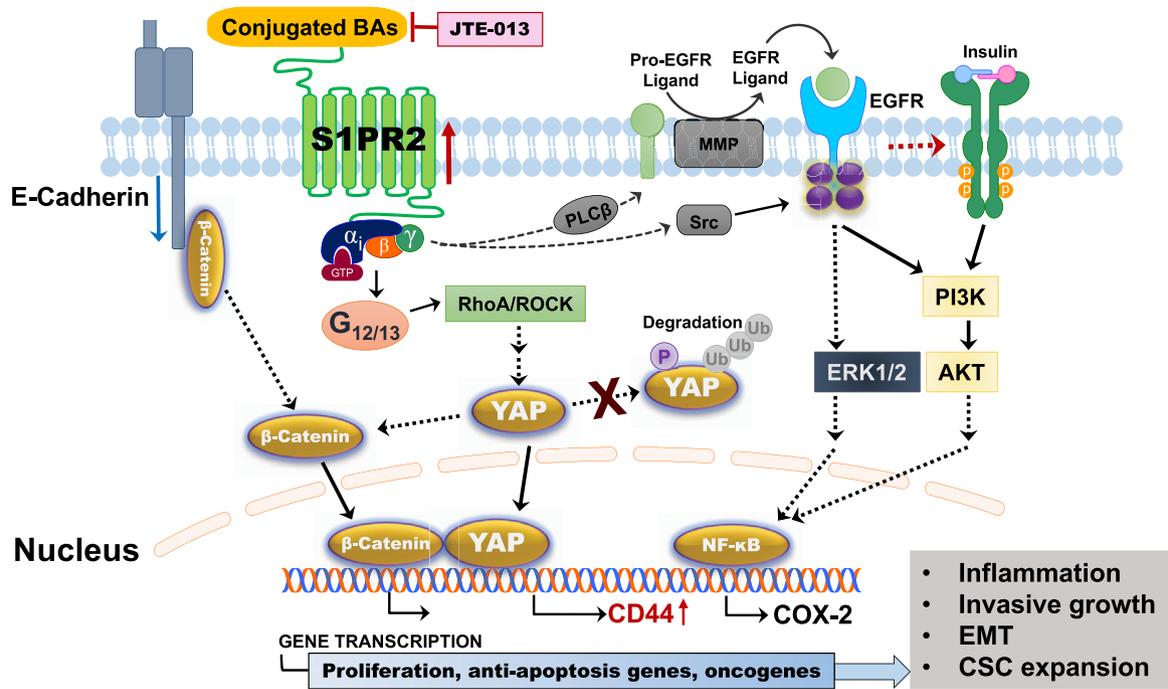
Figure 1 Cell signaling cascade activated by sphingosine1-phosphate receptor 2 (S1PR2) in esophageal adenocarcinoma (EAC). Conjugated bile acids (Bas) activate S1PR2, which then activates the epidermal growth factor receptor (EGFR) via phosphoinositide-specific phospholipase C (PLC)-β and Src, followed by ERK1/2 and AKT phosphorylation. Activation of ERK1/2 and AKT signaling induces cyclooxygenase (COX)-2 expression via NF-κB. S1PR2 activation promotes the nuclear translocation of yes-associated protein (YAP). In addition, down-regulation of E-cadherin results in disassociation and nuclear translocation of β-catenin. The nuclear β-catenin/YAP complex promotes the expression of key genes involved in inflammation, cancer cell proliferation, migration, and cancer stem cell (CSC) expansion. JTE-013 is a chemical antagonist of S1PR2 and may be useful for the treatment of EAC. EMT, epithelial-to-mesenchymal transition; MMP, matrix metalloproteinase; P, phosphorylation; PI3K, phosphatidylinositol 3-kinase; ROCK, Rho-associated protein kinase; Ub, ubiquitination.

acids on COX-2 expression in EAC cells should be investigated in future studies. Understanding the effect of conjugated bile acid signaling on COX-2 expression will help us better understand the signaling mechanisms that regulate inflammation in EAC.

Conclusions and Future Directions

Although the study by Liu et al[5] provides mechanistic insights, it is limited by the lack of *in vivo* data. Future studies on the role of conjugated bile acids and S1PR2 signaling in animal models is necessary. Human cancer cell xenograft models in null mice are not sufficient for studying S1PR2 in the progression of EAC because they do not recapitulate the inflammatory response noted in EAC progression. Currently, the only model that spontaneously develops EAC and recapitulates GERD is a rat esophagojejunostomy-induced reflux BE/EAC model.[11] However, gene manipulation in rats is challenging. In addition, a model of advanced BE/EAC that uses human IL-1β transgenic mice in combination with bile acid supplementation in drinking water has been developed and may be useful for future research.[6] Identifying which animal model will be the best resource for future projects will be a challenge. Another major hurdle is finding an appropriate S1PR2 antagonist

because currently there is not a specific S1PR2 antagonist with reasonable bioavailability. For this reason, downstream targeting of S1PR2 signaling, including YAP activation, may be another option. Overall, this study by Liu et al[5] provides novel insights into the pathogenesis and development of EAC and identifies the role of conjugated bile acids in EAC invasive growth and CSC expansion. As summarized in Figure 1, conjugated bile acid−induced activation of S1PR2 induces nuclear translocation of YAP and subsequent activation of the epidermal growth factor receptor/ERK1/2 and phosphatidylinositol 3-kinase/AKT signaling pathways. Previous work from this group has found that activation of ERK1/2 and AKT induces COX-2 expression via NF-κB.[25] In addition, down-regulation of E-cadherin results in the nuclear translocation of β-catenin, and the nuclear YAP/β-catenin complex promotes inflammation, cancer cell proliferation, and migration. In conclusion, targeting S1PR2-mediated YAP activation may present a novel therapeutic strategy for the treatment of EAC.

Supplemental Data

Supplemental material for this article can be found at *https://doi.org/10.1016/j.ajpath.2018.07.001*.

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