Mitochondrial DNA Expression as Used to Define Metabolic and Immune States in Colorectal Cancer

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Abstract 546164

BACKGROUND

- Colorectal Cancer (CRC) exhibits metabolic reprogramming driven by the Warburg effect; however, active mitochondria and oxidative phosphorylation (OXPHOS) often remain crucial for tumor growth.
- Mitochondrial DNA (mtDNA) encodes critical OXPHOS components and influences the balance between OXPHOS and glycolysis, with effects on tumor microenvironment and response to immune checkpoint inhibitors (ICIs).
- We evaluated whether mtDNA gene expression predicts metabolic phenotype, immune contexture and benefit from ICIs.

METHODS

- 30,887 CRC cases with DNA/RNA sequencing were analyzed from Caris Life Sciences.
- Expression of mtDNA-encoded OXPHOS genes (MT-ND1–6, MT-ND4L, MT-CO1–3, MT-ATP6, MT-CYB) was summarized as a composite Z-score due to correlation (r>0.9).
- Tumors were stratified into quartiles (n=7,722 each), mtDNA-high (MT-H, top quartile) and mtDNA-low (MT-L, bottom quartile) cohorts.
- Overall Survival (OS) was calculated in months (m) from first treatment to last contact. Hazard ratios (HRs) were calculated using Cox proportional hazards models and p-values by log-rank tests.
- Pre-ranked Gene set enrichment analysis (GSEA) was performed to evaluate pathway differences.

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Table 1. Demographics of the mtDNA CRC cohort.

Characteristic	MT-H (n=7,722)	MT-L (n=7,722)	p-value
Age, median (range)	64.0 (19 - 90+)	63.0 (16 - 90+)	0.0019
Gender, % (Pos/Total)			0.1901
Female	45.5% (3513/7722)	44.4% (3432/7722)	
Male	54.5% (4209/7722)	55.6% (4290/7722)	
Site, % (Pos/Total)			0.0134
Primary	56.0% (4325/7722)	58.2% (4493/7722)	
Liver	21.1% (1628/7722)	19.4% (1497/7722)	
Metastatic	21.6% (1667/7722)	20.9% (1614/7722)	
Unclear	1.3% (102/7722)	1.5% (118/7722)	
Sidedness, % (Pos/Total)			0.1781
Left-sided	30.3% (2336/7722)	28.9% (2235/7722)	
Rectum/Anus	25.0% (1928/7722)	25.4% (1965/7722)	
Right-sided	23.2% (1789/7722)	24.3% (1879/7722)	
Transverse	5.0% (383/7722)	4.5% (350/7722)	
Unclear	16.7% (1286/7722)	16.7% (1293/7722)	
CMS, % (Pos/Total)			0
CMS1	14.9% (1136/7610)	17.2% (1303/7596)	
CMS2	**44.2% (3360/7610)**	**21.9% (1661/7596)**	
CMS3	**24.5% (1862/7610)**	**8.7% (664/7596)**	
CMS4	**16.5% (1252/7610)**	**52.2% (3968/7596)**	
Race, % (Pos/Total)			0.4159
Asian or Pacific Islander	2.5% (192/7722)	2.8% (220/7722)	
Black or African American	11.6% (899/7722)	12.3% (951/7722)	
White	3.6% (277/7722)	3.6% (280/7722)	
Other	26.0% (2004/7722)	25.6% (1976/7722)	
Unknown	56.3% (4350/7722)	55.6% (4295/7722)	
Ethnicity, % (Pos/Total)			0.0075
Hispanic or Latino	9.3% (717/7722)	10.6% (816/7722)	
Not Hispanic or Latino	69.5% (5366/7722)	67.4% (5208/7722)	
Unknown	21.2% (1639/7722)	22.0% (1698/7722)	
MSI-H, % (Pos/Total)	5.0% (383/7652)	8.2% (624/7630)	0
TMB-H, % (Pos/Total)	8.5% (624/7327)	12.8% (919/7205)	0

Table 2. Immune microenvironment of mtDNA-H vs mtDNA-L CRC

Immune	All	All	CMS1	CMS2	CMS3	CMS4
IC Genes	*CD274*	-1.164	-1.099	-2.096	-1.383	
	CD276	-1.335	-1.059	-1.192	-1.111	-1.155
	CD80	-1.363	-1.082	-2.448	-1.458	
	CD86	-1.037	-0.787	-1.695	-0.542	
	PDCD1	-1.611	-1.252	-2.269	-1.752	
	PDCD1LG2	-0.844	-0.573	-1.464	-0.841	
	IFNG	-0.813	-0.868	-2.445	-1.788	
	IDO1	-1.107	-1.075	-1.660	-0.827	
	HAVCR2	-1.409	-1.118	-1.799	-0.871	-0.689
	LAG3	-1.353	-1.522	-3.931	-1.804	
	CTLA4	-1.376	-1.197	-3.233	-1.405	
	FOXP3	-1.447	-1.175	-2.828	-1.338	-0.777
MCP	T cell	-1.163	-0.991	-2.020	-1.440	
	T cell CD8+	-1.022	-0.990	-2.053	-1.796	
	cytotoxicity score	-1.087	-0.934	-3.200	-1.387	
	NK cell	-1.100	-0.859	-2.087	-1.851	
	B cell	-1.578	-1.326	-3.220	-1.231	-1.536
	Monocyte	-1.319	-0.966	-2.334	-0.901	-0.759
	Macrophage/Monocyte	-1.319	-0.966	-2.334	-0.901	-0.759
	Myeloid dendritic cell	-1.210	-0.883	-1.844	-0.934	
	Neutrophil	-1.215	-1.117	-1.461	-1.323	-0.795
	Endothelial cell	-1.290	-0.990	-1.829	-1.451	
	CAFs	-1.467	-0.897	-1.909	-1.238	-0.993

RESULTS

Figure 1. Pre-Ranked GSEA in MT-H vs MT-L CRC.

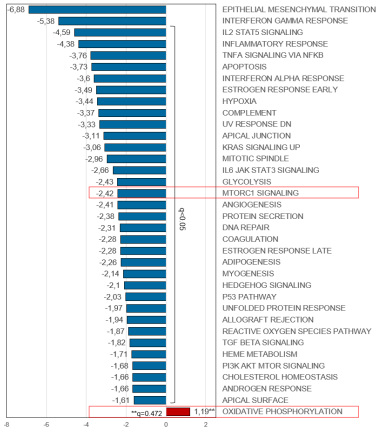
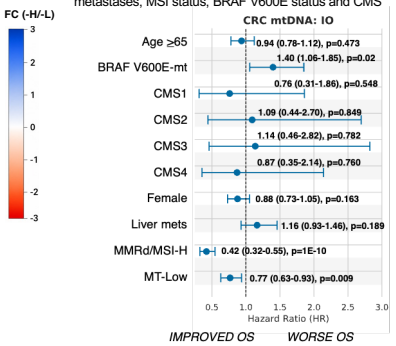
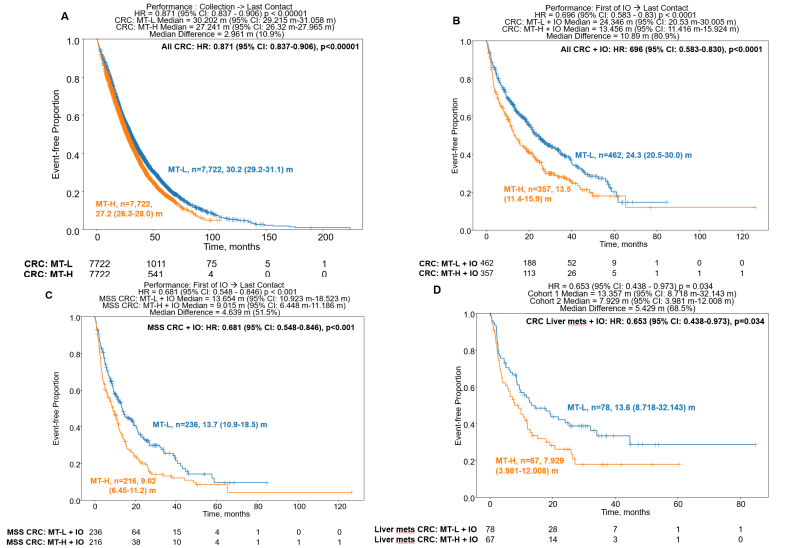


Figure 3. Multi-variate analysis adjusting for age, sex, liver metastases, MSI status, BRAF V600E status and CMS



- Pre-ranked GSEA showed a trend toward higher OXPHOS activity in MT-H tumors (FDR q=0.472), while glycolysis was downregulated (FDR q=0.002), with low inflammatory signaling and immune cell infiltration Table 2. Associations persisted in MSS CRC, all q<0.05.
- **MT-L was prognostic for improved OS vs MT-H** (mOS 30.2 vs 27.2 m; HR 0.87, p<0.00001, Fig 2A).
- **In ICI-treated patients, MT-L showed amplified effect** (mOS 24.3 vs 13.5 m; HR 0.69, p<0.0001, Fig 2B),
  - including in **MSS** (mOS 13.7 vs 9.02 m; HR 0.67, p<0.001, Fig 2C)
  - and in patients with **liver met** (mOS 13.4 vs 7.93 m; HR 0.65, p=0.034, Fig 2D).
- Multivariate analysis adjusting for age, sex, liver metastases, MSI status, BRAF V600E status and CMS confirmed **MT-L to be independently associated with improved OS** in ICI-treated patients (p=0.01, Fig 3).

Figure 2. (A) OS according to mtDNA expression status (MT-L vs MT-H) in all CRC patients (mOS 30.1 vs 27.2 months; HR 0.87, p<0.001), (B) ICI-treated patients (mOS 24.3 vs 13.5 months; HR 0.69, p<0.001), (C) MSS ICI-treated patients (mOS 13.7 vs 9.02 months; HR 0.68, p<0.001), and (D) patients with liver metastases treated with ICIs (mOS 13.4 vs 7.93 months; HR 0.65, p=0.034).



CONCLUSIONS

mtDNA-encoded OXPHOS expression defines biologically distinct CRC subsets with distinct metabolic states and immune infiltration, with MT-L linked to improved OS and enhanced benefit from immunotherapy, including in MSS and liver metastases where ICI sensitivity is limited.

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