A panel of methylation markers for multi-cancer detection from plasma

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Introduction

- Cancer is the leading cause of premature mortality in the world and in dire need of early detection tools (1)
- Measuring cancer-related alterations in the tumour-derived portion of the cell-free DNA (cfDNA) could offer an accurate, non-invasive approach, leading to decreased cancer mortality (2-3)
- We report here a plasma targeted methylation marker panel performance for detection of 4 cancer types – colorectal (CRC), lung (LC), pancreatic (PaC) and breast (BC) cancer

Methods

- Human Methylation 450K data available from The Cancer Genome Atlas (TCGA) consortium was used for initial methylation marker selection (4)
- Candidate methylation marker regions were evaluated in the plasma samples of 101 patients with cancer and 71 age/gender- matching non-cancer controls (Table 1)
- Methylation-sensitive restriction enzyme–qPCR approach was used to target regions of interest in plasma cfDNA
- dCt (delta cycle threshold) values were used for further analysis
- Random forest feature selection algorithm utilizing Monte-Carlo cross-validation over 50 sub-setting was utilized for building and testing marker panels:
  - Panel 1 was used for overall cancer detection
  - Panel 2 was used for localization of tissue of origin.
- Accuracy was defined as the fraction of correct calls.

Table 1 Sample set

	Control	Colorectal Cancer	Pancreatic Cancer	Lung Cancer	Breast Cancer
Characteristics	(n=71)	(n=20)	(n=15)	(n=37)	(n=28)
Age (years, average (IQR))	60 (32-78)	59 (28-77)	60 (50-71)	59 (42-75)	57 (41-77)
Gender (n (%))					
Female	36 (51%)	10 (50%)	7 (47%)	12 (32%)	28 (100%)
Male	35 (49%)	10 (50%)	8 (53%)	25 (68%)	
Stage					
Stage I		6		15	7
Stage II		9		4	8
Stage III		5	15	17	13
Unknown				1	1

Results

- Differential methylation could be observed on single marker level (Figure 1 A)
- 10 methylation marker panel (Panel 1) showed area under curve (AUC) of 89% (Figure 1B), with 79% (80/101) sensitivity for cancer at 90% (64/71) specificity
- 100% of the CRC (20/20), 80% of the PaC (12/15), 75% of the BC (21/28) and 73% of LC (27/37) were correctly identified as cancer patients (Figure 2A)
- Notably, the sensitivity for stage I cancers was 75% (21/28) (Figure 2B).

Figure 1 Performance of methylation markers. A. *dCt values plotted for 4 markers for control samples (red) and cancer samples (blue).* ***B.*** *ROC curve and AUC for 10-marker panel performance (Panel 1)*

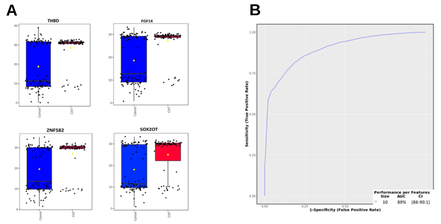
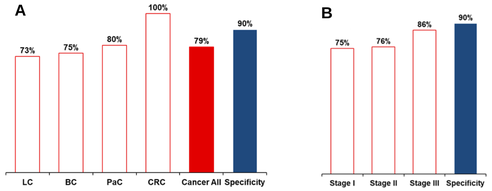


Figure 2 Performance of 10 methylation marker panel (Panel 1). A. *Accuracy values, where red bar represents the overall sensitivity for cancer, red-outlined bars represent sensitivity by cancer type, and blue bar represents specificity.* ***B.*** *Accuracy values, red-outlined bars represent sensitivity by stage and blue bar represents specificity*



- Cancer cases that were correctly separated from control group were further evaluated for their tissue of origin.
- Individual markers showed differential methylation levels between different cancer types (Figure 3)

- 16 methylation marker panel (Panel 2) allowed correct tissue of origin assignment of 80% of CRC (16/20), 78% of LC (21/27), 75% of PaC (9/12) and 62% of BC (13/21) cases (Table 2).

Figure 3 Individual marker performance . A. *dCt values plotted for 4 markers for BC (red), CRC (green), LC (dark blue), Pac (light blue)*

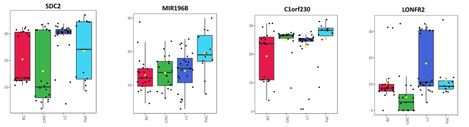


Table 2 Confusion matrix according to 16-marker panel (Panel 2). *Diagonal line indicates the correct classification*

	BC	CRC	LC	PaC	class. error	Correct
BC	**13**	0	4	4	0.38	62%
CRC	0	**16**	2	2	0.2	80%
LC	1	3	**21**	2	0.22	78%
PaC	1	1	1	**9**	0.25	75%

Conclusions

- We show that targeted methylation marker panels have potential for early blood-based detection of multiple cancers with high sensitivity and specificity and further tissue of origin localization.
- This method could serve as the basis for further development of a simple, highly accurate and minimally invasive blood-based multi-cancer screening test.

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