Case report

Clonal lineage of high grade serous ovarian cancer in a patient with neurofibromatosis type 1

Eric J. Norris\textsuperscript{a,⁎,f,1}, Wendell D. Jones\textsuperscript{b}, Marius D. Surlea\textsuperscript{c}, Andrei J. Petrescu\textsuperscript{c}, Darla Destephan\textsuperscript{a}, Qing Zhang\textsuperscript{a}, Issam Hamadeh\textsuperscript{a}, Jeffrey S. Kneisl\textsuperscript{a}, Chad A. Livasy\textsuperscript{d}, Ram N. Ganapathi\textsuperscript{a}, David L. Tait\textsuperscript{a}, Mahrukh K. Ganapathi\textsuperscript{a,⁎,f}

\textsuperscript{a} Levine Cancer Institute, Carolinas HealthCare System, 1021 Morehead Medical Drive, Charlotte, NC 28204, USA
\textsuperscript{b} Department of Bioinformatics, Institute of Biochemistry of the Romanian Academy, Splaiul Independenței 296, Bucharest 060031, Romania
\textsuperscript{c} Q2 Solutions-EA Genomics, 5827 South Miami Boulevard, Morrisville, NC 27560, USA
\textsuperscript{d} Carolinas Pathology Group, 2001 Vail Avenue, Charlotte, NC 28207, USA

A R T I C L E   I N F O

Keywords:
Cancer
Ovarian
Neurofibromatosis
NF1

A B S T R A C T

Neurofibromatosis type 1 (NF1) is caused by mutations in the \textit{NF1} gene encoding neurofibromin, which negatively regulates Ras signaling. NF1 patients have an increased risk of developing early onset breast cancer, however, the association between NF1 and high grade serous ovarian cancer (HGSOC) is unclear. Since most NF1-related tumors exhibit early biallelic inactivation of NF1, we evaluated the evolution of genetic alterations in HGSOC in an NF1 patient. Somatic variation analysis of whole exome sequencing of tumor samples from both ovaries and a peritoneal metastasis showed a clonal lineage originating from an ancestral clone within the left adnexa, which exhibited copy number (CN) loss of heterozygosity (LOH) in the region of chromosome 17 containing \textit{TP53}, \textit{NF1}, and \textit{BRCA1} and mutation of the other \textit{TP53} allele. This event led to biallelic inactivation of \textit{NF1} and \textit{TP53} and LOH for the \textit{BRCA1} germline mutation. Subsequent CN alterations were found in the dominant tumor clone in the left ovary and nearly 100% of tumor at other sites. Neurofibromin modeling studies suggested that the germline \textit{NF1} mutation could potentially alter protein function. These results demonstrate early, biallelic inactivation of neurofibromin in HGSOC and highlight the potential of targeting RAS signaling in NF1 patients.

1. Introduction

Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant disorders affecting approximately 1 in 3500 individuals. The disease is caused by mutations in the \textit{NF1} gene and shows complete penetrance (Yap et al., 2014). \textit{NF1}, which encodes a GTPase-activating protein (neurofibromin) that negatively regulates Ras-signaling pathways, is considered a classical tumor suppressor gene. NF1 patients have an estimated lifetime risk of 59.6% of developing cancer resulting in a decreased life expectancy of 10–15 years (Walker et al., 2006). While NF1 haploinsufficiency may have functional consequences, biallelic inactivation of \textit{NF1} frequently precedes or occurs simultaneously with malignant transformation in NF1-related cancers (Yap et al., 2014; McPherson et al., 2015). Recent evidence suggests that NF1 is associated with an increased incidence of early onset breast cancer and biallelic inactivation of \textit{NF1} is an early event in tumorigenesis (McPherson et al., 2015). An association between NF1 and ovarian cancer is emerging and somatic mutations and copy number alterations (CNA) of \textit{NF1} are frequently observed in high grade serous ovarian cancer (HGSOC) (Cancer Genome Atlas Research N, 2011; Patil and Chamberlain, 2012; Kanchi et al., 2014; Salud et al., 1991; Ceccaroni et al., 2002; Jeon et al., 2015). We present the case of a woman who developed two separate NF1-related malignancies (malignant peripheral nerve sheath tumor (MPNST) and HGSOC) before the age of 44. Whole exome sequencing (WES) of tumor DNA from bilateral ovaries and peritoneal metastasis was performed to investigate tumor evolution and determine whether biallelic inactivation of \textit{NF1} is an early event of ovarian carcinogenesis.

2. Case history

A 44-year-old gravida 5, para 3 African American female presented...
to the emergency department complaining of back and abdominal pain associated with weight loss, constipation, and anemia. A computed tomography scan revealed a 12 × 12 × 10 cm mass with displacement of the uterus, peritoneal carcinomatosis, ascites, and pelvic adenopathy. The CA125 level was 1971 U/mL. Her past medical history was significant for NF1 and two NF1-related tumors: a benign schwannoma excised from the breast 25 years previously and a T2bN0M0 PMNST of the right knee, treated with excision and radiation 7 years previously. A fine needle aspirate biopsy of the pelvic mass showed PAX8 (marker of Müllerian origin) positive HGSOC revealing a new primary gynecological malignancy, not a MPNST recurrence. The patient underwent radical tumor resection with total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy and tumor resection, for optimal cytoreduction. Pathological review was performed per sectioning and extensively examining of the fimbriated end (SEE-FIM) protocols. HGSOC was noted in both ovaries and peritoneal metastasis with < 1% of the right fallopian tube (serosal side) involved. No serous tubal intraepithelial carcinomas were observed. Final pathological diagnosis was HGSOC arising from the ovary, stage IIB. After an uneventful postsurgery recovery, the patient refused adjuvant chemotherapy. Six months after surgery the patient experienced tumor progression and began a course of dose dense carboplatin/paclitaxel. After 9 cycles of chemotherapy, CA125 levels normalized (< 5 U/mL), but a CT scan showed a residual 11 × 12 mm nodule in the pelvic mesentery. At the time of this manuscript, the patient is alive and receiving salvage chemotherapy for platinum-resistant progressive disease.

3. Results and discussion

Since malignant transformation of NF1-related cancers frequently involves early somatic mutation of the wild-type NF1 allele followed by an additional genomic event (e.g. TP53, CDKNA loss), we hypothesized that biallelic NF1 inactivation was an early event in the development of HGSOC in this patient (Upadhyaya et al., 2004). To study tumor evolution, we performed WES on tumor samples obtained from each ovary and one peritoneal metastasis. Sequencing of germline DNA (average depth 130×) revealed a missense mutation (c.7161C > G) in NF1, which leads to substitution of asparagine 2387 to lysine has not been previously reported and is characterized as a variant of unknown significance (VUS). However, based on the patient's clinical diagnosis and a previous report of a pathogenic NF1 mutation involving

---

Fig. 1. Copy Number Alterations. (A) Genomic profile of CNA in tumor samples isolated from the left ovary, right ovary, and peritoneal metastasis. Germline DNA was used as a reference. Regions of interest are shaded as unique to left ovary (grey), unique to right ovary (blue), unique to peritoneal metastasis (yellow), and common between right ovary and peritoneal metastasis (green). (B) A detailed Variant Allele Frequency and Relative Depth Log Ratio plot from the right ovary tumor sample indicating the regions where copy number alterations occurred on chromosomes 14 and 17. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
in-frame deletion of N2387, as well as F2388 (ClinVar, M_001042492.2:c.7159_7164delAACTTT), we postulate that the NF1 VUS reported herein represents a novel pathogenic mutation. Although, the BRCA1 variant (ClinVar, NM_007294.3(BRCA1):c.1846_1848-delTCT (p.Ser616del)) has been previously described in women of African ancestry with early onset breast cancer (Biunno et al., 2014; Tazzite et al., 2012), there are conflicting reports of its pathogenicity. Analysis of the 1000 genomes project revealed this BRCA1 variant (rs80358329) to be a rare variant observed in African sub-populations suggesting that it may be an ethnic specific polymorphism (Supplementary Fig. 1).

Sequencing of tumor samples (average depths 185×) revealed a marked degree of CNA and a frameshift mutation in TP53 (p.T79 fs) in all tumor samples (Fig. 1, Supplementary Table 1). The CNA data, which includes regional consistencies in variant allele frequency (VAF) and comparative levels of sequencing depth, revealed a clonal tumor lineage which originated from an ancestral clone (N1) in the left adnexa (Fig. 2). Initial genomic alterations associated with N1, which accounts for ~40% of the tumor specimen located in the left ovary, included a copy loss event (thus LOH) in a portion of 17p&q (Fig. 1B, region 1) as well as a TP53 mutation in the remaining allele. These events resulted in the N1 tumor clone being homozygous for the NF1 variant and the TP53 mutation, and homozygous for the BRCA1 reference allele. This is consistent with data from The Cancer Genome Atlas database, which demonstrates that somatic inactivation of NF1 in HGSOC is frequently associated with CNA loss, whereas other cancer types exhibit mutational inactivation (Supplementary Fig. 2) (Cancer Genome Atlas Research N, 2011). The bulk of the N1-event associated tumor cells in the left ovary (78%) and possibly 100% of the tumor cells from other sites exhibited an additional copy number neutral LOH event (N2) at 17q (regions 2 and 3) and chromosome 14 (region 3) (Fig. 1B). An additional subclone (N3) was present in the right ovary and included a copy number loss in other regions of chromosome 14 (region 1) that occurred after metastasis but which clonally expanded to be the dominant clone at the time of surgery.

Based on calculation of the fraction of cells within the tumor that harbored key mutational events and potentially pathogenic germline variants (Supplementary Table 2), we concluded that the initial transformative events occurred in the left ovary and involved CN loss in a region of chromosome 17 that resulted in biallelic inactivation of NF1 and loss of one TP53 allele, as well as a TP53 mutation (likely pathogenic) in the second allele. Since chromosome 17 CN loss and TP53 mutation were present in similar cell fractions (i.e., overlapping confidence intervals) of the tumor at all three sites, we were unable to ascertain whether the two events occurred simultaneously or sequentially. Nevertheless, our findings provide evidence that biallelic inactivation of NF1, accompanied by inactivation of TP53, occurred early in the development of HGSOC.

To determine whether the N2387 K mutation located in the C-terminal domain (CTD) of neurofibromin could alter protein function, we performed sequence and structure analysis of the C-terminal region, which comprises of the SEC14, pleckstrin homology (PH) and C-terminal domains. Due to the large size of this region (~1250 amino acids) and availability of the X-ray crystal structure of only the SEC14 and PH
domains we used fold recognition techniques and structure based sequence analysis to identify in importin-β the closest template for building a putative coarse model of CTD using remote homology modeling techniques (Supplementary methods). This model suggested that the N2387K mutation could affect the interaction of the CTD with a highly acidic loop linking SEC14 and PH, thereby changing the relative configuration of these three domains to alter protein function (Fig. 3, Supplementary Methods and Supplementary Fig. 3). This finding provides further support for a pathogenic role of the N2387K mutation.

Forty-five percent of HGSOC show evidence of hyperactive RAS-signaling (Cancer Genome Atlas Research N, 2011). Since neurofibromin is a negative regulator of RAS-signaling, complete loss of NF1, as observed in this patient's tumor, could confer an advantage during the transformative process. Indeed, ovarian cancer cell lines that harbor NF1 defects show increased RAS-mitogen activated protein kinase (MAPK) activation (Sangha et al., 2008). Our findings do not exclude the fact that NF1 haploinsufficiency may contribute to HGSOC development. Notably, in vitro and in vivo studies demonstrated that NF1 heterozygosity is associated with enhanced cellular proliferation and migration, as well as perturbed cellular differentiation. Further, NF1 +/− heterozygosity may modulate the microenvironment during tumorigenesis (Staser et al., 2010).

In summary, we highlight a case of a HGSOC patient with NF1 which supports the concept that females with NF1 should be monitored for ovarian cancer, in addition to breast cancer. The data presented add additional support for a pathogenic role of the N2387K mutation.

Supplementary Methods and Supplementary Fig. 3). This study was provided by the Carolinas Ovarian Cancer Research Fund, UEFISCDI grant PN-II-ID-PCE-2016-0650 (MDS and AJP) and the Romanian Academy programs 1 & 3 of IBAR (MDS and AJP).

Author contributions

EJN, RNG, and MKG designed the research study. EJN, DD, RNG, DT and JK were involved in the clinical management of the patient. CL performed pathological assessment of tumor samples. WDJ performed sequencing experiments. WDJ, IH and OZ performed analysis of sequencing data. MDS and AJP performed protein structural analysis. EJN, WDJ, and MKG wrote the manuscript. All authors edited the final manuscript.

Acknowledgements

First and foremost, the authors would like to thank the patient for her generosity in consenting to the present study. Financial support for this study was provided by the Carolinas Ovarian Cancer Research Fund, UEFISCDI grant PN-II-ID-PCE-2016-0650 (MDS and AJP) and the Romanian Academy programs 1 & 3 of IBAR (MDS and AJP).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gore.2018.01.005.

References

Biunnio, I., Aceto, G., Awadelkarim, K.D., Morgano, A., Elhaj, A., Eltayeb, E.A., et al., 2014. BRCA1 point mutations in premenopausal breast cancer patients from Central Sudan. Familial Cancer 13 (3), 437–444 (Epub 2014/04/15). Cancer Genome Atlas Research N, 2011. Integrated genomic analyses of ovarian carcinoma. Nature 474 (7353), 609–615 (Epub 2011/07/02). Ceccononi, M., Gennardì, M., Legge, F., Lucchi-Cordesco, E., Carrara, S., D’Amico, F., et al., 2002. BRCA1-related malignancies in a family presenting with von Recklinghausen’s disease. Gynecol. Oncol. 86 (3), 375–378 (Epub 2002/09/10). Jeon, Y.W., Kim, R.M., Lim, S.T., Choi, H.J., Suh, Y.J., 2015. Early-onset breast cancer in a family with neurofibromatosis type 1 associated with a germilne mutation in BRCA1. J. Breast Cancer 18 (1), 97–100 (Epub 2015/04/04). Kanchi, K.L., Johnson, K.J., Lu, C., McClean, M.D., Leiserson, M.D., Wendl, M.C., et al., 2014. Integrated analysis of germline and somatic variants in ovarian cancer. Nat. Commun. 5, 3156 (Epub 2014/01/23). Maeterlinx, O., Prenen, H., Debiec-Rychter, M., Wozniak, A., Sciot, R., Pawels, P., et al., 2006. Molecular pathogenesis of multiple gastrointestinal stromal tumors in NF1 patients. Hum. Mol. Genet. 15 (6), 1015–1023 (Epub 2006/02/08). McPherson, J.R., Ong, C.K., Ng, C.C., Rajoegarana, V., Heng, H.L., Yu, W.S., et al., 2015. Whole-exome sequencing of breast cancer, malignant peripheral nerve sheath tumor and neurofibroma from a patient with neurofibromatosis type 1. Cancer Med. 4 (12), 1871–1878 (Epub 2015/10/04). Patil, S., Chamberlain, R.S., 2012. Neoplasms associated with germline and somatic NF1 gene mutations. Oncologist 17 (1), 101–116 (Epub 2012/01/14). Salud, A., Perel, J.M., Capdevila, F., Felip, E., Rovira, M.A., del Campo, J.M., 1991. Ovarian cancer in a female patient with von Recklinghausen’s disease. Med. Clin. 96 (4), 138–140 (Epub 1991/02/02). Sangha, N., Wu, R., Kruik, R., Powers, S., Ma, D., Fiander, D., et al., 2008. Neurofibromin 1 (NF1) defects are common in human ovarian serous carcinomas and co-occur with TP53 mutations. Neoplasia 10 (12), 1362–1372 (following 72. Epub 2008/12/03). Staser, K., Yang, F.C., Clapp, D.W., 2010. Mast cells and the neurofibromatosis microenvironment. Blood 116 (2), 157–164 (Epub 2010/03/18). Tazzite, A., Jouhabi, H., Nadifi, S., Aretoni, F., Falaschi, E., Collavoli, A., et al., 2012. BRCA1 and BRCA2 germline mutations in Moroccan breast/ovarian cancer families: novel mutations and unclassified variants. Gynecol. Oncol. 125 (3), 687–692 (Epub 2012/03/20). Upadhyaya, M., Han, S., Consoli, C., Majounie, E., Horan, M., Thomas, N.S., et al., 2004. Characterization of the somatic mutational spectrum of the neurofibromatosis type 1 (NF1) gene in neurofibromatosis patients with benign and malignant tumors. Hum. Mutat. 23 (2), 134–146 (Epub 2004/01/15). Walker, L., Thompson, D., Eaton, D., Ponder, B., Ponder, M., Frayling, I., et al., 2006. A prospective study of neurofibromatosis type 1 cancer incidence in the UK. Br. J. Cancer 95 (2), 233–238 (Epub 2006/06/21). Yap, Y.S., McPherson, J.R., Ong, C.K., Rozen, S.G., Teh, B.T., Lee, A.S., et al., 2014. The NF1 gene revisited - from bench to bedside. Oncotarget 5 (15), 5873–5892 (Epub 2014/07/16).