Cross-platform gene expression signature for microsatellite instability in colon and gastric cancers

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Objective:

- **To construct a cross-platform gene expression signature for microsatellite instability in both colon and gastric cancers.**

Introduction:

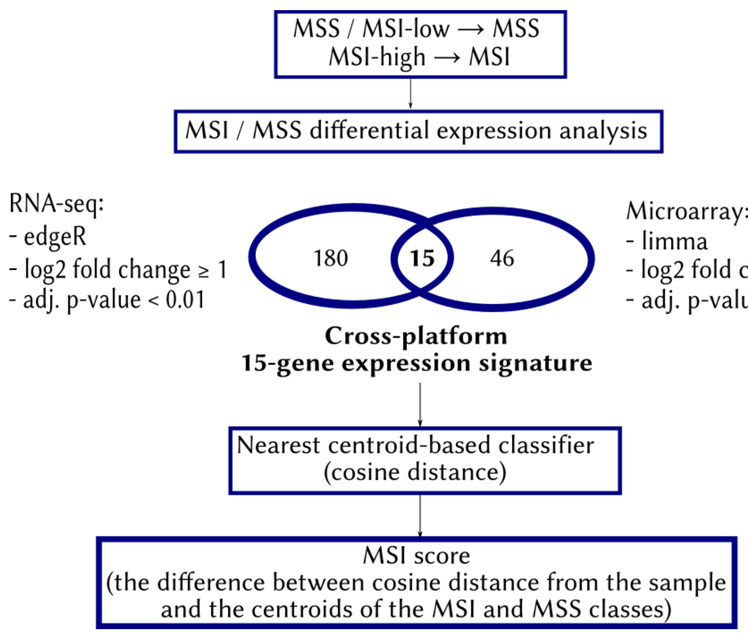
- The dysfunction of DNA mismatch repair system results in microsatellite instability (MSI).
- In colon cacner, MSI is a favourable prognosis marker, despite being associated with resistance to 5-fluorouracil treatment.
- In gastric cancer, its prognostic value is not so well established.
- Recognising the MSI tumours is of clear clinical importance and having **a single cross-platform gene expression signature** for both cancers has immediate practical benefits.

Methods:

Datasets

cohort	N (MSI)	platform	tissue	reference
A1 development	175 (35)	RNA-seq	colon	TCGA
A2 development	369 (56)	microarray	colon	GSE39582
B1 validation	83 (10)	microarray	colon	GSE39582
B2 validation	103 (20)	microarray	colon	GSE41258
C1 validation	335 (54)	RNA-seq	gastric	TCGA
C2 validation	34 (16)	microarray	gastric	GSE13911

Construction of the cross-platform gene expression signature for MSI status



(MSI: microasatellite instability;
MSS: microastellite stability)

Performance evaluation of the cross-platform 15-gene expression signature

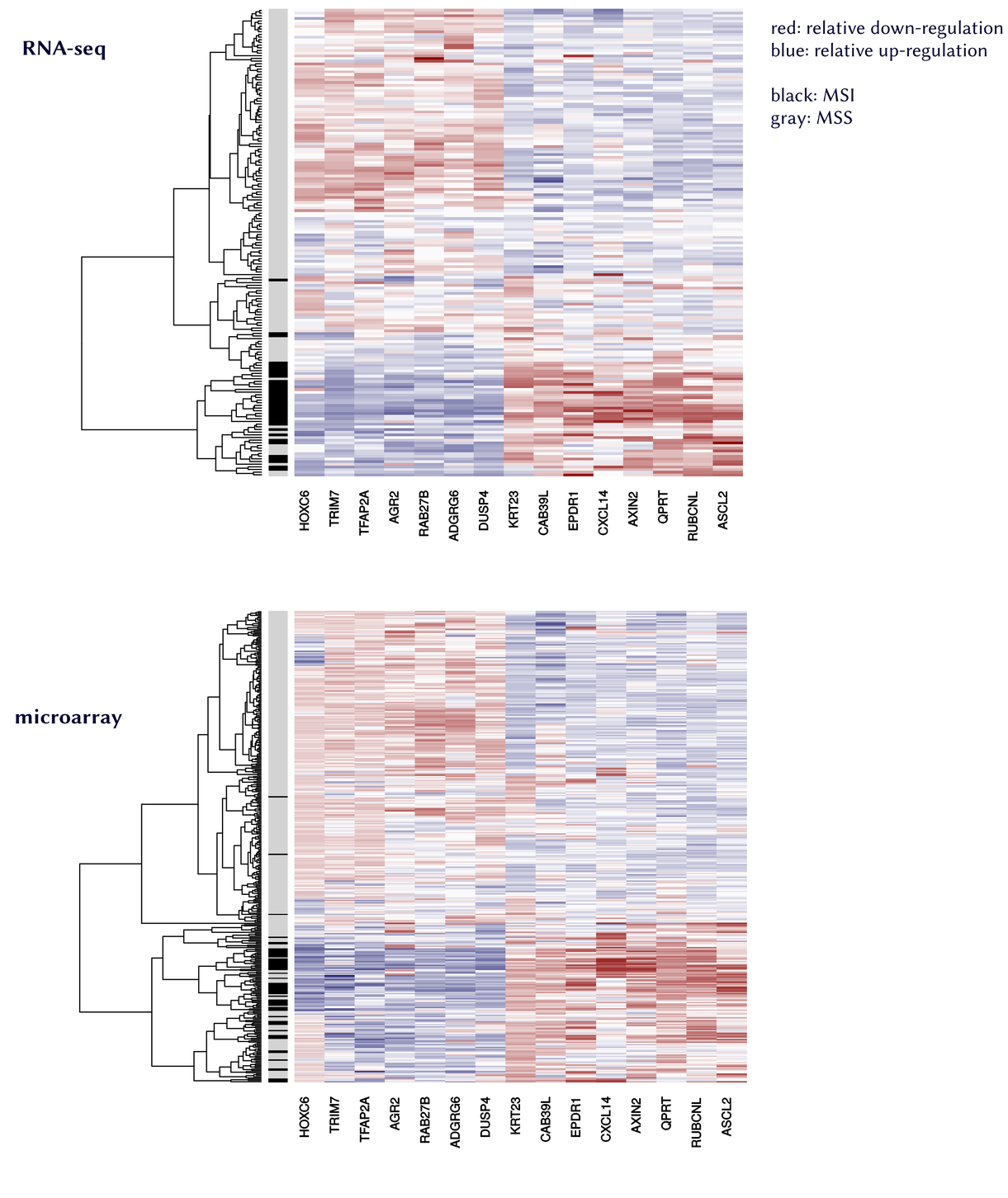
- The performance was estimated using 10-fold cross-validation.
- The performance index ⟶ an area under the receiver operating characteristic curve (AUC); 95 % confidence intervals (CI), normal approximation
- Validation was done using two independent colon cancer datasets (cohort B1 and cohort B2)
- The performance of the cross-platform gene expression signature was also evaluated on 369 **gastric cancer** samples (cohort C1 and cohort C2)
- Comparison with published MSI gene expression signatures trained exclusively on microarray datasets:
  - 8-gene signature[1] (two genes excluded from the nearest centroid classification)
  - 64-gene signature[2] (nine genes excluded from the nearest centroid classification)
- McNemar's test used to compare the accuracy of cross-platform 15-gene expression signature and published MSI gene signatures
- Correlation analysis was performed to detect a great deal of redundancy among genes from signatures (Spearman's correlation)

Pathway enrichment analysis and survival analysis

- The pathway enrichment analysis was performed against MSigDB gene collections
- The prognostic value of the cross-platform 15-gene expression signature was assessed by fitting Cox regression model:
  - relapse-free survival (RFS) in stage II and stage III cohort A2 sub-population (adjusted p-value < 0.1)

Results:

Cross-platform 15-gene expression signature for MSI status



Performance evaluation of the cross-platform 15-gene expression signature

cohort	AUC	95% CI	platform	tissue
A1 development	**0.93**	0.80 - 1	RNA-seq	colon
A2 development	**0.87**	0.62 - 1	microarray	colon
B1 validation	**0.93**	0.83 - 1	microarray	colon
B2 validation	**0.84**	0.73 - 0.96	microarray	colon
C1 validation	**0.86**	0.80 - 0.93	RNA-seq	gastric
C2 validation	**0.87**	0.74 - 1	microarray	gastric

- RNA-seq cohort A1: Significantly higher accuracy of the 15-gene signature than the 8-gene signature (p-value < 0.01)
- RNA-seq cohort A1: The assumptions of McNemar's test was not fulfilled!
  (Significantly higher accuracy of the 64-gene signature than the 15-gene signature (p-value 0.01529))
- 8 genes identified both in the 15-gene signature and in the 64-gene signature
- High redundancy of 64-gene signature in RNA-seq cohort A1 (14 gene-pairs with $|r| > 0.75$)
- Gene AXIN2 (HR=0.66, 95% CI=(0.50 – 0.87)) was significantly associated with RFS (adjusted p-value 0.076).

Pathway enrichment analysis and survival analysis

MsigDB gene set	adj. p-value	genes in overlap
ttgttt foxo4 01 [3]	0.056	DUSP4,ASCL2,KRT23,AGR2,CAB39L,HOXC6,AXIN2,TFAP2A
Ik2 01	0.002	HOXC6,ASCL2,TFAP2A,AXIN2,CXCL14
sansom apc targets [4]	0.010	ASCL2,AXIN2,KRT23,DUSP4
sansom apc targets up [4]	0.054	ASCL2,KRT23,AXIN2
koinuma colon cancer msi dn[5]	0.012	QPRT,AXIN2

Discussion and conclusion:

- We present the cross-platform 15-gene expression signature that yields high accuracy in MSI phenotype prediction in both RNA-seq and microarray studies.
- The pathway enrichment analysis results support the 15-gene expression signature association with colon cancer MSI phenotype.
- Despite being derived from colon cancer datasets, the signature maintained a good performance on gastric cancer datasets.
- From a biologic perspective, this supports the idea that MSI gene expression pattern is comparable across various cancers pointing towards similar regulatory pathways.

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