A challenging case of endometrial cancer low tumour cellularity: utility of alternative methods for variant confirmation

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INTRODUCTION

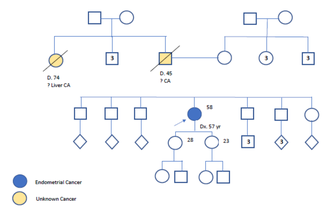
Lynch syndrome is the most common form of hereditary colorectal cancer predisposition, consisting of 2-4% of all colorectal cancer cases. The genes associated with Lynch syndrome include the DNA MMR genes (*MLH1, MSH2, MSH6* and *PMS2*), as well as the *EPCAM* gene. Patients with colorectal cancer typically first undergo testing on a biopsy of their tumour, which may include immunohistochemical (IHC) analysis for MMR protein expression and/or microsatellite instability (MSI) analysis, which is a detectable outcome from disruption of the MMR proteins. If abnormal, additional somatic testing for *MLH1* promoter methylation and/or *BRAF* targeted sequencing is often performed. If the patient's tumour does not show *MLH1* promoter methylation or a *BRAF* mutation, germline testing of *MLH1, MSH2, MSH6, PMS2* and *EPCAM* is often assessed using a peripheral blood sample. In a significant number of patients with MMR deficient and/or MSI tumours, no germline pathogenic variant is identified. The current National Comprehensive Cancer Network (NCCN) guidelines for Lynch syndrome (Version 2.2016) recommend patient's with an MMR deficient tumour sample, which are germline testing negative, should consider somatic tumour testing of the MMR genes inorder to assess for the genetic etiology of their MMR tumour deficiency (1,2). The NCCN recommends that individuals found to have double somatic pathogenic variants in the MMR genes likely do not have Lynch syndrome and management should be based on personal/family history, until such time that further research has emerged on this new subgroup of patients.

AIM

In this discussion, we review a challenging low tumour cellularity endometrial case with IHC MSH6 absent staining and normal *MSH6* constitutional/germline testing.

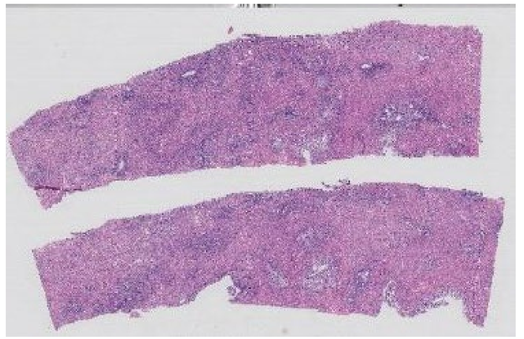
PATIENT

- 58 year old female
- Well differentiated FIGO Grade I endometroid adenocarcinoma diagnosed at age 57
- IHC performed on tumor showed isolated absent MSH6
- Germline hereditary Lynch Panel performed was negative (*MLH1, MSH2, MSH6, PMS2, EPCAM)*
- No reported significant personal or family history suggestive of hereditary cancer syndrome

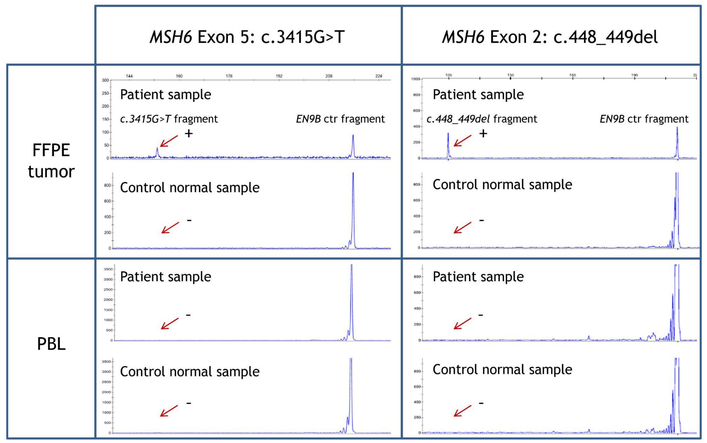


RESULTS

Two somatic variants were identified by NGS at a low mosaic level, each estimated to be present in 8% of the cells tested from this patients FFPE tissue block which was consistent with the predicted low tumour cellularity by pathology. Both variants were assessed by Sanger sequencing; however, only trace levels of both variants were detected above background signal. To confidently confirm or rule-out the NGS findings, unique AS-PCR assays were designed. AS-PCR confirmed the presence of both variants in the patients tumour sample as well as the absence of both variants in the patient's DNA from blood, consistent with previously reported negative germline results.



Detected in tumor	Detected in blood	Gene	Variant	Classification
Yes	No	*MSH6*	c.448_449delCC, p.(Pro150IlefsTer21)	Pathogenic
Yes	No	*MSH6*	c.3415G>T, p.(Gly1139Cys)	VUS



METHOD

Next-generation sequencing (NGS), sanger sequencing and allelic-specific PCR (AS-PCR) were performed for *MSH6* coding regions. Variant classification was assessed using the criteria outlined by the International Society for Gastrointestinal Hereditary Tumours (InSiGHT). Next-generation sequencing (NGS): DNA corresponding to *all MLH1, MSH2, MSH6, PMS2* and *EPCAM* coding regions as well as 25 base pairs (bp) of non-coding flanking DNA was captured using DNA hybridization probes. Captured DNA was sequenced using Illumina sequencing technologies and processed using the Data-Driven Medicine (DDM) Bioinformatics pipeline (Sophia Genetics). Minimum NGS coverage is 1000X for all exons and ±25 bp of flanking intronic sequencing. All regions with coverage that does not meet this threshold are assessed by Sanger sequencing. All pathogenic, likely pathogenic and uncertain NGS variants are confirmed by Sanger sequencing. Deletion/duplication analysis: Multiplex ligation-dependent probe amplification (MLPA) is used to assess for large single or multi-exon deletions and duplications. When required, the following kits by MRC Holland (Amsterdam, Netherlands) are used: SALSA MRC Holland P003-D1 (*MLH1, MSH2, EPCAM*), P248-B1 (*MLH1, MSH2*), P008-C1 (*PMS2*) and P072-C1 (*MSH6, EPCAM*). Note: only exons 3, 8 and 9 of the *EPCAM* gene are assessed when kits P003-D1 and P072-C1 are used.

CONCLUSIONS

This case illustrates the challenges of low tumour cellularity in somatic tumour testing and the value of using alternative confirmation methods aside from conventional Sanger sequencing to provide confidence in variant calling.

REFERENCES

1 - National Comprehensive Cancer Network (NCCN) guidelines for Oncology: Genetic/Familial High-risk Assessment – Colorectal (Version 2.2016). nccn.org

2 - Haraldsdottir et al 2014. Colon and endometrial cancers with mismatch repair deficiency can arise from somatic rather than germline, variants. Gastroenterology. 147(6):1308-1316.

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