A tumour-DNA methylation biomarker to predict response to treatment in patients with ovarian cancer; a multi-centre study

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Methylation of MYLK3 gene promoter region: a biomarker to stratify surgical care in ovarian cancer in a multi-centre study

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8.8% of TOTALLY debulked women progressed or died within 12 months, with median survival of 3 months.
Can we predict poor prognosis post surgery?
DNA methylation

- Addition of CH$_3$ methyl group to cytosine which is paired to guanine (CpG)
- Genetic sequence remains unaltered
- 5-methyl cytosine methylation, can down regulate gene transcription
- Measured easily with pyrosequencing and Illumina beadchip array.

- It is very stable
- Easier to quantify than RNA
- Robust high throughput technology
- Clinically useable
- Potential cell free DNA
- Bed side testing in future
Where to look?

27K Illumina methylation array

Differentially methylated probes

65 probes

Δ methylation >20% adjusted for age, stage, grade, chip

27 probes

Six with largest effect

Validation

FGF4
FGF21
MYLK2
MYLK3
MYL7
ITGAE

Bisulphite pyrosequencing

N=89 tumour DNA

27 probes in adhesion & migration pathways

N=277 tumour DNA
Results MYLK3

Median Overall Survival
Hammersmith high methylation 52.4m
Hammersmith low methylation 33.0m
Charité high methylation 68.0m
Charité low methylation 34.7m
TCGA high methylation 44.5m
TCGA low methylation 34.0m

Charité
Logrank $P=0.05$
Multivariable $P=0.05$
n=47

TCGA
Logrank $P=0.009$
Multivariable $P=0.021$
n=201

Hammersmith
Logrank $P=0.017$
Multivariable $P=0.029$
n=39
All datasets combined

Methylation at cg13247990 (MYLK3) and debulk status

Survival benefit from total debulk appears to be lost in the presence of low methylation.

High methylation improves prognosis for women with residual disease.

Probability of overall survival

- Total debulk, low methylation
- Total debulk, high methylation
- Any residual, low methylation
- Any residual, high methylation

Logrank $P=4.33\times10^{-7}$
Multivariable $P=0.004$

$n=436$
High, intermediate and low methylation

In totally debulked patients there is a continuous effect with increasing methylation

Should we be offering radical surgery to those with the lowest levels of methylation?
Correlation with gene expression

Correlation of MYLK3 Methylation and Expression

\[ P = 0.550 \]
\[ \text{Rho} = -0.042 \]

TCGA: Overall Survival according to expression of MYLK3

Logrank \( P=0.143 \)
Multivariable \( P=0.152 \)
Summary

- *MYLK3* methylation independently affects survival after surgery
- Not fully understood why *MYLK3* has this effect
- High *MYLK3* appears to be beneficial and potentially serves as a useful biomarker
- 8.8% progressed or died within 12 months of total debulk – could *MYLK3* be useful in selecting patients for treatment options?
- *MYLK3* may be one of the first steps in tailoring surgery according to tumour biology
- Is there potential to increase methylation of *MYLK3*?
- Possible to measure cell-free circulating tumour DNA in blood
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