Sub-classification of colorectal cancer using surface antigen antibody microarray and fluorescence multiplexing

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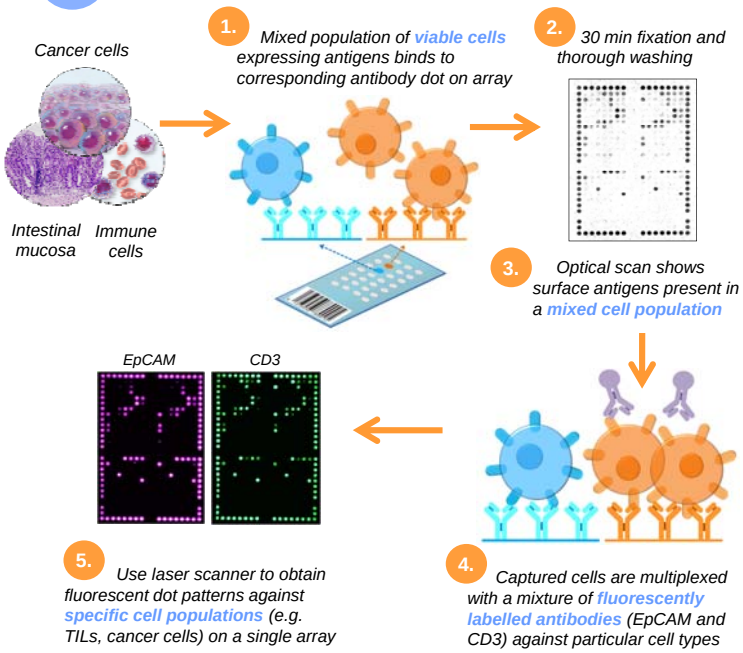
1 Background

Colorectal cancer (CRC) is the second most frequent cause of cancer deaths in Australia. Even after resection up to **50% of patients relapse**. In an attempt to prevent recurrences chemotherapy is administered to high risk patients. However, as few as 10-20% patients genuinely benefit because the clinical course for individuals with **CRC remains difficult to predict**, largely due to prognostically heterogeneous groups within same-stage tumour categories.

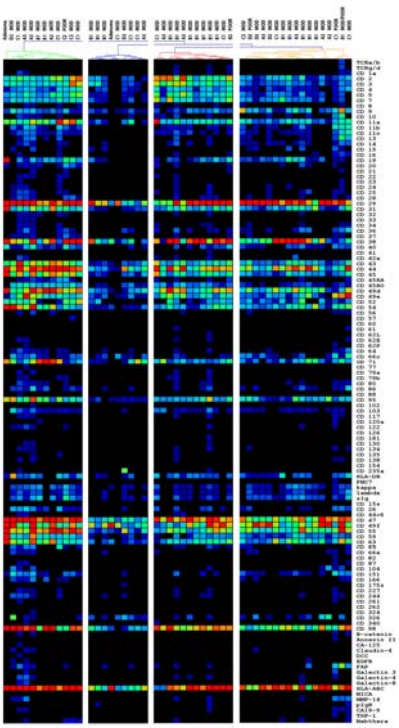
Cancer specific biomarkers have played crucial roles in cancer characterisation and prediction. **Surface molecules (also known as CD antigens)** make ideal biomarkers, as their expression often evolves with tumour progression or interactions with other cell types, such as tumour infiltrating lymphocytes(TILs) and tumour associated macrophages (TAMs).

Our study describes a method for the rapid processing of surgical CRC samples and control intestinal mucosa for the profiling of **122 surface antigens** on CRC cells, intestinal epithelial cells and lymphocytes. The CRC DotScan microarray takes a **molecular signature approach to CRC classification** and should be the prototype for a diagnostic alternative to the anatomically-based CRC staging system.

2 Results



3 Results



Heatmap colours correspond to numerical values of cell binding densities converted using DotScan software
A1-3, B1-3, C1-3 and D1-3 denote Australian Clinico-pathologic Staging (**ACPS**), WELL, MOD, and POOR denote tumour differentiation stages: well moderate and poor, respectively

Figure 1: Heatmaps of CRC optical scan with hierarchical clustering

Table 1: Differentially expressed antigens between control and tumour optical scans (P value< 0.05)

Antigen	Control	Tumour	P-value
CD 2	28	53	0.00001
CD 3	27	47	0.00003
CD 4	12	29	0.00001
CD 5	20	38	0.00001
CD 7	23	39	0.00002
CD 9	55	33	0.00001
CD 11a	41	54	0.01418
CD 11b	6	18	0.00001
CD 11c	5	16	0.00001
CD 36	6	14	0.00001
CD 49e	19	48	0.00001
CD 59	44	34	0.04322
CD 63	67	56	0.05147
CD 66c	3	18	0.00001
CD 98	68	84	0.01852
CD 71	31	47	0.01278
FAP	3	11	0.00006
HLA-DR	8	25	0.00001

CD2, CD3, CD4, CD5, CD7 CD9 CD11a, CD11b, CD11c, CD36, CD98 and HLA-DR are **lymphocytic antigens**

CD9,CD49e, CD59, CD63, CD66c and CD98 are **colorectal cancer differential antigens**

CD71 **Transferrin receptor**

Table 2: Differentially expressed antigens between control and tumour CD3 scans (P value< 0.05)

Antigen	Control	Tumour	P-value
CD 5	63	83	0.04177
CD 9	20	12	0.00807
CD 11c	4	11	0.00376
CD 28	8	17	0.04779
CD 45RO	47	65	0.03656
CD 49e	19	52	0.00007
CD 57	6	15	0.00350
CD 71	11	44	0.00001
CD 95	49	70	0.02099
CD 98	58	90	0.00271
HLA-DR	4	23	0.00001

CD45RO, CD71, CD98, HLA-DR and CD95 **activation markers**

CD5 and CD28 are **T-cell specific markers**

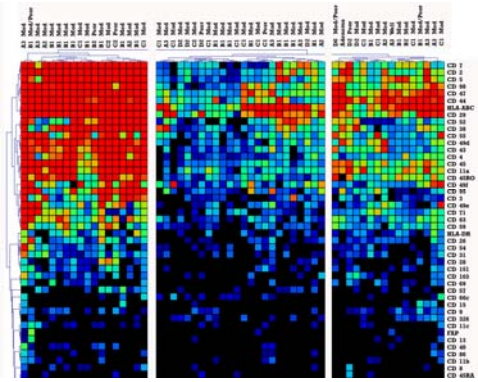


Figure 2: Heatmaps of CRC CD3 multiplexing with hierarchical clustering

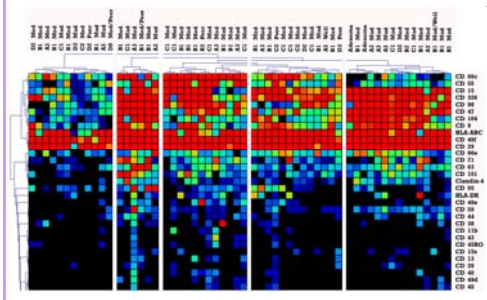


Figure 3: Heatmaps of CRC EpCAM multiplexing with hierarchical clustering

Table 3: Differentially expressed antigens between control and tumour EpCAM scans (P value< 0.05)

Antigen	Control	Tumour	P-value
CD 44	6	13	0.01856
CD 49e	3	19	0.00057
CD 55	42	84	0.00006
CD 66c	21	108	0.00001
CD 71	25	44	0.02660
CD 95	74	39	0.00018
CD 98	34	92	0.00001
CD 151	30	22	0.04540
HLA-DR	5	20	0.00473

CD44, CD55 and CD151 overexpression in CRC associated with **poor prognosis**

In CRC reduced HLA-DR expression correlates with **poor prognosis**

4 Conclusion

- **Surface profiles** determined for **mixed populations** of cells in CRC tissue
- Working towards a **molecular approach** to the sub-classification of CRC
- **Quantification** and profiling of the **T lymphocyte population** within tumours
- **Increased** number of samples should enable **accurate sub-classification** of CRC from surface profiles

Ellmark P, Belov L, Huang P, Lee CS, Solomon MJ, Morgan DK, Christopherson RI. (2006) Multiplex detection of surface molecules on colorectal cancers. Proteomics. 6(6):1791-802.

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Prall, F., Duhrkop, T., Weirich, V., Ostwald, C., Lenz, P., Nizze, H. and Barten, M. (2004) Prognostic role of CD8+ tumor-infiltrating lymphocytes in stage III colorectal cancer with and without microsatellite instability. Hum Pathol 35, 808-16.