Published in final edited form as:

Histopathology. 2019 March ; 74(4): 638–650. doi:10.1111/his.13796.

Somatic genetic alterations in synchronous and metachronous low-grade serous tumours and high-grade carcinomas of the adnexa

Rajmohan Murali¹, Pier Selenica¹, David N. Brown¹, R. Keira Cheetham², Raghu Chandramohan¹, Nidia L. Claros³, Nancy Bouvier³, Donovan T. Cheng⁴, Robert A. Soslow¹, Britta Weigelt¹, and W. Glenn McCluggage⁵

¹Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA;

²Illumina Cambridge Ltd, UK;

³Marie-Josée and Henry R. Kravis Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center, New York, NY, USA;

⁴Illumina Inc, San Francisco CA, USA;

⁵Department of Pathology, Belfast Health and Social Care Trust, Belfast, UK

Abstract

Aims: Low-grade serous carcinomas (LGSCs) and their precursor serous borderline tumours (SBTs) characteristically harbour mutations in *BRAF*, *KRAS* and *NRAS* but rarely in *TP53*, whereas high-grade serous carcinomas (HGSCs) are characterised by frequent *TP53* mutations but rare *BRAF*, *KRAS* and *NRAS* mutations. In a small subset of cases, LGSCs and/or SBTs develop high-grade tumours including HGSCs and poorly differentiated carcinomas (PDCs). Here we sought to define the repertoire of somatic genetic alterations in low-grade serous tumours and synchronous or metachronous high-grade adnexal carcinomas.

Methods and Results: DNA extracted from five SBTs/LGSCs and synchronous or metachronous HGSCs/PDCs and matched normal tissue was subjected to massively-parallel sequencing targeting all exons and selected non-coding regions of 341 cancer-related genes. The low-grade and high-grade tumours from a given case were related, and shared mutations and copy number alterations. Progression from low-grade to high-grade lesions was observed, and involved acquisition of additional mutations and copy number alterations or shifts from subclonal to clonal mutations. Only two (a HGSC and a PDC) of the five high-grade tumours investigated harboured *TP53* mutations, whereas *NRAS* and *KRAS* hotspot mutations were seen in two and one HGSCs, respectively.

Address for correspondence: Dr. Rajmohan Murali, Department of Pathology, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA. Phone: +1-212-639-5905, Fax: +1-646-422-2070, MuraliR@mskcc.org, Dr. Britta Weigelt, Department of Pathology, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA. Phone: +1-212-639-5905, Fax: +1-646-422-2070, weigeltb@mskcc.org.

Author contributions: Design (RM, BW, RAS, WGM); Pathological review (RM, RAS, WGM); Tissue processing and sequencing (NLC, NB, CG); Data analysis (BW, PS, RM, DNB, RKC, DTC, RC); Writing manuscript (RM, BW, WGM, RAS, DNB)

Conflict of interest: RKC and DTC are employees of Illumina. The remaining authors have no conflicts of interest to declare.

Conclusions: Our results suggest that progression from SBT to HGSC may take place in a subset of cases, and that at least some of the rare HGSCs lacking *TP53* mutations may be derived from a low-grade serous precursor.

Keywords

High-grade serous carcinoma; Low-grade serous carcinoma; Massively parallel sequencing
Molecular genetics; Ovary; Serous borderline tumour; Tumour progression

INTRODUCTION

Extrauterine serous carcinomas arising from the adnexal structures (fallopian tubes and ovaries) and peritoneum form two distinct groups that differ in their pathogenetic pathways and clinical behaviour.^{1–3} Low-grade serous carcinomas (LGSCs) often, but not always, arise from precursor lesions (serous borderline tumours, SBTs), which characteristically harbour mutations in *BRAF*, *KRAS*, *NRAS* and *ERBB2*, rarely show *TP53* mutations, and exhibit low cellular proliferation.^{1–7} On the other hand, compelling evidence suggests that the vast majority of extrauterine high-grade serous carcinomas (HGSCs) arise primarily via *de novo* neoplastic transformation of the fallopian tubal epithelium.^{3, 8–12} HGSCs are characterised by the presence of *TP53* mutations in >95% of cases^{13, 14} and high proliferative indices, but only rarely harbour mutations in *BRAF* and *KRAS*.^{1, 4, 5} The five year survival rates for women with advanced LGSC and HGSC are ~62%¹⁵ and 25–50%,¹⁶ respectively, although some studies have reported that the prognosis (especially long-term) in advanced-stage LGSC is similar to that of advanced-stage HGSC.^{15, 17}

The vast majority of extrauterine serous tumours show fidelity with respect to the two pathogenetic groups described above; recurrences in women with low-grade serous tumours (SBT and LGSC) are almost invariably in the form of SBT, non-invasive implants or LGSC (including invasive implants). Very rarely, however, women with LGSC and/or SBT develop high-grade tumours including HGSC and poorly differentiated/high-grade carcinomas including sarcomatoid carcinomas (the latter hereafter referred to as poorly differentiated carcinomas, PDCs), either synchronously^{18–24} or metachronously.^{24–27} When detected synchronously, women may present with high-grade tumour in association with SBT and/or LGSC.^{7, 20, 24, 25} In the metachronous setting, women initially presenting with SBT or LGSC may subsequently recur in the form of high-grade tumour (HGSC or PDC).^{22, 24, 26, 27} Although a single study⁷ reported the presence of SBT in association with ~6% of HGSCs (see discussion), several other studies^{18–27} have found the co-occurrence of low-grade and high-grade serous tumours to be a very infrequent phenomenon, the genetic alterations underlying which are poorly understood.

In this study, we sought to investigate the somatic genetic alterations in the different tumour components in a small cohort of women who developed both low-grade serous tumours and high-grade carcinomas, either synchronously or metachronously.

MATERIALS AND METHODS

Case selection

We identified 5 women who had combinations of the following ovarian tumours: low-grade serous tumours (SBT, LGSC) with synchronous or metachronous high-grade carcinomas (HGSC or PDC). The study was approved by the MSKCC institutional review board (protocol 16–1684, approved 23 December 2016).

Histopathological classification of tumours

Tumour slides were reviewed by 3 experienced gynaecological pathologists (RM, RAS, WGM). Histopathological classification of tumours as SBT, LGSC and HGSC was based on the criteria in the World Health Organization Classification of Tumours of Female Reproductive Organs.²⁸ High-grade carcinomas composed of polygonal or spindle-shaped cells exhibiting marked cytological atypia, high mitotic indices, variable necrosis and generally sheet-like architecture were classified as PDC.

DNA extraction

From representative formalin-fixed, paraffin-embedded (FFPE) tissue blocks, one 5µm-thick section was stained with hematoxylin and eosin (H&E). Areas of non-neoplastic tissue (uninvolved ovary spatially distant from tumour areas), low-grade serous tumour (SBT and/or LGSC), and high-grade carcinoma were mapped. Unstained sections (10µm) were deparaffinised, and tumour and normal tissues were manually microdissected following the mapped H&E slides as guides. Genomic DNA from the dissected tissue samples was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Targeted capture massively parallel sequencing

DNA obtained from the microdissected FFPE tumour and matched normal tissue specimens was subjected to targeted capture massively parallel sequencing at the Memorial Sloan Kettering Cancer Center (MSKCC) Integrated Genomics Operation (IGO) using the MSK-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) assay, targeting all exons and selected non-coding regions of 341 cancer-related genes, as previously described.^{29, 30}

MSK-IMPACT sequencing data were analyzed as previously described.^{30, 31} In brief, paired-end reads were aligned to the human reference genome GRCh37 using the Burrows-Wheeler Aligner (BWA 0.7.15).³² Local realignment, duplicate removal and quality score recalibration were performed using the Genome Analysis Toolkit (GATK 3.7).³³ Somatic mutations were identified using MuTect (1.1.7) for single nucleotide variants (SNVs),³⁴ and Strelka (1.0.15) and VarScan2 (2.3.7) for small insertions and deletions (indels)^{35, 36}. Variants with a mutant allelic fraction (MAF) of <1% and/or variants supported by <5 reads and/or covered by <10 reads at a given locus were discarded. Additionally, variants whose MAF in the tumour was <5 times that in the matched normal sample were disregarded, as were variants, which were present at >5% minor allele frequency in dbSNP (Build 137).³¹ Mpileup files generated from SAMtools mpileup (1.2.1)³⁷ for each sample were used to

determine whether a given mutation detected from the pipeline exists in the BAM file of the corresponding matched SBT, LGSC, HGSC or PDC from the same individual.

Copy number alterations and loss of heterozygosity (LOH) were identified using FACETS³⁸ as previously described.^{31, 39} The cancer cell fraction (CCF) for each mutation was inferred using ABSOLUTE (1.0.6)⁴⁰ as previously described.^{31, 39} Mutations for which the probability of being clonal was >50%⁴¹ or those for which the lower bound of the 95% confidence interval of CCF was >90%⁴² were classified as clonal.^{31, 39} A combination of mutation function predictors was employed to define the potential effect of each non-synonymous SNV, as described previously.^{39, 43} Mutation hotspots were defined according to Chang *et al.*⁴⁴

RESULTS

Summary of findings in study cohort

We included five women with combinations of the following ovarian tumours: low-grade serous tumours (SBT and/or LGSC) with synchronous or metachronous high-grade tumours (HGSC or PDC) (Table 1). The age of the women ranged from 35–82 years at initial diagnosis. In four women, the low-grade and high-grade tumours were diagnosed synchronously at initial presentation (cases 1, 3, 4 and 5). One woman (case 2) was initially diagnosed with SBT and LGSC, and five years later, developed a recurrence that comprised HGSC (Table 1).

We subjected the low-grade and high-grade tumours and matched normal tissues from the five cases to massively parallel sequencing targeting 341 cancer genes (using the MSK-IMPACT assay²⁹), at a median depth of coverage of 971x (range 791x-1256x) for tumour and of 523x (range 326x-628x) for normal samples (Supplementary Table 1). This analysis revealed that the low-grade and high-grade tumours in all cases were related at the molecular genetic level, and shared somatic mutations and/or gene copy number alterations. Furthermore, the tumours from all but one of the cases harboured mutations affecting the RTK/RAS pathway, including *KRAS* or *NRAS* hotspot mutations or *NFI* loss-of-function mutations. In addition, the tumours from two of the five cases harboured pathogenic *TP53* mutations (Figs 1–5; Supplementary Table 2).

Case 1

A 35-year-old woman presented with synchronous LGSC and HGSC (Table 1; Fig. 1). Based on the 341 genes tested, the LGSC and HGSC were found to harbour only a single likely pathogenic non-synonymous somatic missense mutation in *PIK3C2G* (p.G1085V) affecting the PI3K pathway. This mutation was subclonal in the LGSC and clonal in the HGSC, suggesting progression from the low-grade to high-grade tumour. This was further supported by the observation that the high-grade tumour displayed higher levels of copy number alterations compared to the case-matched LGSC (Fig. 1).

Case 2

A 52-year-old woman presented with synchronous SBT and LGSC; five years later, she presented with a recurrent tumour, which comprised HGSC (Table 1; Fig. 2). The SBT and HGSC shared a clonal *EIF1AX* p.G6D mutation associated with loss of heterozygosity (LOH) of the wild-type allele, a clonal *NRAS* p.Q61R hotspot mutation and a clonal *SETD2* p.X1529 splice site mutation as well as an *EGFR* p.G378C mutation, which was subclonal in the SBT but clonal in the HGSC. In addition, somatic mutations private to either the SBT or HGSC were identified; the SBT harboured a *CDKN2A* hotspot mutation, and the HGSC showed *SMARCA4* and *CREBBP* loss-of-function mutations coupled with LOH as well as *EP300* and *MYCL1* missense mutations (Fig. 2). Of note, *SETD2*, *EP300* and *SMARCA4* are chromatin-remodeling genes, aberrations in which play an important role in carcinogenesis.^{45–47} These data suggest that whilst the SBT and HGSC of case 2 are clonally related, parallel evolution took place and the lesions acquired private driver alterations.

Case 3

An 82-year-old woman presented with synchronous SBT, LGSC and PDC (Table 1; Fig. 3). The PDC was a high-grade sarcomatoid carcinoma composed of spindle and epithelioid cells. The level of copy number alterations was higher in the PDC compared to the synchronous SBT/LGSC (Fig. 3). We observed that all components harboured a clonal frameshift *NFI* mutation coupled with LOH and a clonal *ATR* nonsense mutation. Consistent with the increase in copy number alterations, a pathogenic frameshift *TP53* mutation coupled with LOH was absent in the SBT, subclonal in the LGSC and clonal in the PDC, providing evidence to suggest that progression from SBT to LGSC and PDC took place in this case. Additional mutations private to the LGSC and PDC were present, including subclonal mutations in genes associated with chromatin remodeling (*SPOP* and *KMT2D*, respectively; Fig. 3).

Case 4

A 41-year-old woman presented with synchronous SBT, LGSC and HGSC (Table 1; Fig. 4). The SBT, LGSC and HGSC showed only few copy number alterations, with a high-level *PAX8* amplification (chromosome 2q14.1) and a low-level *ETV6* amplification (chromosome 12p13.2) restricted to the SBT (Fig. 4). Only one clonal mutation was shared by all three tumour components, an *NRAS* Q61R hotspot mutation (Fig. 4). The HGSC harboured an additional *NRAS* T50I mutation.

Case 5

A 67-year-old woman presented with synchronous SBT and HGSC (Table 1; Fig. 5). The HGSC showed a considerably larger number of copy number gains and losses than the SBT, including a focal amplification of *MYC* (chromosome 8q24.21; Fig. 5). Both the SBT and HGSC shared a clonal *KRAS* G12V hotspot mutation. Consistent with the increase in genomic instability accompanying the progression from SBT to HGSC, we identified a subclonal *TP53* Q136E mutation in the SBT, which became clonal in the HGSC (Fig. 5). Of note, rather than a *MYC* amplification (c-Myc), the SBT of case 5 was found to harbour a

frameshift mutation in *MYCN* (n-Myc, chromosome 2p24.3), another member of the Myc family of oncogenes, providing evidence of a convergent phenotype (Fig. 5).

DISCUSSION

Here we report that low-grade tumours (SBT and/or LGSC) and high-grade tumours from a given individual share mutations and gene copy number alterations, and observed that progression from low-grade to high-grade tumours takes place either through acquisition of additional genetic alterations (mutations/copy number alterations) or shifts from subclonal to clonal mutations.

Two hypotheses for the observed co-occurrence of SBT and HGSC have been proposed: 1) SBT is rarely a precursor of HGSC, with *TP53* mutation being an early event that occurs in a small subset of SBT and determines its progression to HGSC; or 2) The SBT-like component is not a precursor of HGSC, but rather represents a morphologically better differentiated component of the HGSC that mimics SBT but harbours molecular alterations characteristic of HGSC.

With respect to the latter hypothesis, Emmanuel *et al.*⁷ reported the presence of SBT in association with invasive serous carcinomas in ~12% (16/136 cases with pathological review of a full set of diagnostic slides) of cases; approximately half of the carcinomas were classified as HGSC based on molecular and pathological findings. Their reported 6% frequency of co-occurrence of low-grade serous and high-grade tumours in the same individuals⁷ is substantially higher than that reported in other studies, which found this to be a very infrequent phenomenon.^{18–27} This discrepancy is possibly due to the fact that areas classified as SBT by Emmanuel *et al.*⁷ may represent areas of HGSC with a ‘borderline-like’ architecture that morphologically mimic SBT but represent an unusual growth pattern of HGSC and harbour molecular alterations characteristic of HGSC; this was acknowledged by the authors.⁷ In other studies, such areas were correctly interpreted as HGSC rather than as SBT.

RAS-mutant SBTs and co-occurring HGSCs with and without *TP53* mutations have been rarely described in the literature.^{7, 48, 49} In our series, we found that only 2 (a HGSC and a PDC) of the 5 high-grade tumours harboured *TP53* mutations, which are generally characteristic of HGSCs,^{13, 14} whereas *NRAS* and *KRAS* hotspot mutations, characteristic of SBTs,^{6, 7} were present in 2 and 1 HGSCs, respectively. Only 1 of the 3 high-grade tumours with *KRAS* or *NRAS* mutations also had a concurrent *TP53* mutation, and the other *TP53*-mutant case harboured a concurrent clonal *NF1* loss-of-function mutation coupled with LOH. In addition, we observed progression in each of our five cases, in the form of shifts from subclonal in the SBT/LGSC to clonal mutations in the HGSC/PDC (*PIK3C2G* in case 1, *EGFR* in case 2, *TP53* in cases 3 and 5) or the acquisition of additional mutations/copy number alterations (all cases). These data provide evidence to suggest that progression from SBT to HGSC may take place in a small subset of cases. Furthermore, we hypothesise that at least a subset of the small proportion of HGSCs lacking *TP53* mutations are derived from a low-grade serous precursor.

In the context of understanding the progression from low-grade to high-grade serous cancers, consistent with the notion that acquisition of genetic complexity would be expected to match histologic progression, we identified an additional *NRAS*T50I mutation in the HGSC in case 4. The *NRAS*T50I mutation is not a canonical activating (i.e. hotspot) mutation acquired in the context of somatic genetic alterations in human cancers. It should be noted, however, that in the cBioPortal⁵⁰ and COSMIC⁵¹ databases, somatic *NRAS*T50I mutations are predicted to be likely pathogenic and gain-of-function. Importantly, the *NRAS*T50I mutation has also been reported to activate MAPK signaling in cells and, when acquired in the germline setting, to be causative of Noonan Syndrome.⁵² This *NRAS* mutation may not necessarily be causative of the progression in this case; in fact, this mutation may have merely constituted an indication of additional genetic complexity in the HGSC. However we cannot rule out the alternative hypothesis that this mutation may have contributed to the progression, given that acquisition of non-canonical *RAS* and *PIK3CA* mutations are known to be involved in the progression of other solid malignancies.

It is possible that progression from low-grade to high-grade tumours would be observed more frequently over time in women with untreated low-grade serous tumours, but the natural course of disease is interrupted in the vast majority of individuals by diagnosis and treatment (involving surgical removal and sometimes chemotherapy) prior to the occurrence of the transformative genetic events. Alternative possibilities are that some women diagnosed with high-grade tumours may have had an antecedent or coexisting low-grade tumour which: 1) was not recognised, for example due to its being present only focally and/or sampling bias; 2) was overgrown by the high-grade tumour, and/or 3) underwent early, rapid clonal evolution and progression to a high-grade tumour that subsequently dominated the tumour burden. As discussed earlier, there is now compelling evidence that a large majority of extrauterine HGSCs arise from the fallopian tube, especially the fimbria, while low-grade serous tumours have an ovarian origin.^{3, 8–12} It could be hypothesized that some HGSCs with grossly normal tubes and no mucosal tubal lesion after examination by a detailed SEE-FIM (Sectioning and Extensively Examining the FIMbria) protocol arise from low-grade serous precursors. Interestingly in our cases, no tubal lesions were identified, although in most of the cases, the tubes were not examined using a SEE-FIM protocol.

The low-grade and high-grade tumours in cases 2 and 3 harboured likely pathogenic alterations in genes involved in chromatin remodeling, including *SETD2* and *KMT2D* (which mediate methylation of histone H3^{53, 54}); *EP300* (responsible, in conjunction with *CREBBP*, for acetylation of histone H3⁵⁵); *SMARCA4* (a component of the SWI/SNF complex⁵⁶); and *SPOP* (which encodes an E3 ubiquitin ligase protein⁵⁷). Ovarian tumours commonly exhibiting alterations in chromatin-remodeling genes include clear cell and endometrioid carcinomas and small cell carcinomas of hypercalcemic-type.^{47, 56, 58, 59} Alterations in several genes involved in chromatin remodeling have also been reported in serous carcinomas, more commonly in those arising in the endometrium (including *CHD4*, *EP300*, *ARID1A*, *FBXW7* and *SPOP*)^{46, 60, 61} than in the adnexa.^{47, 62} TCGA analyses identified *SMARCA4* alterations in a minority of ovarian HGSC, a small subset of which lacked identifiable *TP53* mutations.¹⁴ Our finding of clonal mutations in *SETD2*, *EP300* and *SMARCA4* in the HGSC in case 2 adds to the list of reported mutations in chromatin remodeling genes in adnexal HGSC, including *CARM1*⁴⁷ and *RSF1*.⁶² The HGSC in case 2

exhibited a predominantly solid architecture but did not show any unusual morphological features. In contrast, the high-grade tumour in case 3 showed a monomorphic spindled/sarcomatoid appearance, the neoplastic cells showing marked cytological atypia and exhibiting diffuse cytokeratin positivity; WT1, a marker which is positive in most adnexal HGSCs, was negative but was positive in the HGSC component of the other cases. This tumour contained a subclonal mutation of *KMT2D*, given the frequent presence of mutations in chromatin remodeling genes in gynaecological carcinosarcomas⁶³ and mammary phyllodes tumours,⁶⁴ and the recent identification of a *KMT2D-BCOR* fusion in a pelvic sarcoma characterised by round-to-short spindle cells,⁶⁵ the question of whether these mutations play a role in the development of sarcomatoid morphology in gynaecological malignancies merits further study.

Our study has several limitations. Given the rarity of the coexistence of low-grade serous and high-grade tumours^{20, 25, 26}, the number of cases analyzed here is small. As all tissue material analyzed in this study was formalin-fixed and paraffin-embedded and the amount of tumour and normal tissue was limited, we subjected the different tumour components to targeted massively parallel sequencing focusing on cancer-related genes rather than to whole-exome or whole genome sequencing. Therefore, we cannot exclude the possibility that additional genes not studied here may be involved, however, most of the genes described to be recurrently altered in SBTs and HGSCs are captured by our sequencing panel, and mutations were identified in each of the cases analyzed. In addition, the number of somatic mutations identified in the different tumour components was too low to assess the mutational signatures, and further studies are warranted to define whether there is a shift in the mutational signatures in the progression from low-grade serous tumours to high-grade carcinomas.

Despite these limitations, our results suggest that progression from SBT/LGSC to HGSC may take place in a subset of cases. Based on our findings, we hypothesise that at least some of the rare HGSCs lacking *TP53* mutations may be derived from a low-grade precursor and that a sarcomatoid phenotype in high-grade tumours may be associated with mutations in chromatin remodeling genes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We are most grateful to the following individuals and institutions for their assistance: Jennifer Becq (Bioinformatics at Illumina); the Marie-Josée and Henry R. Kravis Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center.

Funding: This work was supported in part by the National Cancer Institute at the National Institutes of Health (Cancer Center Support Grant P30 CA008748).

REFERENCES

1. Shih Ie M, Kurman RJ. Ovarian tumorigenesis: A proposed model based on morphological and molecular genetic analysis. *Am J Pathol* 2004;164:1511–1518. [PubMed: 15111296]

2. Kaldawy A, Segev Y, Lavie O, Auslender R, Sopik V, Narod SA. Low-grade serous ovarian cancer: A review. *Gynecol Oncol* 2016;143:433–438. [PubMed: 27581327]
3. Singh N, McCluggage WG, Gilks CB. High-grade serous carcinoma of tubo-ovarian origin: Recent developments. *Histopathology* 2017;71:339–356. [PubMed: 28477361]
4. Dansonka-Mieszkowska A, Ludwig AH, Kraszewska E, Kupryjanczyk J. Geographical variations in tp53 mutational spectrum in ovarian carcinomas. *Ann Hum Genet* 2006;70:594–604. [PubMed: 16907706]
5. Kurman RJ, Shih Ie M. Pathogenesis of ovarian cancer: Lessons from morphology and molecular biology and their clinical implications. *Int J Gynecol Pathol* 2008;27:151–160. [PubMed: 18317228]
6. Boyd J, Luo B, Peri S et al. Whole exome sequence analysis of serous borderline tumors of the ovary. *Gynecol Oncol* 2013;130:560–564. [PubMed: 23774303]
7. Emmanuel C, Chiew YE, George J et al. Genomic classification of serous ovarian cancer with adjacent borderline differentiates ras pathway and tp53-mutant tumors and identifies nras as an oncogenic driver. *Clin Cancer Res* 2014;20:6618–6630. [PubMed: 25316818]
8. Lee Y, Miron A, Drapkin R et al. A candidate precursor to serous carcinoma that originates in the distal fallopian tube. *J Pathol* 2007;211:26–35. [PubMed: 17117391]
9. Singh N, Gilks CB, Wilkinson N, McCluggage WG. Assignment of primary site in high-grade serous tubal, ovarian and peritoneal carcinoma: A proposal. *Histopathology* 2014;65:149–154. [PubMed: 24660659]
10. Eckert MA, Pan S, Hernandez KM et al. Genomics of ovarian cancer progression reveals diverse metastatic trajectories including intraepithelial metastasis to the fallopian tube. *Cancer Discov* 2016;6:1342–1351. [PubMed: 27856443]
11. Singh N, Gilks CB, Hirschowitz L et al. Primary site assignment in tubo-ovarian high-grade serous carcinoma: Consensus statement on unifying practice worldwide. *Gynecol Oncol* 2016;141:195–198. [PubMed: 26827965]
12. Singh N, Gilks CB, Hirshowitz L, Wilkinson N, McCluggage WG. Adopting a uniform approach to site assignment in tubo-ovarian high-grade serous carcinoma: The time has come. *Int J Gynecol Pathol* 2016;35:230–237. [PubMed: 26977579]
13. Ahmed AA, Etemadmoghadam D, Temple J et al. Driver mutations in tp53 are ubiquitous in high grade serous carcinoma of the ovary. *J Pathol* 2010;221:49–56. [PubMed: 20229506]
14. Cancer Genome Atlas Research N. Integrated genomic analyses of ovarian carcinoma. *Nature* 2011;474:609–615. [PubMed: 21720365]
15. Okoye E, Euscher ED, Malpica A. Ovarian low-grade serous carcinoma: A clinicopathologic study of 33 cases with primary surgery performed at a single institution. *Am J Surg Pathol* 2016;40:627–635. [PubMed: 26900814]
16. Heintz AP, Odicino F, Maisonneuve P et al. Carcinoma of the ovary. FIGO 26th annual report on the results of treatment in gynecological cancer. *Int J Gynaecol Obstet* 2006;95 Suppl 1:S161–192.
17. Ali RH, Kalloger SE, Santos JL, Swenerton KD, Gilks CB. Stage ii to iv low-grade serous carcinoma of the ovary is associated with a poor prognosis: A clinicopathologic study of 32 patients from a population-based tumor registry. *Int J Gynecol Pathol* 2013;32:529–535. [PubMed: 24071867]
18. McCullough J, Batman P, Parker D. Sarcoma-like nodules in cystic ovarian tumours. *J Clin Pathol* 1988;41:1028–1029. [PubMed: 3192749]
19. De Rosa G, Donofrio V, De Rosa N, Fulciniti F, Zeppa P. Ovarian serous tumor with mural nodules of carcinomatous derivation (sarcomatoid carcinoma): Report of a case. *Int J Gynecol Pathol* 1991;10:311–318. [PubMed: 1917278]
20. Dehari R, Kurman RJ, Logani S, Shih Ie M. The development of high-grade serous carcinoma from atypical proliferative (borderline) serous tumors and low-grade micropapillary serous carcinoma: A morphologic and molecular genetic analysis. *Am J Surg Pathol* 2007;31:1007–1012. [PubMed: 17592266]
21. Andrews TD, Dutton PM, Beattie G, Al-Nafussi A. Sarcomatoid carcinoma arising within a serous borderline ovarian tumour: A case report and practical approach to differential diagnosis. *Histopathology* 2008;52:233–238. [PubMed: 17903197]

22. Quddus MR, Rashid LB, Hansen K, Sung CJ, Lawrence WD. High-grade serous carcinoma arising in a low-grade serous carcinoma and micropapillary serous borderline tumour of the ovary in a 23-year-old woman. *Histopathology* 2009;54;771–773. [PubMed: 19438755]
23. Gungor T, Altinkaya SO, Akbay S, Bilge U, Mollamahmutoglu L. Malign mural nodules associated with serous ovarian tumor of borderline malignancy: A case report and literature review. *Arch Gynecol Obstet* 2010;281;485–490. [PubMed: 19597831]
24. Boyd C, McCluggage WG. Low-grade ovarian serous neoplasms (low-grade serous carcinoma and serous borderline tumor) associated with high-grade serous carcinoma or undifferentiated carcinoma: Report of a series of cases of an unusual phenomenon. *Am J Surg Pathol* 2012;36;368–375. [PubMed: 22082603]
25. Malpica A, Deavers MT, Lu K et al. Grading ovarian serous carcinoma using a two-tier system. *Am J Surg Pathol* 2004;28;496–504. [PubMed: 15087669]
26. Parker RL, Clement PB, Chervocky DJ, Sornarajah T, Gilks CB. Early recurrence of ovarian serous borderline tumor as high-grade carcinoma: A report of two cases. *Int J Gynecol Pathol* 2004;23;265–272. [PubMed: 15213603]
27. Garg K, Park KJ, Soslow RA. Low-grade serous neoplasms of the ovary with transformation to high-grade carcinomas: A report of 3 cases. *Int J Gynecol Pathol* 2012;31;423–428. [PubMed: 22833081]
28. Kurman RJ, Carcangiu M-L, Herrington CS, Young RH. World health organization classification of tumours of female reproductive organs. Lyon: IARC Press, 2014.
29. Cheng DT, Mitchell TN, Zehir A et al. Memorial sloan kettering-integrated mutation profiling of actionable cancer targets (msk-impact): A hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. *J Mol Diagn* 2015;17;251–264. [PubMed: 25801821]
30. Schultheis AM, Ng CK, De Filippo MR et al. Massively parallel sequencing-based clonality analysis of synchronous endometrioid endometrial and ovarian carcinomas. *J Natl Cancer Inst* 2016;108;djv427. [PubMed: 26832770]
31. DeLair DF, Burke KA, Selenica P et al. The genetic landscape of endometrial clear cell carcinomas. *J Pathol* 2017;243;230–241. [PubMed: 28718916]
32. Li H, Durbin R. Fast and accurate short read alignment with burrows-wheeler transform. *Bioinformatics* 2009;25;1754–1760. [PubMed: 19451168]
33. DePristo MA, Banks E, Poplin R et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet* 2011;43;491–498. [PubMed: 21478889]
34. Cibulskis K, Lawrence MS, Carter SL et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat Biotechnol* 2013;31;213–219. [PubMed: 23396013]
35. Koboldt DC, Zhang Q, Larson DE et al. VarScan 2: Somatic mutation and copy number alteration discovery in cancer by exome sequencing. *Genome Res* 2012;22;568–576. [PubMed: 22300766]
36. Saunders CT, Wong WS, Swamy S, Becq J, Murray LJ, Cheetham RK. Strelka: Accurate somatic small-variant calling from sequenced tumor-normal sample pairs. *Bioinformatics* 2012;28;1811–1817. [PubMed: 22581179]
37. Li H, Handsaker B, Wysoker A et al. The sequence alignment/map format and samtools. *Bioinformatics* 2009;25;2078–2079. [PubMed: 19505943]
38. Shen R, Seshan VE. Facets: Allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. *Nucleic Acids Res* 2016;44;e131. [PubMed: 27270079]
39. Ng CKY, Pisuoglio S, Geyer FC et al. The landscape of somatic genetic alterations in metaplastic breast carcinomas. *Clin Cancer Res* 2017;23;3859–3870. [PubMed: 28153863]
40. Carter SL, Cibulskis K, Helman E et al. Absolute quantification of somatic DNA alterations in human cancer. *Nat Biotechnol* 2012;30;413–421. [PubMed: 22544022]
41. Landau DA, Carter SL, Stojanov P et al. Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. *Cell* 2013;152;714–726. [PubMed: 23415222]
42. Pisuoglio S, Ng CK, Murray MP et al. The genomic landscape of male breast cancers. *Clin Cancer Res* 2016;22;4045–4056. [PubMed: 26960396]

43. Martelotto LG, Ng CK, De Filippo MR et al. Benchmarking mutation effect prediction algorithms using functionally validated cancer-related missense mutations. *Genome Biol* 2014;15;484. [PubMed: 25348012]
44. Chang MT, Asthana S, Gao SP et al. Identifying recurrent mutations in cancer reveals widespread lineage diversity and mutational specificity. *Nat Biotechnol* 2016;34;155–163. [PubMed: 26619011]
45. Wilson BG, Roberts CW. Swi/snf nucleosome remodellers and cancer. *Nat Rev Cancer* 2011;11;481–492. [PubMed: 21654818]
46. Okawa R, Banno K, Iida M et al. Aberrant chromatin remodeling in gynecological cancer. *Oncol Lett* 2017;14;5107–5113. [PubMed: 29113150]
47. Zhang R, Fukumoto T, Magno E. Swi/snf complexes in ovarian cancer: Mechanistic insights and therapeutic implications. *Mol Cancer Res* 2018.
48. Bashashati A, Ha G, Tone A et al. Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling. *J Pathol* 2013;231;21–34. [PubMed: 23780408]
49. Hunter SM, Anglesio MS, Ryland GL et al. Molecular profiling of low grade serous ovarian tumours identifies novel candidate driver genes. *Oncotarget* 2015;6;37663–37677. [PubMed: 26506417]
50. Cbioportal for cancer genomics.
51. Cosmic: Catalogue of somatic mutations in cancer.
52. Cirstea IC, Kutsche K, Dvorsky R et al. A restricted spectrum of nras mutations causes noonan syndrome. *Nat Genet* 2010;42;27–29. [PubMed: 19966803]
53. Li J, Duns G, Westers H, Sijmons R, van den Berg A, Kok K. Setd2: An epigenetic modifier with tumor suppressor functionality. *Oncotarget* 2016;7;50719–50734. [PubMed: 27191891]
54. Froimchuk E, Jang Y, Ge K. Histone h3 lysine 4 methyltransferase kmt2d. *Gene* 2017;627;337–342. [PubMed: 28669924]
55. Attar N, Kurdistani SK. Exploitation of ep300 and crebbp lysine acetyltransferases by cancer. *Cold Spring Harb Perspect Med* 2017;7.
56. Jelinic P, Mueller JJ, Olvera N et al. Recurrent smarca4 mutations in small cell carcinoma of the ovary. *Nat Genet* 2014;46;424–426. [PubMed: 24658004]
57. Mani RS. The emerging role of speckle-type poz protein (spop) in cancer development. *Drug Discov Today* 2014;19;1498–1502. [PubMed: 25058385]
58. Wiegand KC, Shah SP, Al-Agha OM et al. Arid1a mutations in endometriosis-associated ovarian carcinomas. *The New England journal of medicine* 2010;363;1532–1543. [PubMed: 20942669]
59. Witkowski L, Carrot-Zhang J, Albrecht S et al. Germline and somatic smarca4 mutations characterize small cell carcinoma of the ovary, hypercalcemic type. *Nat Genet* 2014;46;438–443. [PubMed: 24658002]
60. Le Gallo M, O'Hara AJ, Rudd ML et al. Exome sequencing of serous endometrial tumors identifies recurrent somatic mutations in chromatin-remodeling and ubiquitin ligase complex genes. *Nat Genet* 2012;44;1310–1315. [PubMed: 23104009]
61. Zhao S, Choi M, Overton JD et al. Landscape of somatic single-nucleotide and copy-number mutations in uterine serous carcinoma. *Proc Natl Acad Sci U S A* 2013;110;2916–2921. [PubMed: 23359684]
62. Sheu JJ, Choi JH, Guan B et al. Rsf-1, a chromatin remodelling protein, interacts with cyclin e1 and promotes tumour development. *J Pathol* 2013;229;559–568. [PubMed: 23378270]
63. Jones S, Stransky N, McCord CL et al. Genomic analyses of gynaecologic carcinosarcomas reveal frequent mutations in chromatin remodelling genes. *Nat Commun* 2014;5;5006. [PubMed: 25233892]
64. Tan J, Ong CK, Lim WK et al. Genomic landscapes of breast fibroepithelial tumors. *Nat Genet* 2015;47;1341–1345. [PubMed: 26437033]
65. Kao YC, Owosho AA, Sung YS et al. Bcor-ccnb3 fusion positive sarcomas: A clinicopathologic and molecular analysis of 36 cases with comparison to morphologic spectrum and clinical behavior of other round cell sarcomas. *Am J Surg Pathol* 2018;42;604–615. [PubMed: 29300189]

Figure 1. Gene copy number alterations and somatic mutations identified in the low-grade serous carcinoma (LGSC) and high-grade serous carcinoma (HGSC) of case 1. Representative haematoxylin and eosin stained sections of the LGSC (x200) and HGSC (x200) are shown on the top left. Chromosome plots are shown on the right, with the Log₂-ratios plotted on the y-axis according to their genomic coordinates on the x-axis. On the bottom, non-synonymous somatic mutations (left) and the cancer cell fractions of the mutations (right) are shown. Only one likely pathogenic non-synonymous somatic missense mutation in *PIK3C2G* was identified; this mutation was subclonal in the LGSC and clonal in the HGSC. HGSC = high-grade serous carcinoma; LGSC = low-grade serous carcinoma; SNV = single nucleotide variant.

Figure 2. Gene copy number alterations and somatic mutations identified in the serous borderline tumour (SBT) and high-grade serous carcinoma (HGSC) of case 2. Representative haematoxylin and eosin stained sections of the SBT (x100; inset x400) and HGSC (x200) are shown on the top left. Chromosome plots are shown on the right, with the Log₂-ratios plotted on the y-axis according to their genomic coordinates on the x-axis. On the bottom, non-synonymous somatic mutations (left) and the cancer cell fractions of the mutations (right) are shown. The SBT and HGSC of case 2 shared a clonal *EIF1AX* mutation associated with loss of heterozygosity (LOH) of the wild-type allele, a clonal *NRAS* hotspot mutation and a clonal *SETD2* splice site mutation as well as a *EGFR* mutation (subclonal in the SBT and clonal in the HGSC). In addition, somatic mutations private to either the SBT (*CDKN2A*) or HGSC (*SMARCA4* and *CREBBP* mutations coupled with LOH as well as *EP300* and *MYCL1* missense mutation) were found. HGSC =

high-grade serous carcinoma; Indel = small insertion and deletion; SBT = serous borderline tumour; SNV – single nucleotide variant.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 3. Gene copy number alterations and somatic mutations identified in the serous borderline tumour (SBT), low-grade serous carcinoma (LGSC) and poorly differentiated carcinoma (PDC) of case 3.

Representative haematoxylin and eosin stained sections of the SBT (x100), LGSC (x200; inset x400) and PDC (x200) are shown on the top left. Chromosome plots are shown on the right, with the Log₂-ratios plotted on the y-axis according to their genomic coordinates on the x-axis. On the bottom, non-synonymous somatic mutations (left) and the cancer cell fractions of the mutations (right) are shown. All components harboured a clonal frameshift *NF1* mutation coupled with LOH and a clonal *ATR* nonsense mutation. A pathogenic frameshift *TP53* mutation coupled with LOH was clonal in the PDC, subclonal in the LGSC and absent in the SBT. Additional subclonal mutations private to the LGSC and PDC were present, including mutations in genes associated with chromatin remodeling (*SPOP* and

KMT2D). The PDC shows a conspicuously greater number of copy number alterations. Indel = small insertion and deletion; LGSC = low-grade serous carcinoma; PDC = poorly differentiated carcinoma; SBT = serous borderline tumour; SNV – single nucleotide variant.

Figure 4. Gene copy number alterations and somatic mutations identified in the serous borderline tumour (SBT), low-grade serous carcinoma (LGSC) and high-grade serous carcinoma (HGSC) of case 4.

Representative haematoxylin and eosin stained sections of the SBT (x100), LGSC (x200) and HGSC (x200) are shown on the top left. Chromosome plots are shown on the right, with the Log₂-ratios plotted on the y-axis according to their genomic coordinates on the x-axis. On the bottom, non-synonymous somatic mutations (left) and the cancer cell fractions of the mutations (right) are shown. Only one clonal *NRAS* hotspot mutation was shared by all three tumour components. The HGSC harboured an additional *NRAS* mutation. The SBT showed a high-level *PAX8* amplification and a low-level *ETV6* amplification. HGSC = high-grade serous carcinoma; LGSC = low-grade serous carcinoma; SBT = serous borderline tumour; SNV – single nucleotide variant.

Figure 5. Gene copy number alterations and somatic mutations identified in the serous borderline tumour (SBT) and high-grade serous carcinoma (HGSC) of case 5. Representative haematoxylin and eosin stained sections of the SBT (x200; including a focus of adjacent HGSC on the left) and HGSC (x200) are shown on the top left. Chromosome plots are shown on the right, with the Log₂-ratios plotted on the y-axis according to their genomic coordinates on the x-axis. On the bottom, non-synonymous somatic mutations (left) and the cancer cell fractions of the mutations (right) are shown. Both the SBT and HGSC shared a clonal *KRAS* G12V hotspot mutation. A subclonal *TP53* mutation in the SBT became clonal in the HGSC. The HGSC showed a considerably larger number of copy number gains and losses than the SBT, including a focal amplification of *MYC* (chromosome 8q24.21), whereas the SBT harboured a *MYCN* mutation. HGSC = high-grade serous carcinoma; Indel = small insertion and deletion; SBT = serous borderline tumour; SNV – single nucleotide variant.

Table 1.

Clinico-pathological features of low-grade serous tumours and high-grade serous carcinomas

Case ID	Age at diagnosis (years)	FIGO stage at diagnosis	Low-grade tumour	High-grade carcinoma	Salient histopathological features	Outcome
Case 1	35	IIIC	LGSC	HGSC	Areas of LGSC and HGSC involving both ovaries, with HGSC involving omentum, uterine serosa and appendiceal serosa (Fig. 1) Fallopian tubes unremarkable	Deceased 20 months after diagnosis
Case 2	52 (SBT, LGSC); 57 (HGSC)	IIIC	SBT, LGSC	HGSC *	Ovaries containing SBT exhibiting microinvasion, and foci of solid proliferation amounting to LGSC; LGSC also involving omentum. Fallopian tubes unremarkable. Recurrent para-vertebral and para-aortic tumour masses composed of HGSC (Fig. 2)	Recurrence as HGSC 60 months after diagnosis of SBT +LGSC Deceased 67 months after diagnosis
Case 3	82	N/A	SBT, LGSC	PDC	Ovary containing SBT, invasive LGSC and areas of PDC; the latter was diffusely cytokeratin-positive (Fig. 3)	Lost to follow-up
Case 4	41	IIIC	SBT, LGSC	HGSC	Bilateral ovaries containing SBT and LGSC; in addition, areas of HGSC were present (Fig. 4) Omentum with LGSC and HGSC Fallopian tubes unremarkable	Deceased 3.5 months after diagnosis
Case 5	67	IA	SBT	HGSC	SBT in both ovaries, with one ovary exhibiting areas of intracystic non-invasive HGSC immediately adjacent to areas of SBT (Fig. 5) Fallopian tubes unremarkable	Alive and well at 30 months after diagnosis

SBT = serous borderline tumour; LGSC = low-grade serous carcinoma; HGSC = high-grade serous carcinoma; PDC = poorly differentiated carcinoma.

* Low-grade and high-grade tumours in cases 1, 3, 4 and 5 were diagnosed synchronously; in case 2, the tumours were diagnosed metachronously