Landscape of endocytosis pathway in colorectal cancer

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Introduction

Recent proteogenomic analyses of colorectal cancer (CRC) revealed that driver gene alterations are enriched in the endocytosis pathway [1]. Endocytosis is a cellular system involving post-translational modification of plasma membrane proteins through internalization, intracellular trafficking, degradation, and recycling. Clathrin-mediated endocytosis (CME) is the main endocytic portal, and endosomal sorting complexes required for transport (ESCRT) play a critical role in the lysosomal degradation pathway [2-3]. Besides the well-known function of endocytosis attenuating signaling pathways through receptor clearance from the cell surface, the opposite function contributing to signal maintenance has also been reported [4]. However, the clinical implications of the endocytosis pathway alterations in CRC are largely unclear.

Methods

- We retrospectively reviewed CRC patient samples (n=15025) submitted to a commercial CLIA-certified laboratory (Caris Life Sciences, Phoenix AZ).
- Next-generation sequencing of DNA and RNA (whole-transcriptome sequencing) and immunohistochemistry (IHC) were performed.
- CME-related (47 genes) and ESCRT-related (35 genes) expression signatures were calculated as composite z-scores and compared between subgroups stratified by *RAS/BRAF* mutation status, MSS/MSI status, tumor sidedness, and consensus molecular subtype (CMS). CME- and ESCRT-related genes are listed below.
- *VPS4A/VPS4B* expression correlation with major oncogenic pathway signatures (composite z-scores) was assessed.
- *CMTM6/CMTM4/HIP1R* expression association with PD-L1+ IHC (defined as tumors with moderate/strong staining in ≥5% of tumor cells) was also assessed.

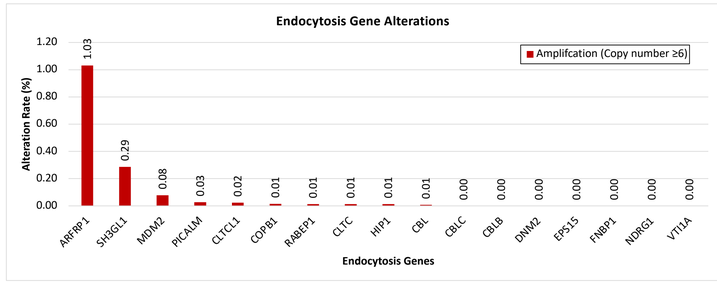
CME-related genes (47 genes)	ESCRT-related genes (35 genes)
CLTA, CLTB, CLTC, FCHO1, FCHO2, AP2A1, AP2A2, AP2B1, AP2M1, AP2S1, EPS15, EPS15L1 (EPS15R), ITSN1, ITSN2, SNAP91, PICALM, EPN1, EPN2, AMPH (AMPH1), BIN1, SNX9, DNM1, DNM2, DNM3, DNAJC6, GAK, HSPA8, LDLRAP1, DAB2, STON1, STON2, AGFG1, NECAP1, NECAP2, NUMB, ARRB1, ARRB2, SYNJ1, SYNJ2, INPPL1 (SHIP2), OCRL (OCRL1), AAK1, SCYL2, DYRK1A, HIP1, HIP1R, CTTN	*HGS (HRS), STAM (STAM1), STAM2, TSG101, VPS28, VPS37A (HCRP1), VPS37B, VPS37C, VPS37D, MVB12A, MVB12B, UBAP1, VPS25 (EAP20), SNF8 (EAP30), VPS36 (EAP45), PDCD6IP (ALIX), PTPN23 (HDPTP), BROX, CHMP1A, CHMP1B, CHMP2A, CHMP2B, CHMP3, CHMP4A, CHMP4B, CHMP4C, CHMP5, CHMP6, CHMP7, IST1, VPS4A, VPS4B, VTA1 (LIP5), USP8, MITD1*

Results

Cohort demographics

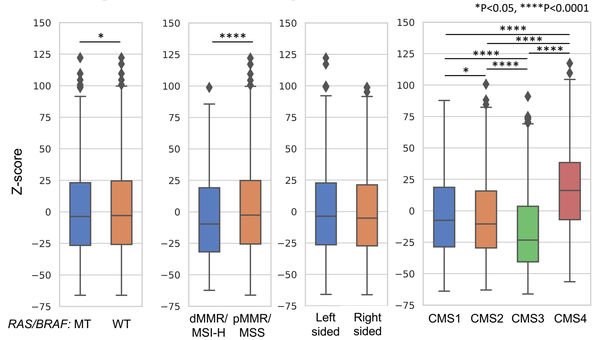
Median Age (range)		62.0 (14 - 90+)
Gender	Male	54.8% (8236/15025)
	Female	45.2% (6789/15025)
Primary Tumor Sidedness	Left	55.0% (8264/15025)
	Right	30.8% (4632/15025)
	Unknown	14.2% (2129/15025)
Biopsy site	Primary/Local	55.5% (8334/15025)
	Metastatic	42.4% (6380/15025)
	Unknown	2.1% (311/15025)

Gene alteration profile in endocytosis pathway

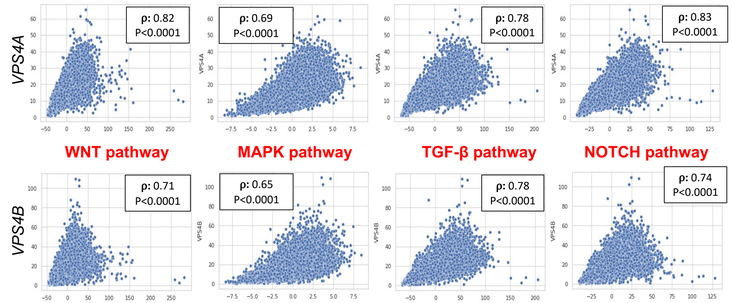


No pathogenic/likely pathogenic mutations were identified.

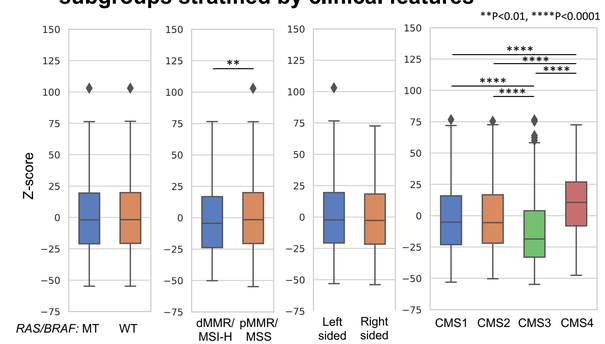
CME pathway signature compared between subgroups stratified by clinical features



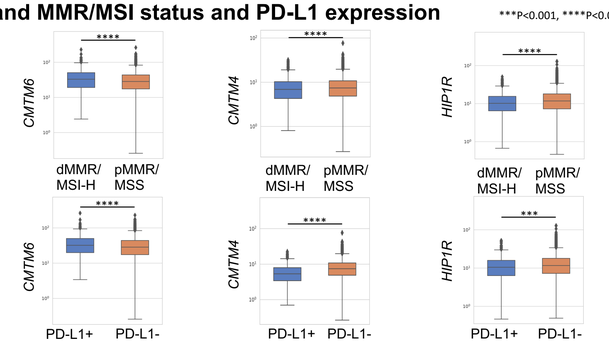
Association between *VPS4A/VPS4B* expression levels and oncogenic pathway signatures



ESCRT pathway signature compared between subgroups stratified by clinical features



Association between *CMTM6/CMTM4/HIP1R* expression levels and MMR/MSI status and PD-L1 expression



Summary

- Among 17 endocytosis-related genes, no pathogenic/likely pathogenic mutations were identified.
- The CME signature was increased in *RAS/BRAF* wild type vs. mutant (0.93 z-score difference, p=0.04) and MSS vs. MSI-high (6.0 z-score difference, p<0.01), while the ESCRT signature was higher in MSS compared to MSI-high (2.7 z-score difference; p<0.01). No differences between tumor sidedness were observed in both CME and ESCRT signatures (0.81 and 1.17 z-score differences, respectively). CMS4 had the highest expression of both signatures, while CMS3 had the lowest, of both CME and ESCRT signatures (each >20 z-score difference, p<0.01).
- *VPS4A* and *VPS4B* expression had a strong positive correlation with WNT, MAPK, TGF-β, and Notch pathway signatures (0.65-0.83 Spearman, all p<0.01).
- *CMTM6* expression was positively associated with PD-L1+ IHC (1.2-fold increase vs PD-L1-negative, p<0.01), while *CMTM4* and *HIP1R* expression showed a negative association (0.7- and 0.9-fold decrease, respectively, p<0.01).

Conclusions

This large study indicates endocytosis pathway expression is positively associated with oncogenic pathway signaling (WNT, MAPK, TGF-β, NOTCH) in CRC. Further analysis of *RAS/BRAF* wild type, MSS, and CMS4 patient subgroups are warranted to determine the efficacy of targeting endocytosis pathways in CRC.

References

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