Transcriptomic-Based Prediction of Therapeutic Response in Metastatic *RAS*-Mutant Colorectal Cancer

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Background

- Metastatic colorectal cancer (CRC) remains a leading cause of cancer-related mortality, with 5-year survival rates below 20%. Optimization of systemic therapy is essential to improve clinical outcomes.
- *RAS* mutations, present in ~40% of CRC, are associated with lower response rates compared with wild-type (WT) tumors.
- However, predictive biomarkers for therapeutic response in *RAS*-mutant (mt) CRCs have not yet been established.
- This study aimed to identify clinical and transcriptomic predictors of first-line treatment response in metastatic *RAS*-mt CRCs, and to develop a machine-learning model for predicting response using baseline RNA-seq data.

Methods

- **Cohort:** 328 patients with unresectable/metastatic CRC from the SCRUM-Japan MONSTAR-SCREEN-2, a nationwide prospective observational study (UMIN000043899), including **156 *RAS/BRAF* WT** and **172 *RAS*-mt** cases (Table 1).
- **Response classification:** Patients were classified based on first-line treatment outcomes.

Responders (R) = CR/PR; Non-responders (NR) = SD/PD

- **Clinical analysis:** Multivariate logistic regression using baseline clinical data.
- **Transcriptomic analysis:** Baseline whole transcriptome testing was performed (Caris Life Sciences, Phoenix AZ); differentially expressed gene (DEG) analysis was performed, and gene set enrichment analysis (GSEA) was conducted using (i) MSigDB Hallmark gene sets combined with published *RAS*-related signatures[1)], and (ii) Gene Ontology Biological Process (GO-BP) terms in *RAS*-mt CRCs.
- **Prediction model:** The *RAS*-mt cohort was randomly split into training (n = 120) and test (n = 52) sets (7:3). Machine-learning model was developed using PyCaret based on selected DEGs, and externally validated with The Cancer Genome Atlas (TCGA) dataset.

1) Klomp JA, et al. Defining the KRAS- and ERK-dependent transcriptome in KRAS-mutant cancers. *Science*. 2024;384(6700):eadk0775.

Table 1. Baseline Characteristics

Variable		*RAS/BRAF* WT N = 156	*RAS*-mt N = 172	*p* value
Age	Mean (SD)	59.2 (13.1)	61.9 (13.0)	0.064
Gender	F / M	57 / 99	91 / 81	0.0042
Sidedness	Right / Left	28 / 128	54 / 118	0.0073
Onset	Metachronous / Synchronous	33 / 123	43 / 129	0.49
Metastasis	0 / 1 / 2 / 3-5	5 / 84 / 46 / 21	5 / 86 / 59 / 22	0.83
Met-Liver	No / Yes	47 / 109	62 / 110	0.31
Met-Lung	No / Yes	106 / 50	96 / 76	0.032
Met-Peritoneum	No / Yes	126 / 30	132 / 40	0.45
CMS	1 / 2 / 3 / 4	6 / 89 / 16 / 45	20 / 53 / 46 / 53	< 0.001
Molecular-targeted drug	No / Yes	9 / 147	21 / 151	0.067
Chemotherapy	Mono / Doublet / Triplet	9 / 140 / 7	6 / 142 / 24	0.010

CMS; Consensus molecular subtypes

Results – Clinical analysis

- ***RAS*-mt** negatively predicted response (response rate: 49.4% vs 67.3%; odds ratio [OR] = 0.50, $p = 0.0062$) (Table 2).
- **CMS analysis** revealed that CMS3 was independently associated with non-response compared to CMS2 (40.3% vs 66.2%; OR = 0.42, $p = 0.013$), supporting the role of transcriptomic features in predicting response (Table 3).

Table 2. Multivariate Logistic Regression for Response

Variable	Group	Responders	Total	(%)	OR	95%CI	*p* value
	***RAS / BRAF* WT**	105	156	67.3	ref		
	***RAS*-mt**	85	172	49.4	0.50	(0.31-0.82)	0.0062
Age	20-50	34	63	54.0			
	50-70	107	170	62.9	1.01	(0.99-1.03)	0.49
	70-90	48	94	51.1			
Gender	F	78	148	52.7	ref		
	M	112	180	62.2	1.36	(0.84-2.22)	0.21
Sidedness	Right	38	82	46.3	ref		
	Left	152	246	61.8	1.89	(1.05-3.43)	0.035
Onset	Metachronous	40	76	52.6	ref		
	Synchronous	150	252	59.5	1.53	(0.85-2.74)	0.15
Metastasis	0-1	104	180	57.8	ref		
	2-5	86	148	58.1	2.25	(1.10-4.59)	0.026
Met-Liver	No	65	109	59.6	ref		
	Yes	125	219	57.1	0.49	(0.26-0.93)	0.028
Met-Lung	No	124	202	61.4	ref		
	Yes	66	126	52.4	0.46	(0.24-0.88)	0.019
Met-Peritoneum	No	152	258	58.9	ref		
	Yes	38	70	54.3	0.64	(0.32-1.32)	0.23
Molecular-targeted drug	No	12	30	40	ref		
	Yes	178	298	59.7	2.17	(0.95-4.96)	0.065
Chemotherapy	Mono	8	15	53.3	1.13	(0.37-3.46)	0.83
	Doublet	159	282	56.4	ref		
	Triplet	23	31	74.2	3.70	(1.49-9.19)	0.0049

Table 3. Multivariate Logistic Regression including CMS

Variable	Group	Responders	Total	(%)	OR	95%CI	*p* value
	***RAS / BRAF* WT**	105	156	67.3	ref		
	***RAS*-mt**	85	172	49.4	0.58	(0.35-0.97)	0.038
CMS	CMS1 (MSI immune)	13	26	50	0.58	(0.23-1.46)	0.25
	CMS2 (Canonical)	94	142	66.2	ref		
	CMS3 (Metabolic)	25	62	40.3	0.42	(0.22-0.84)	0.013
	CMS4 (Mesenchymal)	58	98	59.2	0.81	(0.45-1.47)	0.49

Other clinical variables were included in the analysis, but only *RAS/BRAF* status and CMS are displayed.

Results – Transcriptomic analysis

RAS-mut CRC 172 cases: Responders 85 vs Non-responders 87

- **DEG analysis** in *RAS*-mt CRCs identified 132 significant genes (false discovery rate [FDR] < 0.1) (Figure 1).
- **GSEA** revealed that *RAS*-mt responders showed downregulation of RAS signaling (NES = 1.39, $q = 0.033$), whereas non-responders exhibited enrichment of RAS-activated signatures (NES = –1.56, $q < 0.001$) and upregulation of pathways including MTORC1 signaling (NES = –2.08, $q < 0.001$), E2F targets (NES = –1.96, $q < 0.001$), G2M checkpoint (NES = –1.89, $q < 0.001$), and MYC targets (NES = –1.68, $q < 0.001$) (Figure 2).
- Distinct alterations in immune response and the tumor microenvironment genes were also observed between response groups (Figure 3).

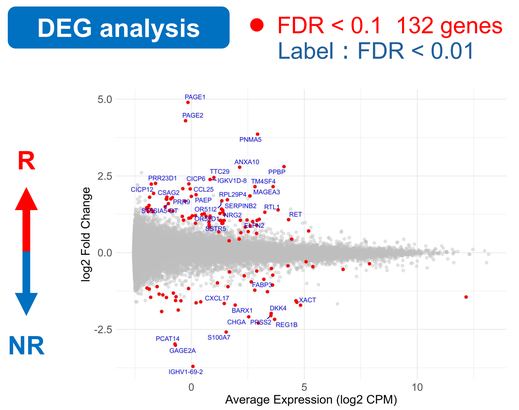

Figure 1. MA plot of DEGs (responders vs non-responders)

GSEA

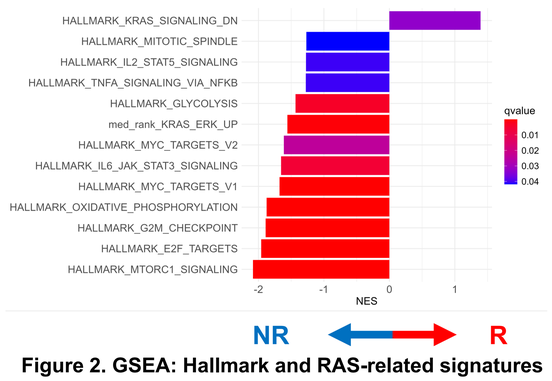

Figure 2. GSEA: Hallmark and RAS-related signatures

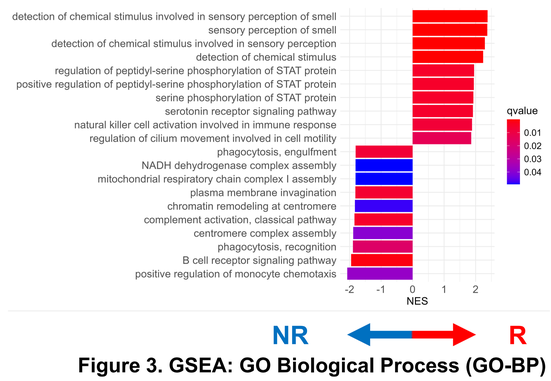

Figure 3. GSEA: GO Biological Process (GO-BP)

Results – Prediction model and Conclusions

Figure 4. MA plot of 26 selected DEGs
(FDR < 0.1 with feature selection)

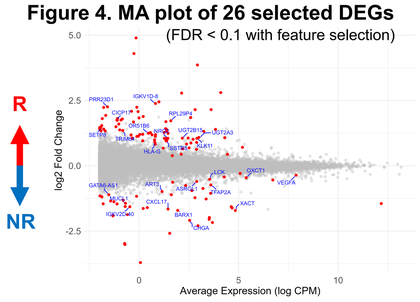

Figure 5. PCA plot

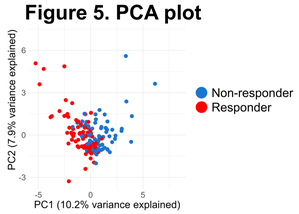

Figure 6. Discovery cohort (n = 52, test set)

Model	Accuracy	AUC	Recall	Prec.	F1	Kappa	MCC
Logistic Regression	0.7692	0.7737	0.7692	0.7692	0.7692	0.5385	0.5385

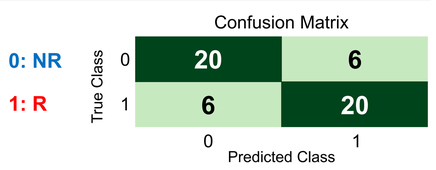

Figure 7. External validation (n = 22, TCGA)

Model	Accuracy	AUC	Recall	Prec.	F1	Kappa	MCC
Logistic Regression	0.7727	0.8118	0.8000	0.5000	0.6154	0.4660	0.4920

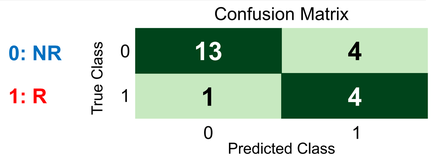

Table 4. Representative DEGs by functional category

Tumor progression & microenvironment	KLK11, VEGFA, CHGA, CXCL17
Transcription factors & signaling	BARX1, TFAP2A
Metabolism (energy & drug)	ART3, OXCT1, UGT2A3, UGT2B15
Immune-related genes	IGKV, LCK, HLA-G

- PCA of 26 DEGs, selected from 132 candidates (FDR < 0.1) using PyCaret, demonstrated clear separation between responders and non-responders (Figures 4 and 5).
- The predictive model achieved **76.9% recall and 76.9% precision** in the discovery cohort (Figure 6).
- **External validation** using TCGA data confirmed robust performance, correctly classifying **80.0% (4/5) of responders and 76.5% (13/17) of non-responders** (Figure 7).
- Functional categorization of the selected DEGs highlighted roles in tumor progression, signaling, metabolism, and immunity (Table 4).
- **In conclusion**, this transcriptomic analysis established a clinically relevant model for predicting therapeutic response in metastatic *RAS*-mt CRC.
- Future directions include integrating clinical parameters with transcriptomic features to further improve predictive accuracy and enhance their value for personalized treatment strategies.

R. Yokoi has no conflicts of interest to declare.