Malignant pleural mesothelioma (MPM)-specific DNA methylation patterns in patients using liquid biopsies

S. Schmid[1,2*], S. Ul Haq[1*], L. Zhan[1], M. C. Brown[1], D. Patel[1], F.A. Shepherd[1], N.B. Leighl[1], A.G. Sacher[1], M. de Perrot[1], B.C. J. Cho[1], F. Zaeimi[1], B.H. Lok[1], S.V. Bratman[1], M.S. Tsao[1], M. Früh[3], P.A. Bradbury[1], G. Liu[1] .

*Contributed equally, [1]Princess Margaret Cancer Centre, University of Toronto; [2]Inselspital, University Hospital Berne,University of Berne, Switzerland; [3]Cantonal Hospital St.Gallen, Switzerland

Background

- The Cancer Genome Atlas (TCGA) show DNA methylation patterns are distinct between tumors and normal tissue
- DNA methylation profiles are highly tissue-specific and can identify tissue-of-origin from cell free DNA (cfDNA).
- Circulating tumor (ct)DNA profiling in MPM has been limited by molecular heterogeneity
- No clear recurrent mesothelioma-specific mutations
- Methylome profiling could identify underlying mesothelioma-specific patterns
- Cell-free methylated DNA immunoprecipitation sequencing (cfMeDIP-seq) can be used on plasma cfDNA
- cfMeDIP-seq on MPM and healthy non-cancer controls (NCC) plasma samples can identify epigenetic changes, overcoming molecular heterogeneity

Methods

- cfMeDIP-seq was applied to pre-treatment plasma samples of 39 MPM patients and 16 NCCs
- cfMeDIP-seq libraries sequenced at a depth of 70 million reads, paired-end, on the NovaSeq 6000.
- For all analyses, chromosomes 1-22 were binned into 300-bp windows (total of 9.6e6 genome-wide windows) and reads were tallied per bin
- Differential methylated regions (DMRs) were examined using DESeq2
- Signficant DMRs (log2FC > 1, p-adj < 0.05) were characterized for MPM and NCC samples for CpG and gene body features
- KEGG pathway analysis was also done on signficant DMRs

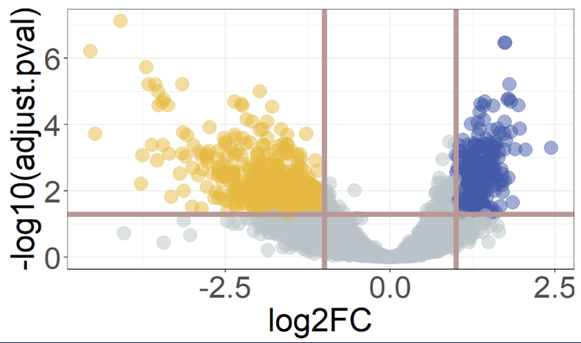
Results

Baseline characteristics

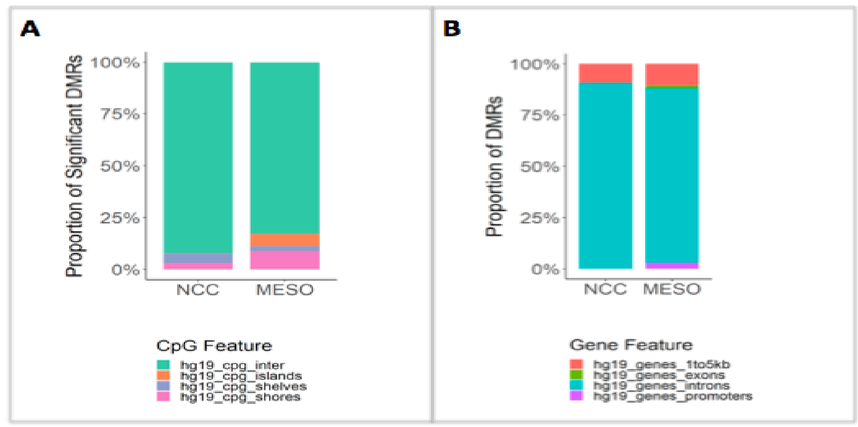
Covariate	Category	All MPM patients
Total N (%)		N = 39
Sex	Female	6 (15.4)
	Male	33 (84.6)
Age at diagnosis (years)	Median [IQR]	70.4 [65.1-73.8]
Clinical stage at initial diagnosis	I	6 (16.7)
	II	17 (47.2)
	III	7 (19.4)
	IV	6 (16.7)
	(Missing)	3
Histology	Biphasic	6 (15.4)
	Epithelioid	30 (76.9)
	Sarcomatoid	3 (7.7)
Smoking history	Ever smoker	15 (38.5)
	Never smoker	24 (61.5)
Asbestos exposure	Yes	28 (80.0)
	No	7 (20.0)
	(Missing)	4
ECOG performance status at diagnosis	0	12 (33.3)
	1	21 (58.3)
	>=2	3 (8.3)
	(Missing)	3

Volcano plot of significant DMRs in Meso versus NCC plasma samples

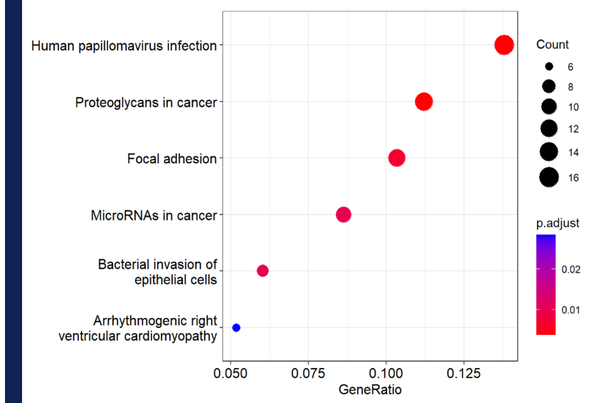
Each dot represents a 300bp window. Horizontal line corresponds to p-adj = 0.05. Vertical lines are +/- 1 log2 fold-change.
Yellow: Significant DMRs in Meso. **Blue**: Significant DMRs in NCC.
Grey: Non-significant DMRs



Characterizing DMRs in Meso and NCC samples by: A. Differences in CpG features B. Differences in gene features



KEGG pathway analysis of significant DMRs in Meso DMRs



Several promoters regions were significantly hypermethylated in MPM:

- RPS27
- USP39
- ZUP1
- ADAP1
- NCAPG2
- BRSK2
- TMEM216
- KLHL11

These genes are implicated in other cancers

Summary and Conclusion

- Meso samples are enriched for tumor-specific CpG islands and prpmoter regions. KEGG-pathway analysis in MPM samples identified several important pathways. Several promotors significantly hypermethylated in MPM have also been implicated in other cancers
- ctDNA global methylome profiling is a promising tool to identify novel biological pathways and targets in MPM. There is potential for future use of cfMeDIP-Seq in MPM screening, minimal residual disease, and therapeutic monitoring.

Abstract #4125

Sabine.schmid@insel.ch