Medicine

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Analysis of loss of heterozygosity by whole-exome sequencing yields insights into pseudomyxoma peritonei

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Introduction

Pseudomyxoma peritonei (PMP) is a clinical syndrome characterized by gross mucinous ascites originating from a disseminated intra-peritoneal neoplasm. Although typically confined to the abdomen, mortality is high if untreated[1]. Biomarkers, including genetic mutation profiles, may aid treatment selection and decision making. Next generation sequencing approaches such as whole-exome-sequencing (WES) can be applied to gain a broad view of the genome of these neoplasia.

Aims

To investigate the exome wide mutational profile of PMP in a pilot project.

Patient samples

All patients (A-E; Table 1) underwent debulking surgeries at the Peritoneal Malignancy Institute, Basingstoke, and tissue was archived as FFPE blocks (Figure 1).

Patient ID	A	B	C	D	E
Age (at diagnosis)	65	35	50	65	45
Sex	Female	Female	Male	Female	Female
PCI	28	21	34	28	18
Serum CEA (U/mL)	34	11	20	15	14
Serum CA 125 (U/ml)	79	49	13	74	50
Serum CA 19-9 (U/mL)	122	23	373	891	39
Follow-up (Months)	58	56	53	53	53

Table 1 | Clinical characteristics of sequenced patients

PCI: Peritoneal cancer index (range 0-39); CEA: Carcinoembryonic antigen (reference range 0-5); CA 125: Cancer antigen 125 (reference range 0-35); CA 19-9: Cancer antigen 19-9 (reference range 0-33).

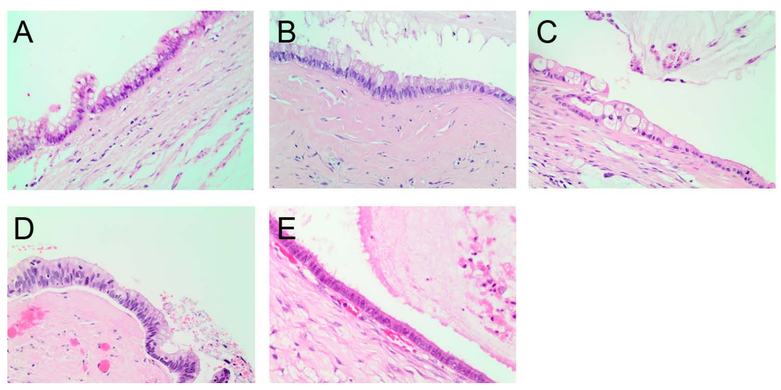

Figure 1 | H&E sections showing representative cells

Methods

FFPE blocks underwent laser capture micro-dissection to obtain cellular material of neoplastic and normal origin, from which DNA was extracted. WES was performed, aiming to generate an average depth 50X and 100X for the normal and neoplastic material respectively, data was analysed as outlined in Figure 2.

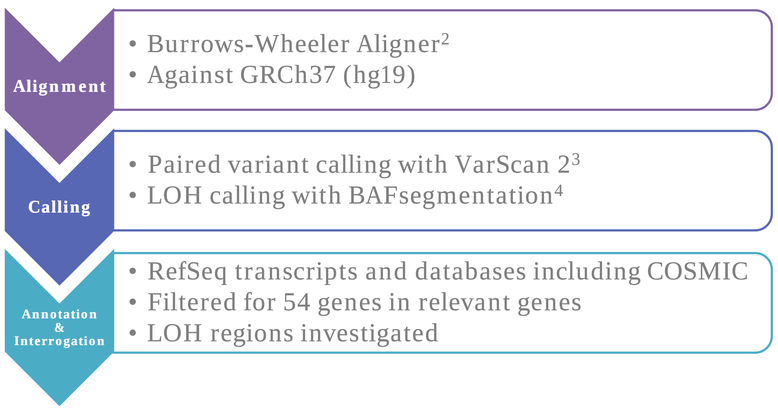

Figure 2 | Bioinformatic workflow for data analysis

Results

Good quality sequence data was obtained for all samples, with an average read depth of 68X and 113X for the normal and neoplastic material respectively. All samples had a relatively low somatic mutation burden across the exome (Table 2), and contained the frequent *KRAS* and *GNAS* mutations, with none containing *TP53* mutations.

	A	B	C	D	E
Frameshift	0	32	9	14	16
Nonsynonymous	75	44	71	55	62
Splicing	7	53	60	35	30
Stopgain	4	2	4	4	6
Synonymous	26	21	29	26	28
Sum	**112**	**152**	**173**	**134**	**142**

Table 2 | Somatic mutation classes and counts

The overall mutation profiles of the samples was assessed (Figure 3), revealing a profile consistent with methylcytosine deamination as a dominant mutational mechanism[5].

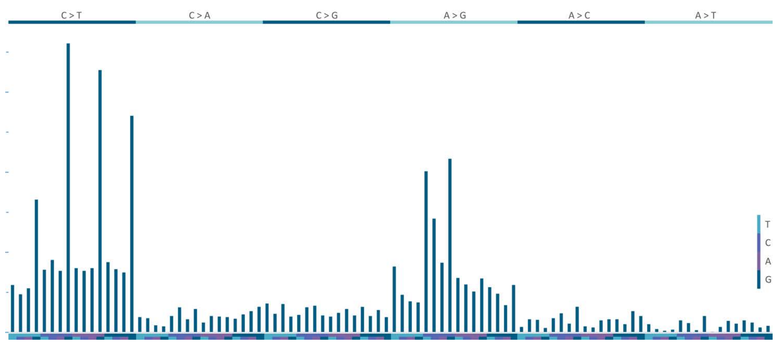

Figure 3 | Mutation profile of all sequenced samples

Percentage of mutations identified for each triplet context is shown. Top row of x-axis represents 5' nucleotide, bottom row 3'. An excess of C>T transitions are seen, consistent with methylcytosine deamination as a mutational mechanism.

Loss of heterozygosity (LOH) events were observed in four of the samples (Table 3). 86% of these event contained mutations of potential interest.

	Region	Somatic variants	Novel/Clinical somatic variants in region[a]		
			Gene	Variant type	BAF[b]
A	Chr20q13.13-qter	2	*GNAS*	nonsynonymous	24%
			RGS19	nonsynonymous	20%
B	Chr18q12.3-qter	1	*SMAD2*	stopgain	63%
C	Chr8	4	*ST18*	nonsynonymous	51%
			C8orf34	nonsynonymous	12%
			COL22A1	nonsynonymous	31%
	Chr20	4	*FERMT1*	nonsynonymous	57%
			C20orf203	nonsynonymous	41%
			GNAS	nonsynonymous	54%
E	Chr1p36.13-p36.11	0			
	Chr12pter-p11.21	6	*ERGIC2*	nonsynonymous	64%
			KRAS	nonsynonymous	71%
	Chr17q11.2-qter	8	*EZH1*	nonsynonymous	65%
			RNF43	stopgain	70%
			RAB37	nonsynonymous	75%

Table 3 | LOH and encompassed mutations

[a]Not present in 1000 Genomes or dbSNP 135, or previously reported; [b]Frequency of alternate allele in read data.

Patient 248 exhibited an apparent two-stage LOH event (Figure 4), with the distal q-arm undergoing an initial event earlier in the clonal evolution of the cell population.

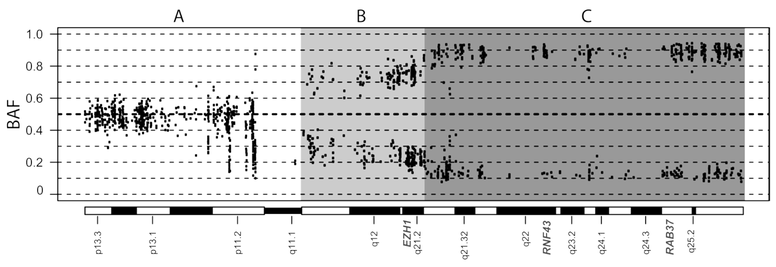

Figure 4 | LOH plot showing two-stage event in E

Whilst the LOH event spans chr17q11.2-qter, there is a marked increase in the allele skewing in the right section (dark grey background), demonstrating a more substantial LOH in this region.

Conclusions

We have successfully applied WES to PMP samples, achieving high purity of neoplastic cells from FFPE. LOH appears to be an important event in the pathogenesis of some PMP cases[6].

Investigating genes present within recurrent regions of LOH in PMP would provide a powerful way to identify new genes involved in disease processes, and thus potential novel treatment targets.

Future work

We are currently undertaking targeted sequencing a large scale project to sequence ~400 fresh frozen PMP samples. This will allow us to combine the deep clinical phenotypes with genomic data, further developing our understanding of PMP.

Our follow-up study is utilising a bespoke panel tailored to PMP genomics, providing optimal coverage and data quality. These data will then be combined with deep clinical phenotyping of the cohort in order to provide robust testing for prognostic and treatment response predictors.

References

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