Table 1. Patient characteristics

		Number of Patients (%)
		N = 257
Demographics	Male	107 (41.6)
	Female	150 (58.4)
	Age, median (Q1-Q3)	63 (55.9 - 70.0)
	BMI, mean, (SD)	27.7 (6.9)
Risk Factors	Current Smoker	18 (7.0)
	Former Smoker	99 (38.5)
	Never Smoker	137 (53.3)
	Not Reported	3 (1.2)
	Alcohol Use	75 (29.2)
	Family History of Gastric Cancer	27 (10.0)
Race/Ethnicity	White	93 (36.2)
	Black	39 (15.2)
	Asian/Pacific Islander	24 (9.4)
	Hispanic	35 (13.6)
	Other/Unknown	66 (25.6)

SD, standard deviation; *Q1*, first quartile; *Q3*, third quartile

Table 2. Endoscopic evaluation of GIM

		Number of Patients (%)		
		Index endoscopy (N = 257)	First follow up EGD (N = 139)	Final follow up EGD* (N = 60)
Common Indications*	GERD	65 (25.3)	23 (16.5)	
	Abdominal Pain/Dyspepsia	58 (22.6)	13 (9.4)	
	Surveillance/Screening (Non-GIM)	49 (19.1)	38 (27.4)	
	Surveillance of GIM	N/A	23 (16.5)	
H pylori	Detected	53 (20.6)		
	Eradication regimen prescribed	48/53 (90.5)		
	Confirmed eradication	22/53 (41.5)		
	Stool Test	9/22 (42.8)		
	Endoscopic Biopsy	11/22 (52.3)		
	Breath Test	2/22 (4.8)		
Biopsy Location	Antrum	207 (80.5)		
	Body	149 (58.0)		
	Antrum and Body	138 (53.7)		
	Incisura	24 (9.3)		
	Fundus	53 (20.6)		
	Cardia	11 (4.3)		
	Not Reported	39 (15.2)		
Location of GIM	Body	64 (24.9)		
	Antrum	160 (62.3)		
	Incisura	17 (6.6)		
	Fundus	17 (6.6)		
	Cardia	3 (1.9)		
	Not Reported	59 (23.0)		
Extent of GIM	Extensive	51 (19.8)		
	Limited	160 (62.3)		
	Not reported	46 (17.9)		
GIM severity	Complete	102 (39.7)		
	Incomplete	70 (27.2)		
	Not reported	85 (33.1)		
Dysplasia	No Dysplasia	211 (96.8)		
	Low Grade	7 (3.2)		
	High Grade	0 (0)		
Risk assessment for cancer progression	OLGIM reported	0	0	0
	Abbreviated Sydney Protocol Followed	21 (9.6)	37 (37.4)	32 (53.3)
Number of follow up EGDs	0	118 (45.9)	**Time to first follow up EGD, median months (Q1-Q3)**	**Time to final follow up EGD, median months (Q1-Q3)**
	1	56 (21.8)		
	2	28 (10.9)		
	3	15 (5.8)	17.5 (7.3 - 36.1)	28.1 (7.3 – 62.4)
	4	15 (5.8)		
	≥4	25 (9.7)		

*Commonly more than one indication for EGD; * Most recent follow up EGD if more than 1 follow up EGD; Q1, first quartile; Q3, third quartile*

Sa1143

EVALUATING THE TRα1-DEPENDENT CHARACTERISTICS OF COLON CANCER STEM CELL BIOLOGY

Maria Virginia Giolito, Leo Claret, Diana Farhat, Michelina Plateroti

Background. The thyroid hormone T3 and its nuclear receptor TRα1 control gut development and homeostasis through the modulation of intestinal crypt cell proliferation (rev. in Skah et al, Dev. Biol_2017; Sirakov et al, CMLS_2014). The translational potential of our studies in mouse models has been proved in human colorectal cancer (CRC) patients as we observed increased TRα1 expression in CRC and, from a molecular point of view, we showed a significant correlation between TRα1 levels and Wnt activity (Uchuya-Castillo et al. 2018). TRα1 loss-of-function (LOF) and gain-of-function (GOF) in human adenocarcinoma Caco2 cell lines not only confirmed that TRα1 levels control Wnt activity but also demonstrated the role of TRα1 in regulating cell proliferation and migration. Altogether these data strongly suggested an involvement of TRa1 in cancer stem cell biology, similar to what we previously observed in normal intestinal stem cells (SC) (Godart et al, Dev. 2021). ***Aims.*** (1) define the basis of TRα1 upregulation in CRCs and (2) its impact in CSC biology. ***Methods.*** We analyzed the activity of *THRA* gene promoter (1) and used tumor spheroids based on Caco2 cells (Giolito et al, JoVE 2021) with altered TRα1 expression (2). ***Results.*** By *THRA* promoter analysis, we demonstrated the presence of binding sites for transcription factors involved in intestinal homeostasis and SC/CSC biology that are also altered in CRC, such as TCF7L2 (Wnt pathway), RBPJ (Notch pathway) and CDX2 (intestinal epithelial cell identity). In-depth analysis of the Wnt pathway allowed us to recapitulate the regulation of *THRA* transcription and TRα1 expression by this signaling pathway in human adenocarcinoma cell lines as well as mouse enteroids (Giolito et al, submitted). We studied the impact of altering TRα1 levels in tumor spheroids and observed that TRα1 up-regulation increases while TRα1 KD decreases the size of spheres, strongly acting on their growth capacity. Specific CSC populations are altered, as observed by IF and cytometry. We are in the process of evaluating the response to chemotherapy depending on T3/TRα1 by using drugs employed in CRC patients (FOLFOX, FOLFIRI). ***Conclusion and perspectives.*** Our work describes, for the first time, the regulation of the *THRA* gene in specific cell and tumor contexts. We also gained insights into the T3/TRα1-dependent tumor cell behavior, eventually including their response to chemotherapy. These studies are currently enlarged to mouse models and to human organoids.

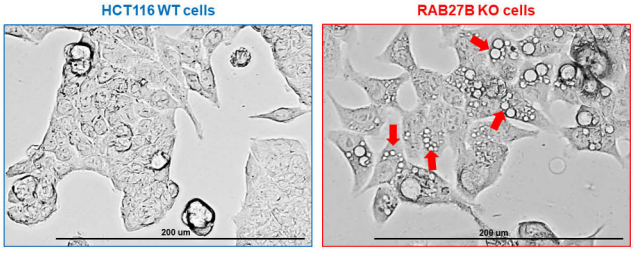
Sa1144

OVEREXPRESSION OF THE SMALL GTPASE RAB27B PROMOTES TUMOR GROWTH BY REGULATING AUTOPHAGY FLUX IN COLORECTAL CANCER

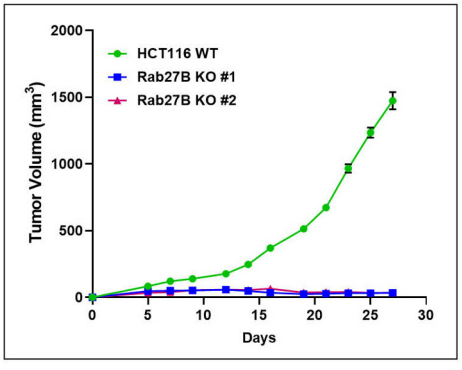
Sahida Afroz, Ranjan Preet, Vikalp Vishwakarma, Dan A. Dixon

Autophagy is a dynamic, multi-step process that cells use to degrade damaged, abnormal, and potentially harmful cellular substances. While autophagy is maintained at a basal level in all cells, it is activated at a higher level in many cancer cells and promotes tumor growth, anti-tumor immune response, and resistance to cancer therapy. As a result, autophagy is increasingly being recognized to have an important role in cancer progression and emerging as a potential target for cancer therapy. We recently discovered that small GTPase Rab27B, a well-known regulator of vesicle trafficking and extracellular vesicle secretion is overexpressed in colorectal cancer (CRC) and regulates autophagy process. Depletion of Rab27B in CRC cells by CRISPR-Cas9 and siRNA resulted in an abnormal accumulation of autophagy vesicles and an increase in autophagy markers LC3-II and p62, indicating a defect in the autophagy flux. To characterize this autophagy defect, we used EGFP-mCherry-LC3 reporter, lysotracker, and Cyto-ID staining and identified that autophagy flux is blocked through inhibition of autophagosome and lysosome fusion when Rab27B is silenced. As autophagy has been shown to have a pro-survival role in tumor growth and stress response, we hypothesized that this defect in autophagy flux resulting from Rab27B deletion will impact cellular stress response and reduce tumor growth. Indeed, Rab27B knockout resulted in a 60% reduction in cell viability in response to starvation and a 94% reduction in colony formation in soft agar assay. Rab27B deletion also prevented spheroid formation *in vitro*. Finally, to analyze the effect of Rab27B deletion in tumor formation *in vivo*, we performed a xenograft study with wildtype and Rab27B knockout CRC cells HCT116 and observed that Rab27B deletion resulted in a significant reduction of tumor size ($p<0.0001$). Next, to analyze the therapeutic potential of Rab27B inhibition in CRC, we sought to identify small-molecule inhibitors of Rab27B GTPase activity *in vitro* through high-throughput drug screening from a panel of ~2,100 compounds contained within the KU-HTSL chemical library. This screening resulted in the identification of three compounds (BTB00809, BTB03584, and KM01532) that demonstrated Rab27B GTPase activity inhibition. Interestingly, inhibition of Rab27B in CRC cells with the compound km01532 resulted in a similar increase of LC3-II in CRC cells HCT116 and SW480 as seen in the Rab27B knockout. Taken together, these results demonstrate a new role of Rab27B in the autophagy trafficking process in CRC. Future studies will focus on characterizing the role of Rab27B in CRC tumor formation and growth *in vivo* using Rab27B knockout mouse model, as well as whether Rab27B can be targeted as a potential therapeutic strategy.

Rab27B deletion results in accumulation of autophagic vesicles in CRC cells (HCT116)



Deletion of Rab27B suppresses tumor growth in HCT116 cells *in vivo*



Sa1145

N6-METHYLADENINE DNA DEMETHYLASE ALKBH1 PROMOTES GASTRIC CARCINOGENESIS VIA DISRUPTING NRF1 BINDING CAPACITY

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Background and Aims: DNA N^6-methyladenine (6mA) is a recently discovered epigenetic modification and it regulates numerous biological processes. However, the role of DNA 6mA in gastric cancer (GC) remains unclear. We profiled genome-wide DNA 6mA modifications in GC, and explored the functional and molecular mechanism of ALKBH1 as a 6mA demethylase in gastric carcinogenesis. **Methods:** DNA 6mA was profiled by 6mA-IP-seq and LC-MS/MS. Downstream effectors and signalling pathways were analyzed by 6mA-IP-PCR, ChIP-qPCR, and RNA-seq. Gene expression was examined by RT-PCR, western blot, and immunostaining. The biological function of ALKBH1 was studied *in vitro and in vivo*. The clinical implication of ALKBH1 was determined in two human GC cohorts (n=412). **Results:** GC cell lines and primary GC tumors displayed the marked reduction in 6mA levels compared to normal gastric tissues and normal gastric GES1 cells. Consistently, 6mA-IP-seq showed that 6mA peaks in GC cells (112-2,967) were drastically reduced compared to normal gastric epithelial cell line GES1 (33,199). 6mA is enriched around transcription start sites with consensus motifs of GGAT, GAGG, and GAAG, and 6mA modification was significantly associated with transcriptional activation. To identify underlying molecular mechanisms deregulated 6mA in GC, we profiled the expression of 6mA regulators, revealing that ALKBH1, the 6mA demethylase, was up-regulated in GC compared to adjacent normal tissues (n=32, $P<0.05$). Moreover, high ALKBH1 expression was associated with poor survival in GC patients in our two GC cohorts (cohort I: n=276, $P=0.0093$; cohort II: n=136, $P=0.001$) and in TCGA dataset, implying that ALKBH1 might promote GC by suppressing 6mA. In line with this notion, ALKBH1 knockout increased 6mA, while suppressing GC cell growth, clonogenicity, G1-S cell cycle progression, and cell migration/invasion *in vitro*. Conversely, ALKBH1 overexpression induced GC growth *in vitro* and *in vivo*. Overlap of 6mA-IP-seq and RNA-seq datasets identified gene target NRF1 of ALKBH1-mediated 6mA demethylation in GC, whose interaction was validated by ChIP-qPCR. *In silico* screening of