BRAF wild-type, PTEN mutant malignant uveal melanoma arising within a mature ovarian teratoma: A case report and review of the literature.

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ABSTRACT

Mature cystic teratomas (MCT) are common in women of all ages however malignant transformation within them is rare and difficult to diagnosis preoperatively.

Primary melanoma of the ovary is exceptionally rare and only occurs in relation to a teratoma where it can originate from sporadic somatic mutagenesis within epidermal junctional melanocytes, through malignant transformation of a benign naevus formed within the MCT or from other well differentiated pigment containing structures such as the uvea.

We present a case of primary malignant melanoma arising within a mature cystic teratoma in a young patient, who ultimately developed widespread metastasis necessitating systemic therapy. Our case highlights the role of molecular medicine not only in forming an understanding the origin of the melanoma, but also guiding targeted systemic therapies.

Alongside the case we present a review of the literature describing the incidence of molecular aberrations within melanoma as well as the established and emerging techniques and cytotoxic agents for malignant melanoma.

INTRODUCTION

Ovarian teratomas are common germ cell neoplasms and all contain mature or immature pluripotent cells arising from one or more dermal layers. Mature ovarian cystic teratomas (MCT) are the most common benign tumours found in women of all ages and are composed of mature histologic structures from at least two of the three germ layers: ectoderm, mesoderm and endoderm. They classically contain well differentiated tissue types, giving them a characteristic appearance.

Malignant transformation within MCT is rare with an incidence of 0.6 - 2%. Of these, squamous cell carcinomas are the most common accounting for 80% of cases [1]. Ovarian malignant melanoma was first described in 1901 [2] with most reports being only of single cases [1, 3-8]. Pre-operative diagnosis of malignant transformation within MCTs is difficult with radiological detection of solid components being the only non-specific indicator. Additionally, encountering a melanoma in the ovary presents a particular diagnostic dilemma given the challenges in differentiating primary disease from secondary metastasises, taking into account the high degree of mimicry seen [9].

We report a case of malignant ovarian melanoma arising within an MCT treated initially with fertility preservation surgery. We herein present this case with detailed immunohistochemistry and genetic profiling alongside a brief literature review.

CASE REPORT

A 39-year-old nulliparous woman presented with sudden onset abdominal pain. Ultrasound examination demonstrated a septated left adnexal cyst, measuring 130 x 60 x 100 mm. With clinical suspicion of ovarian torsion she underwent emergency laparoscopic left ovarian cystectomy in the secondary care unit. Intraoperatively there was evidence of preoperative cyst rupture but no evidence of surface or extraovarian disease.

The disrupted cystectomy specimen contained semi-solid material with enmeshed hair. Two solid areas were preferentially sampled and initial review suggested a torted mature cystic teratoma composed of skin, lamellar bone and fibrofatty tissue. In keeping with rupture there was a florid foreign body giant cell reaction to broken hair shafts. An area of small round blue cells was observed raising the possibility of lymphoma or an immature Sertoli Leydig cell tumour, and this prompted referral to the tertiary pathology centre. Further systematic sampling of the surgical material demonstrated no dysplasia or increase in melanocytes in the squamous lined epithelium. Towards the deep dermis and subcutis, and involving fibroconnective tissue elsewhere, was a small round blue cell tumour arranged in a nested pattern. In areas the tumour was composed of larger epithelioid cells with moderate eosinophilic cytoplasm, eccentric nuclei and irregular nuclear outlines, coarse chromatin and occasional intranuclear inclusions. Admixed with the larger cells there were diffuse sheets of relatively monomorphic small blue cells with increased cellular density around vascular structures and a hint of nesting in areas. They possessed basophilic small round, and in places irregular, nuclei with condensed chromatin, Figure 1. Mitoses were present but not brisk. Brown granular pigment was seen, positive for Fontana Masson stain (Figure

2A) and negative for Perls stain for iron, confirming it to be melanin pigment. Due to the fragmented and haemorrhagic nature of the specimen, architecture and relationship of the tumour to the skin and background benign structures was difficult to assess but there was no evidence of an intraepidermal or junctional melanocytic component.

Immunohistochemistry demonstrated strong diffuse positivity for S100 (Figure 2B), Melan A (Figure 2C), vimentin, and NSE. The tumour showed weak patchy positivity for synaptophysin and CD117. Ki67 proliferation fraction was variable with 5% proliferative activity in the larger cells with organoid areas and approximately up to 20-30% in the diffuse small cell areas. A wild type p53 staining pattern was seen throughout.

Consideration of the morphology alongside the immunoprofile favoured a diagnosis of malignant melanoma arising in a mature cystic teratoma.

Post-operative cross-sectional imaging demonstrated no lymphadenopathy or extraovarian disease and a full clinical Dermatology review excluded a primary cutaneous source.

Following regional Melanoma multidisciplinary (MDT) review the patient opted to undergo fertility-sparing completion staging surgery with left salpingo-oophorectomy, bilateral pelvic and para-aortic node dissection, omentectomy and peritoneal washings. Peritoneal fluid cytology demonstrated cells suspicious of melanoma and histology confirmed malignant melanoma, similar in morphology and immunoprofile to that of the cystectomy specimen, present in the stroma, blood vessels and serosal surfaces of the ipsilateral fallopian tube and residual ovarian tissue. The tumour infiltrated the tissues in subtle linear arrays without eliciting any inflammatory reaction and without distorting background structures. All other specimens were negative with an assigned FIGO Stage of 2A [10].

Further whole body nuclear imaging excluded metastasis and the patient underwent definitive surgical excision with hysterectomy, right salpingo-oopherectomy and biopsies of bladder peritoneum and pelvic side wall, noted to be newly pigmented at the time of surgery. Histology demonstrated malignant melanoma within the contralateral ovary and peritoneal biopsies.

DNA extracted from the primary FFPE tissue underwent mutational testing demonstrating wild type BRAF and NRAS with further testing demonstrating microsatellite stability but a loss of PTEN with deletions on exons 1 and 2.

Six weeks after definitive surgery repeat radiological investigations demonstrated lung, liver parenchymal and bone metastases, as well as probable peritoneal disease. The patient received four cycles of combination immunotherapy with ipilimumab (monoclonal antibody to cytotoxic T-lymphocyte associated antigen 4 [CTLA-4]) and nivolumab (monoclonal antibody to programmed death 1 [PD-1]). One cycle of maintenance nivolumab was also given but follow-up cross-sectional imaging demonstrated progressive disease. Immunotherapy was consequently stopped and second-line treatment with dacarbazine commenced. She received three cycles and further widespread disease progression was evident at subsequent radiological assessment.

Best supportive care was provided as she continued to deteriorate and she died 14 months following her initial emergency presentation.

DISCUSSION

Primary ovarian malignant melanomas are exceptionally rare and as the ovary does not contain melanocytes, can only arise as part of a teratoid lesion. The frequency of the precise site of origin of ovarian melanomas within MCT is not known. The current case supports a diagnosis of primary melanoma in line with Cronje and Woodruff's established diagnostic criteria which included: 1) absence of another primary melanoma; 2) unilateral ovarian tumour with an associated teratoid element; 3) correlation of clinical findings with those in the literature; and 4) demonstration of melanocytic junctional activity (although not mandatory for diagnosis) [11]. This case highlights a few areas of particular interest. Malignant melanomas typically arise de novo from the dermal-epidermal junction, however in this case there was no intraepidermal or junctional activity. It is therefore possible that this lesion originated either within a benign nevus or from another pigment-containing component of the MCT, for example the uveal epithelium. Retrospective review of the original cystectomy material revealed that the lesion was seen to be in close association with structures with ocular differentiation, (Figure 3A/B). The pattern of dissemination seen with the described case is in keeping with this site of origin. Uveal melanomas show a high rate of haematogenous metastasis with a propensity for liver [12], and due to lack of lymphatic drainage in the uvea, they do not spread to regional lymph nodes, in keeping with the negative nodes and disseminated disease seen in our case. This site of origin is further supported by the molecular profile of the tumour with literature reporting universal wildtype BRAF status of uveal melanomas [13]. Furthermore, loss of the tumour-suppressor gene PTEN, has been shown to be prevalent in uveal melanoma with loss of cytoplasmic

PTEN expression negatively associated with disease free survival [14]. Mitsutaku et al describe a case of malignant melanoma arising within a MCT which was BRAF WT and PDL-1 negative in whom there was no response to immune checkpoint inhibitors [15]. Furthermore, Tate et al describe another case of ovarian malignant melanoma found to be BRAF WT with loss of homology (LOH) of PTEN. Following paired mutational analysis of the benign MCT from which it arose they infer that LOH of PTEN may be a molecular alteration of the MCT with a further KIT mutation, found in the melanoma, acting as a promotional event associated with oncogenesis [16]. The lack of response to immunotherapy in the described case would also be in keeping with uveal origin, as it is known the uveal melanomas are generally not responsive to immune check-point inhibitors.

Cancer of the ovary carries the highest mortality of all gynaecological malignancies and additionally melanomas arising in unusual sites are accepted to be associated with a poor prognosis. Specifically, in a collective series of 31 cases of primary ovarian melanoma, McNeilage *et al* reported that 43% of patients died of disease within 18 months of diagnosis [6]. Five of these patients received adjuvant platinum-based chemotherapy with only one patient receiving platinum combined with immunotherapy.

In line with the management of epithelial ovarian cancer, surgery has historically formed the cornerstone of treatment of ovarian melanoma [4], and it is often necessary for an accurate diagnosis to be reached. Published literature demonstrates that the pattern of spread of primary ovarian melanoma (uveal or cutaneous origin) can replicate that of epithelial ovarian cancer but, in contrast to epithelial ovarian cancer, metastases more frequently

occur through lymphatic and haematogenous routes, giving rise to distant metastases in lymph nodes, lung, liver and bone.

The combination of surgical cytoreduction and systemic therapy may confer a significant benefit in this rare disease but the role of adjuvant treatment is not established.

Until a few years ago, melanoma patients had few effective systemic treatment options and historically, response rates to conventional chemotherapy and interleukin-2 or interferon-gamma, have been low at only 5–19% [17]. New therapeutic options include treatments targeted to genetic mutations within tumours as well as immune modulators.

Approximately 35-50% of all cutaneous melanomas harbour a *BRAF* gene mutation [18], resulting in a distinct phenotype, and the use of selective inhibitors of BRAF kinase alone [19] or in combination with inhibitors of the downstream MEK kinase has resulted in dramatic improvements in survival [20]. Patients with BRAF WT tumour may however experience paradoxical stimulation of the MAPK pathway resulting in tumour promotion if treated with a BRAF inhibitor [21] thus making molecular testing for BRAF mutations a priority to determine the course of therapy. More recently immunotherapy with immune check point inhibitors has also demonstrated a significant improvement in survival for patients with BRAF mutant and wild type cutaneous melanoma [22], however response rates are significantly lower in uveal or mucosal melanomas.

The PTEN tumour suppressor gene is one of the most frequently inactivated tumour suppressor genes in sporadic cancers with an estimated frequency of 7.3% and 15.2% in primary and metastatic melanomas [23]. PTEN modulates protein synthesis, cell cycle, migration, growth, DNA repair, and survival signalling by regulating phosphoinositide-3-

kinase (PI3K) and the protein-Ser/Thr kinase (AKT) signalling pathway [24]. Loss of function mutations in PTEN occur in only a fraction of PTEN-deficient tumours hence the need to determine PTEN status by protein quantification and DNA sequencing [25]. Previous studies have shown frequent co-occurrence of *BRAF* mutations and *PTEN* mutations or deletions [26]. However, in the TCGA's proposed classification of melanoma into four genomic subtypes (BRAF, RAS, NF1 and triple WT), a higher frequency of amplifications and overexpression of AKT3 is seen in *RAS, NF1*, and Triple-WT melanomas, which may support the use of combination MEK and PI(3)K/AKT/mTOR pathway inhibitors in such subtypes [18].

This case report is an example of an aggressive malignant melanoma within an MCT, showing resistance to first- and second-line therapies. In view of pathological features, we have found lack of mitotic figures and low proliferation fraction can be misleading in the diagnosis of malignant melanoma. In relation to immunohistochemical stains, it is also important to note the synaptophysin and CD117 positivity in this case. This is an interesting finding, as it may be seen in immature neural components of a teratoma. Alongside this, molecular testing offers insight into the site of origin as well as provide vital information to determine therapeutic paths.

FIGURE LEGENDS

Figure 1. H and E of initial cystectomy specimen. Nests of melanoma cells deep in the subcutis with no connection to overlying surface squamous epithelium or adnexal structures. X1 magnification.

Figure 2. . Immunohistochemistry of initial cystectomy specimen. Five micron-thick paraffin sections of initial cystectomy specimen were cut onto slides, deparaffinised in xylene and rehydrated in descending gradients of thanol. Endogenous peroxidase and non-specific binding were blocked before addition of primary antibodies on an automated Ventana stainer:

(A) Fontana-Masson melanin stain, where the melanin granules reduce ammonia-silver nitrate and turn black.

(B) S100 (1:500). Diffuse and dense cytoplasmic and nuclear staining of S100 was seen denoting proliferation of melanoma cells. Strongly positive cells are seen interspersed throughout the tissue sample;

(C) Melan A stain (1:12.5), a specific melanocyte lineage marker. Diffuse cytoplasmic staining is seen.

Figure 3. H and E of initial cystectomy specimen. Basal layer of the double layered uveal epithelium giving rise to small melanoma cells, invading the richly vascularised connective tissue below. X10 magnification.

(A) foci of brown melanin pigment signifying melanoma cells arising from conjunctival/uveal tissue.

(B) Melanoma cells abutting conjunctival epithelium. Uveal epithelium with melanoma cells arising from the basal layer of the left edge of the image undermining adjacent epithelium and invading underlying connective tissue.

REFERENCES

1. Hurwitz, J.L., Fenton, A., McCluggage, W. G., et al, *Squamous cell carcinoma arising in a dermoid cyst of the ovary: a case series.* BJOG, 2007. **114**(10): p. 1283-7.
2. Andrews, H., *Primary Melanotic sarcoma of the ovary.* Trans Obstet Soc (Lond), 1901. **43**: p. 228-31.
3. Kim, M.J., Kim, N. Y., Lee, D. Y., et al, *Clinical characteristics of ovarian teratoma: age-focused retrospective analysis of 580 cases.* Am J Obstet Gynecol, 2011. **205**(1): p. 32 e1-4.
4. Vimla, N., Kumar, L., Thulkar, S., et al, *Primary malignant melanoma in ovarian cystic teratoma.* Gynecol Oncol, 2001. **82**(2): p. 380-3.
5. Moehrle, M., Fischbach, H., Nuessle, B., et al *Primary malignant melanoma arising in a cystic necrotic ovarian teratoma.* Eur J Obstet Gynecol Reprod Biol, 2001. **99**(2): p. 268-71.
6. McNeilage, L.J., Morgan, J., Constable, J., et al, *Metastatic malignant melanoma arising in a mature ovarian cystic teratoma: a case report and literature review.* Int J Gynecol Cancer, 2005. **15**(6): p. 1148-52.
7. Moon, J.W., Kim, J.Y., Shin, Y.J., et al, *Primary malignant melanoma arising from ovarian mature cystic teratoma: a case report and literature review.* Korean J Obstet Gynecol, 2010. **53**: p. 846-850.
8. Hyun, H.S., Mun, S. T., *Primary malignant melanoma arising in a cystic teratoma.* Obstet Gynecol Sci, 2013. **56**(3): p. 201-4.
9. McCluggage, W.G., Bissonnette, J. P., Young, R. H., *Primary malignant melanoma of the ovary: a report of 9 definite or probable cases with emphasis on their morphologic diversity and mimicry of other primary and secondary ovarian neoplasms.* Int J Gynecol Pathol, 2006. **25**(4): p. 321-9.
10. Prat, J., Figo Committee on Gynecologic Oncology, *Staging classification for cancer of the ovary, fallopian tube, and peritoneum.* Int J Gynaecol Obstet, 2014. **124**(1): p. 1-5.
11. Cronje, H.S., Woodruff, J. D., *Primary ovarian malignant melanoma arising in cystic teratoma.* Gynecol Oncol, 1981. **12**(3): p. 379-83.

12. Group, C.O.M.S., *Assessment of metastatic disease status at death in 435 patients with large choroidal melanoma in the Collaborative Ocular Melanoma Study (COMS): COMS report no. 15.* Arch Ophthalmol, 2001. **119**(5): p. 670-6.
13. Cruz, F., Rubin, B. P., Wilson, D., et al, *Absence of BRAF and NRAS mutations in uveal melanoma.* Cancer Res, 2003. **63**(18): p. 5761-6.
14. Abdel-Rahman, M.H., Yang, Y., Zhou, X. P., et al, *High frequency of submicroscopic hemizygous deletion is a major mechanism of loss of expression of PTEN in uveal melanoma.* J Clin Oncol, 2006. **24**(2): p. 288-95.
15. Mitsutake, Y., Yuri, A., Tadaaki, N., et al, *Immune checkpoint inhibitors of CTLA4 and PD-1 for malignant melanoma arising in ovarian cystic teratoma: A case report*. Medicine, 2018. **97**(43):e12937.
16. Tate, G., Tajiri, T., Suzuki, T., et al, *Mutations of the KIT gene and loss of heterozygosity of the PTEN region in a primary malignant melanoma arising from a mature cystic teratoma of the ovary. Cancer Genet Cytogenet,* 2009. **190**(1): p. 15-20.
17. Parker, B.S., Rautela, J., Hertzog, P. J., *Antitumour actions of interferons: implications for cancer therapy.* Nat Rev Cancer, 2016. **16**(3): p. 131-44.
18. Network, C.G.A., *Genomic Classification of Cutaneous Melanoma.* Cell, 2015. **161**(7): p. 1681-96.
19. Bradish, J.R., Cheng, L., *Molecular pathology of malignant melanoma: changing the clinical practice paradigm toward a personalized approach.* Hum Pathol, 2014. **45**(7): p. 1315-26.
20. Long, G.V., Stroyakovskiy, D., Gogas, H., et al, *Dabrafenib and trametinib versus dabrafenib and placebo for Val600 BRAF-mutant melanoma: a multicentre, double-blind, phase 3 randomised controlled trial.* Lancet, 2015. **386**(9992): p. 444-51.
21. Cheng, L., Lopez-Beltran, A., Massari, F., et al, *Molecular testing for BRAF mutations to inform melanoma treatment decisions: a move toward precision medicine.* Mod Pathol, 2018. **31**(1): p. 24-38.
22. Larkin, J., Chiarion-Sileni, V., Gonzalez, R., et al *Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma.* N Engl J Med, 2015. **373**(1): p. 23-34.
23. Aguissa-Toure, A.H., Li, G., *Genetic alterations of PTEN in human melanoma.* Cell Mol Life Sci, 2012. **69**(9): p. 1475-91.
24. Hopkins, B.D., Hodakoski, C., Barrows, D., et al *PTEN function: the long and the short of it.* Trends Biochem Sci, 2014. **39**(4): p. 183-90.
25. Dillon, L.M., Miller, T. W., *Therapeutic targeting of cancers with loss of PTEN function.* Curr Drug Targets, 2014. **15**(1): p. 65-79.
26. Tsao, H., Chin, L., Garraway, L. A., et al, *Melanoma: from mutations to medicine.* Genes Dev, 2012. **26**(11): p. 1131-55.