Novel Non-Invasive Markers for Colorectal Cancer and Adenoma Detection

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Disclaimer

• I am one of the inventors of novel bacterial gene markers (M3 CRC) in CUHK and a scientific co-founder of GenieBiome Ltd

• My lecture will focus on the scientific basis and my personal view on the clinical applications of stool microbiome test for CRC/adenoma

• I will not discuss commercial activities related to M3CRC
Outline

Unmet need for colorectal cancer (CRC) screening?

Role of microbiome in CRC?

Utilising microbial biomarkers for detection and monitoring of CRC and adenomas
I need Courage.
I need a heart.
I need a brain.

I'm over 50.
I need a colorectal exam.
CRC Biomarkers

**Blood-based**
- Diagnostic (early-stage, premalignant): SEPT9 methylation, SFRP2 methylation, miR-21, miR-92a, miR-29a
- Diagnostic (late-stage, malignant): SDC2 methylation, SEPT9 methylation, H3K27me, miR-21, miR-92a, miR-221
- Prognostic: HPP1 methylation, HLFY methylation
- Predictive: miR-155, miR-106

**Blood-based biomarkers from endoscopically resected lesions**

**Stool-based Biomarkers**
- Diagnostic (early-stage, premalignant): ITG4 methylation, SFRP2 methylation, ColoGuard, miR-21, miR-92a, miR-135b
- Diagnostic (late-stage, malignant): SFRP2 methylation, VIM methylation, TP53 methylation, miR-21, miR-92a, miR-223

**Stool-based**
- Methylation markers

**Tissue-based**
- Methylation markers in surgical specimens
  - Prog nostic and/or predictive: H3K4me2, H3K27me2, miR-34a

**Primary tumour**
- Tissue-based biomarkers in surgical specimens
  - Prognostic: Cdkn2a methylation, Chfr methylation, Igf2 methylation, H3K27me2, H3K56a, H4K16ac, miR-31
  - Predictive: MGMT methylation, Tafap2a methylation, miR-106, miR-19a

**Metastasis**
- Tissue-based biomarkers in surgical specimens

Jung G et al, Nat Rev Gastroenterol Hepatol 2020
## Limitations

<table>
<thead>
<tr>
<th>Colonoscopy</th>
<th>&gt;90% sensitivity but inconvenient, costly and invasive</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIT</td>
<td>&lt;10% sensitivity</td>
</tr>
<tr>
<td>Stool DNA</td>
<td>&lt;18% sensitivity</td>
</tr>
<tr>
<td>Molecular tests (blood)</td>
<td>x</td>
</tr>
</tbody>
</table>

FIT, fecal immunochemical test  
nAA, non-advanced adenoma  
AA, advanced adenoma  
CRC, colorectal cancer  

Thrumurthy et al, BMJ2016
Removal of adenomas lowers risk of colorectal cancer

Recurrence is common

Risk of Recurrence
Up to 60%

Lieberman et al, Gastroenterology, 2012

American Cancer Society Guideline
People with adenomas removed
Should get a colonoscopy again after 3 years*

*Earlier (or later) depends on the type, size, and number of the adenomas
Microbiome and its role in CRC?
We discovered that gut dysbiosis leads to adenoma/CRC.
Is there substantial overlap of bacterial gene markers between CRC patients and healthy individuals?
Comparative Metagenomics in Colorectal Cancer

Microbial communities in human gut mucosa are different across stages of tumorigenesis.

Taxonomically defined microbial consortium is implicated in the development of CRC.

Correlations of bacterial taxa indicate early signs of dysbiosis in adenoma, and co-exclusive relationships are subsequently more common in cancer.

Gavage of Fecal Samples From Patients With Colorectal Cancer Promotes Intestinal Carcinogenesis in Germ-Free and Conventional Mice

Sunny H. Wong, Liuyang Zhao, Xiang Zhang, Geicho Nakatsu, Juqiang Han, Weiqi Xu, Xue Xiao, Thomas N. Y. Kwong, Ho Tsoi, William K. K. Wu, Benhua Zeng, Francis K. L. Chan, Joseph J. Y. Sung, Hong Wei, and Jun Yu

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See editorial on page 1475.
Imbalance of gut microbiota leads to CRC

Evidence suggests gut microbiome is linked to the development of colorectal cancer

Only 12-35% of CRC can be explained by hereditary factors\(^4\)

Gut microbiota dysbiosis in colorectal carcinogenesis has been increasingly recognized\(^5,6\)

The gut microbiome of CRC patients is different from that of healthy people\(^7\)

The transfer of gut microbiota from CRC patients to germ-free mice promotes tumorigenesis in the latter\(^5\)

Problematic gut microbiota potentially triggers inflammatory cascades and oncogenic signalling

Nakatsu et al. Nat Commun. 2015
Wong et al, Gastroenterology. 2017
Dai et al, Microbiome 2018
Wong et al, Nat Rev Gastroenterol Hepatol. 2019
Utilising microbiome as biomarker for cancer detection
qPCR-based microbial test for CRC and adenoma

Tested in Hong Kong and Shanghai subjects

Liang Q, ..., Ng SC, Chan FK, et al. Gut 2019
Liang Q, ..., Ng SC, Chan, FK, et al. Clinical Cancer Research 2017
M3 CRC Test – a combination of microbial gene markers
Diagnostic Performance of **M3 CRC** for CRC Detection

AUROC Specificity Sensitivity

<table>
<thead>
<tr>
<th></th>
<th>AUROC</th>
<th>Specificity</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRC</td>
<td>0.9763</td>
<td>85%</td>
<td>94%</td>
</tr>
</tbody>
</table>

JQ Liang,…SC Ng,…FKL Chan,…J Yu. Gut 2020

Linear trend $P<0.0001$

Total sample size

> 1,174

(Mar 2021)
**M3 CRC** test vs. FIT for early colorectal cancer

Use as a **FIRST LINE screening test** for early colorectal cancer

* Use as a FIRST LINE screening test for early colorectal cancer

* *p*<0.05 and **p*<0.001

JQ Liang,…SC Ng,…FKL Chan,…J Yu. Gut 2020
Our New Research Findings

**M3 CRC** can predict adenoma recurrence (90% sensitivity)

Our novel model involving baseline and follow-up stools
Detection of Recurrent Adenomas

M3 CRC Test

Sensitivity: 90%
Specificity: 87%

Fecal Immunochemical Test (FIT)

Sensitivity: 90%
Specificity: 87%

AUC = 0.551
P = 0.380

Sensitivity: 8.3%

AUC = 0.950
P < 0.001
Our markers show Population robustness valid across different geography

Core network across populations

Dai et al, Microbiome 2018
Dai et al, Bioinformatics 2018
M3 CRC Test
At-home Stool Kit
M3 CRC Technology

DNA extraction

qPCR on multiple markers

Quantification

Modeling algorithm

Marker 1

Marker 2

Marker 3

Marker 4

Data analysis & reporting

Within 4 Hours

Test score
Stool-based Microbiome Markers

Colorectal Adenoma Risk Index*

<table>
<thead>
<tr>
<th>Risk categories</th>
<th>Low</th>
<th>Medium</th>
<th>Medium-high</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 33.3</td>
<td>33.4 - 58.2</td>
<td>58.3 - 65.0</td>
<td>85.1 - 100</td>
<td></td>
</tr>
</tbody>
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Colorectal Cancer Risk Index*

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<th>Low</th>
<th>Medium</th>
<th>Medium-high</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 5.5</td>
<td>5.6 - 15</td>
<td>15.1 - 33.3</td>
<td>33.4 - 100</td>
<td></td>
</tr>
</tbody>
</table>
Dietary recommendations + *Bacterial Gene Markers test* to modulate your risk of colorectal cancer

透過飲食建議及*細菌基因測試*調節患大腸癌的風險

Colorectal adenoma risk index

瘜肉風險指數

Colorectal cancer risk index

大腸癌風險指數

low

high
Stool microbial gene markers test:
- Helps triage patients according to their risk indices
- Identify the optimal timing of colonoscopy for the right patient
- Offers hope to modulate CRC/adenoma risk
Conclusion

Stool-based microbial biomarkers could screen unscreened (high sensitivity and specificity)

First non-invasive approach to detect recurrence of adenoma

Cost effective, rapid, non-invasive

Potential to modulate gut microbiota to reduce cancer risk