The morphological features of colorectal tumors that are associated with the appearance of methylated DNA in plasma

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Introduction

- Blood-based colorectal cancer (CRC) screening tests that detect circulating tumor-associated methylated DNA such as *SEPT9*, *BCAT1* or *IKZF1* provide an additional strategy for CRC screening [1-3] (Figure 1).
- Acceptance of these new modalities by screening providers could be enhanced through a better understanding of the biology and mechanism(s) that result in the presence of these tumor biomarkers in blood.
- We recently conducted a clinical evaluation of a two-gene (methylated *IKZF1* and *BCAT1*) blood test in volunteers scheduled for colonoscopy or tumor resection.

Aim

- To determine whether the presence of methylated *BCAT1* and *IKZF1* in plasma correlates with morphological features of the colorectal cancer.

Methods

- The study population consisted of volunteers undergoing colonoscopy for any indication (n = 1777).
- Blood was collected immediately prior to colonoscopy or before resection of the tumor.
- Levels of methylated *BCAT1* and *IKZF1* in bisulphite-converted DNA isolated from 4mL of plasma were measured using a multiplexed methylation specific PCR assay (Clinical Genomics Pty Ltd, Australia).

Methods (cont..)

- Any sample containing detectable levels of either methylated gene was considered positive.
- Cancer histopathological assessment to determine cancer stage and features were undertaken by an expert pathologist.
- Characteristics including T stage (TNM staging), nature of the tumor margin, degree of differentiation, perivascular or perineural invasion and metastasis were determined.
- Univariate (Chi^2 test) and multivariate logistic regression analysis were performed to determine the factors that are associated with test positivity. A Spearman rank correlation was performed to assess the correlation of biological features to the levels of methylated *BCAT1* and *IKZF1* in the plasma. A p value less than 0.05 was considered statistically significant.

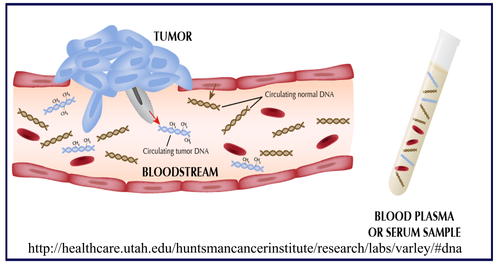

Figure 1. Proposed mechanism of genetic material from tumor entering into circulation

Results

- A total of 127 patients with cancer were identified (median age 68yrs, 62% male)
- Distribution of cancer stages (AJCC) were stage I (n=33), Stage II (n=37), Stage III (n=35), Stage IV (n=22) with three cancers unstaged.
- Univariate analysis revealed advanced cancer stage (AJCC), presence of vascular invasion, presence of nodal metastasis and distant metastatic stage were significantly associated with presence of markers in the blood ($p < 0.05$, Table 1 and Figure 2).
- Following adjustment for the biological features, multivariate logistic regression analysis revealed *depth of invasion* (T3: OR 69.84, 95% CI 5.65-863.12; T4: OR 187.17 95% CI 9.94-3525.43) and *distal location* (OR 5.99, 95% CI 1.42-25.32) were significantly associated with detectable marker ($p<0.001$)
- Concentration of *BCAT1* and *IKZF1* in plasma was strongly associated with depth of invasion ($r=0.461$, $r = 0.481$, respectively, $p < 0.0001$) and overall tumor size ($r=0.348$, $r = 0.463$, respectively, $p < 0.0001$). .

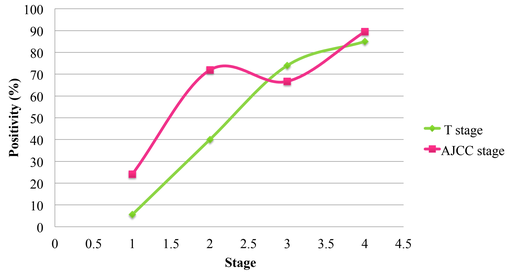

Figure 2. Positivity rate at the different T and AJCC stages

Biological variable	Total number	% Positive	P value
T Stage	119		P<0.0001
T1	20	8%	
T2	17	35.3%	
T3	56	75.0%	
T4	26	88.0%	
AJCC Stage	124		P<0.0001
I	33	21.2%	
II	37	75%	
III	33	69.7%	
IV	21	90.5%	
Nodal stage	113		P=0.002
N0	69	52.1%	
N1	29	65.5%	
N2	15	100.0%	
Distant metastasis	92		P=0.003
M0	74	51.3%	
M1	18	88.9%	
Vascular invasion	114		P=0.005
No	83	50.8%	
Yes	31	80.6%	

Table 1- Significant biological variables on univariate analysis

Conclusion

- Plasma concentrations of methylated *IKZF1* and *BCAT1* correlate positively with depth of cancer invasion, advanced stage and presence of nodal or distant metastases.
- The appearance of tumor-associated DNA in the circulation appears to be dependent on depth of invasion and extent of spread.

References

1. deVos T, *et al*. Clin Chem 2009;55:1337–1346
2. Church TR, *et al*. Gut 2014;63:317–325
3. Pedersen, *et al*. PLoS One. 2015;10(4):e0125041