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KRAS AND CTLA-4 EXPRESSION PROFILING IN CIRCULATING TUMOR CELLS IN PATIENTS WITH COLORECTAL CANCERS

Sharmin Aktar[a], Tracie Cheng[b], Sujani MK Gamage[c], Farhadul Islam[d], Vinod Gopalan[a], Alfred King-yin Lam[a]

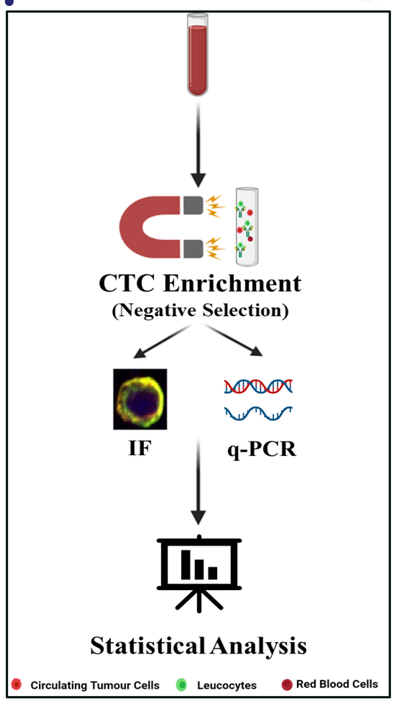
[a]Cancer Molecular Pathology, School of Medicine and Dentistry, Griffith University, QLD, Australia, [b]School of Nursing and Midwifery - Nursing, Griffith University, QLD, Australia, [c]Faculty of Health Sciences & Medicine, Bond University, QLD, Australia, [d]Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi, Bangladesh

BACKGROUND

Globally, colorectal cancer (CRC) is one of the most prevalent and deadly cancers (1). **Circulating tumour cells (CTCs)** may develop **immune-suppressive networks** in the tumour microenvironment and acquire resistance to immunotherapy by acquiring genetic alterations in oncogenes which may affect the expression of immune regulatory molecules (PD-L1, PD-L2, CTLA-4, CD47) in cancer. To date, it has been discovered that KRAS activation can upregulate PD-L1 molecules, which may aid in tumour immune evasion (2, 3). However, the regulatory effects of KRAS on the expression of CTLA-4 in patients with CRC have yet to be explored.

Thus, for the first time, we aimed to investigate **KRAS and CTLA-4 mRNA expression profiling and their correlations in CTCs** from patients with CRC. Additionally, the correlations of the presence of higher CTCs with various clinical and pathological parameters in patients with CRC were analyzed.

METHODOLOGY



RESULTS AND DISCUSSION

Enumeration of CTCs and counts in patients with CRC

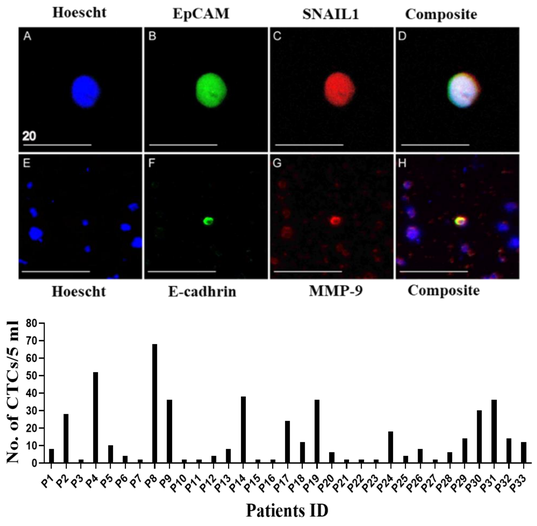


Figure 1: The cells, which were positive for at least one of the cell surface biomarkers (EPCAM, SNAIL-1, E-Cadherin and MMP-9), along with enlarged nucleus and cell size >8µm were considered as CTCs. 66% (n=33/50) of patients with CRC were CTC-positive. Images were captured by Olympus Fluoview FV1000 Confocal Microscope (Scale bar: 20 µm).

KRAS and CTLA-4 gene expression in CTC neg vs CTC pos groups

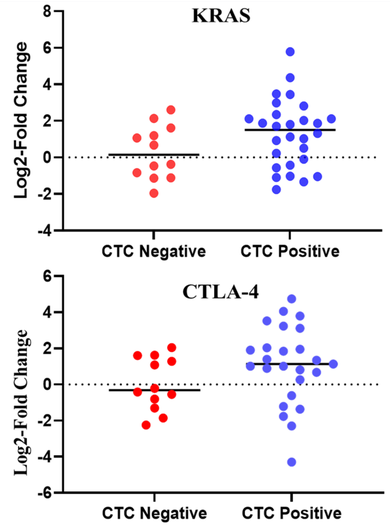


Figure 2: A high expression of KRAS and CTLA-4 genes was noted. These genes play a significant role in the immune escape pathways of CTCs in circulation.

This study has first time found a higher expression level of CTLA-4 in CTCs which may suggest that CTLA-4 can also be expressed in CTCs like other immune checkpoint molecules such as PD-L1 and might be involved in CRC development by promoting immune evasion.

Correlation of the mRNA expression level between CTLA-4 and KRAS

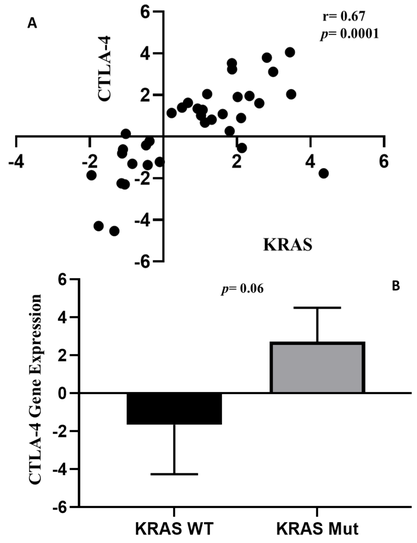


Figure 3: Positive correlation between CTLA-4, and KRAS expression (r=0.67, p=0.0001) (Spearman rank test). CTLA-4 gene expression level tended to be higher in CTCs fractions from patients with KRAS mutation (p<0.06) (From clinical data analysis) which further strengthen the idea that **activation of oncogenes may aid CTCs in evading immune surveillance by modifying the expression of immune genes** (2, 3, 4, 5).

Clinicopathological features of the presence of CTCs in patients with CRC

Characteristics	Total (n=50)	CTC=0 (n=12)	CTC<10 (n=19)	CTC≥10 (n=19)	p-value
Size					
≤ 50mm	33	8	16	9	**0.051**
> 50mm	17	4	3	10	
Subtype					
Conventional	39	9	19	11	**0.019**
Mucinous	11	3	1	7	
Overall stage					
I & II	33	11	14	8	**0.006**
III-IV	17	1	5	11	

Table 1

- Late-stage cancers more often had higher CTCs when compared to early-stage cancers (p=0.0064).
- Patients having higher CTCs were more likely to have mucinous adenocarcinoma (p=0.019),
- Similarly, individuals with higher CTCs were more likely to have larger tumour sizes (>50mm, p=0.051).
- Monitoring **CTCs counts** have a significant role in **predicting the clinicopathological outcomes (especially tumour progression and aggressiveness)** in patients with CRC (6-9).

CONCLUSION

To conclude, this study has investigated **the role of KRAS on CTLA-4 gene expression in CTCs**, and it has the potential to aid in the selection of therapeutic targets in patients with an inhibitory immune gene signature. Future studies can focus on analyzing mutational profiles and the interaction of these genes with different tumour-infiltrating immune cells to better understand the potential role of these genes in CRC pathways.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest

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sharmin.aktar@griffithuni.edu.au