Report: Grant Ref No: SGS 2012 10 11 - Is pseudomyogenic haemangioendothelioma characterised by a recurrent oncogenic gene rearrangement?

Background

PMH is a recently described tumour of soft tissue. In the largest series to date, the tumour was described as an indolent, multicentric, spindle cell tumour that was present in all tissue planes of limbs, particularly of male patients. In the 31 patients with follow up 58% showed local recurrence, 1 patient with regional lymph node metastasis, 1 patient with distant metastasis (Hornick and Fletcher, 2011). A follow up report demonstrated that in a series of 10 PMHs there was a balanced t(7;19) translocation in one case and an unbalanced der (7) (t(7;19) in another case making this a recurrent translocation albeit with ~20% frequency (Trombetta et al., 2011).

Tumours with recurrent translocations such as Ewing sarcoma and synovial sarcoma lack significant degrees of histological heterogeneity compared to non-translocation sarcomas e.g. osteosarcoma. In this regard, PMH is similar to translocation positive sarcomas in that it lacks significant histological heterogeneity (Hornick and Fletcher, 2011). In a series of four cases at the RNOH, using the FISH probes from the Trombetta et al. paper we found that 2 cases were negative for Chr 7 and Chr19 rearrangements, 1 case was equivocal and one not done. We therefore hypothesized that PMH may harbour another recurrent translocation which may account for the remainder of cases or the probes used did not localize to the correct breakpoint. We therefore sought to investigate this using whole genome sequencing.

Material and methods

1) Reviewed histology and confirmed diagnoses by three pathologists.
2) Frozen material retrieved from biobank, frozen sections cut, stained and reviewed to ensure >80% tumour content.
3) High quality DNA extracted.
4) Whole genome sequencing – UCL genomics – up to a sequencing depth of 10x on Illumina HiSeq.
5) SNP array – Illumina CytoSnp 850k Chip array for copy number analysis.
6) Whole genome sequencing analysis – rearrangement analysis performed using BRASS algorithm (Stephens et al., 2012). As no matching normal tissue was sequenced, the tumour was mapped using the reference genome Hg19 and filtered against a tumour panel (breast carcinoma, prostate carcinoma) to *******
7) Copy number analysis – performed using ASCAT 2.0 implemented in R (Van Loo et al., 2010).
Results

1) Whole genome sequencing – Quality metrics: All samples passed QC thresholds and resulted in >95% uniquely mapping reads with an average of 12x coverage (Table 1).

Table 1: Sequencing metrics

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mapped</th>
<th>Unique Mapped</th>
<th>Sequencing Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD14744a</td>
<td>0.925959</td>
<td>0.975721089</td>
<td>12.60340458</td>
</tr>
<tr>
<td>PD14745a</td>
<td>0.892371</td>
<td>0.958954889</td>
<td>12.11290597</td>
</tr>
<tr>
<td>PD14746a</td>
<td>0.906474</td>
<td>0.975573424</td>
<td>11.44154998</td>
</tr>
<tr>
<td>PD14747a</td>
<td>0.881424</td>
<td>0.970319774</td>
<td>11.6043577</td>
</tr>
</tbody>
</table>

2) 360 total rearrangements in four samples (Table 2).

Table 1: Rearrangement count in four samples of PMH

<table>
<thead>
<tr>
<th>Samples</th>
<th>PD14744a</th>
<th>PD14745a</th>
<th>PD14746a</th>
<th>PD14747a</th>
<th>Grand Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count of predicted rearrangements</td>
<td>115</td>
<td>110</td>
<td>65</td>
<td>70</td>
<td>360</td>
</tr>
</tbody>
</table>

The t(7;19) rearrangement was not identified in any of the four samples and no recurrent translocations or likely fusion genes were identified.

3) Copy number analysis – shows that all samples have a normal ploidy of 2. One sample (PD14744a) shows evidence of consanguinity with multiple runs of homozygosity and one sample show a hemizygous deletion on chr 19.
Discussion

The whole genome sequencing analysis did not reveal a recurrent rearrangement nor confirm the t(7;19) translocation.

In a recent early online paper (ahead of print), the authors of t(7;19) paper showed through a combination of RNA-sequencing, FISH and RT-PCR that 10 of 12 PMHs demonstrated a recurrent t(7;19) involving the SERPINA1A and FOSB genes (Walther et al., 2013). In light of this, the WGS analysis was repeated, this time using less stringent thresholds. The t(7;19) rearrangement was still not identified.

There might be a few reasons for this:

1) Sequencing depth is too low and the rearrangement is only present in a small allelic fraction. This may be plausible a large number of cases of t(7;19) detected on FISH is in the region of 20-30% of cells (Walther et al., 2013).

2) The filters used are too stringent for the detection of this rearrangement.

3) Not all cases tested in the J.Pathol paper (Walther et al., 2013) are positive for the rearrangement.

Future plan:

My plan is to screen the samples which have undergone WGS using RT-PCR (design published in J Pathol paper (Walther et al., 2013)). These primers have been ordered and are in hand to confirm if the fusion gene is present in these samples.

Order FISH probes as described in paper to use as a complementary test to RT-PCR.

The aim is to replicate the findings of the recent J Pathol paper (Walther et al., 2013) and confirm if the set of samples we have analysed have the translocation. The aim would be to publish these findings as a letter or reply in J Pathol.

Timeframe 3-6 months – whereupon an additional report will be submitted to the Pathological Society.
References


