Identification of novel cyclin gene fusion transcripts in endometrioid ovarian carcinomas

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Key words: ovarian carcinoma, fusion transcript, NRG4, cyclin, CCNL2

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Malignant epithelial tumors (carcinomas) are the most common ovarian cancers and also the most lethal gynecological malignancies. Based on histopathology and genetic profiling, ovarian carcinomas are divided into five main types: high-grade serous (HGSC) (representing 70% of the malignancies), endometrioid (EC) (10%), clear cell (10%), mucinous (3%), and low-grade serous carcinomas (LGSC) (<5%); the relative frequencies refer to data from Western countries; together they account for over 95% of ovarian malignant tumors.

Each of these histotypes differs in what is their precursor lesion(s), oncogenesis, response to chemotherapy, and prognosis. HGSC harbor TP53 and BRCA mutations, whereas their low-grade counterparts often carry KRAS and BRAF mutations. KRAS and HER2 mutations are frequent in mucinous carcinomas, whereas in EC and clear cell carcinomas, ARID1A is frequently mutated.

Several studies have focused on the identification of fusion genes in ovarian carcinomas. Although >700 samples have been analyzed so far, only a few recurrent transcripts were found, and always with a low rate of recurrence (0.5–5%). Two studies used the genomic data produced by the Cancer Genome Atlas project to find that three fusion transcripts were recurrent in HGSC carcinomas: CCDC6-ANK3 (found in 4 samples or 1% of the tumors), and COL14A1-DEPTOR and KAT5B-ADK (each found in 2 samples or 0.5%). Patch et al. analyzed 114 samples from chemoresistant HGSC and found promoter swapping affecting the SLC25A40-ABCB1 transcript in six samples (5%). Earp et al. identified a cyclin gene fusion transcript, which involves a member of the cyclin family, was found recurrently in four of the 18 cancers; 22%). We also found three additional fusion transcripts involving genes belonging to the cyclin family: ANXA5-CCNA2 and PDE4D-CCNB1 were detected in two endometrioid carcinomas, whereas CCNY-NRG4 was identified in a clear cell carcinoma. The recurrent involvement of CCNL2 in four fusions and of three other genes of the cyclin family in three additional transcripts hints that deregulation of cyclin genes is important in the pathogenesis of ovarian carcinomas in general but of endometrioid carcinomas particularly.

Formation of fusion genes is pathogenetically crucial in many solid tumors. They are particularly characteristic of several mesenchymal tumors, but may also be found in epithelial neoplasms. Ovarian carcinomas, too, may harbor fusion genes but only few of these were found to be recurrent with a rate ranging from 0.5 to 5%. Because most attempts to find specific and recurrent fusion transcripts in ovarian carcinomas focused exclusively on high-grade serous carcinomas, the situation in the other carcinoma subgroups remains largely uninvestigated as far as fusion genes are concerned. We performed transcriptome sequencing on a series of 34 samples from ovarian tumors that included borderline, clear cell, mucinous, endometrioid, low-grade and high-grade serous carcinomas in search of fusion genes typical of these subtypes. We found a total of 24 novel fusion transcripts. The PCMTDI-CCNL2 fusion transcript, which involves a member of the cyclin family, was found recurrently involved but only in endometrioid carcinomas (4 of 18 tumors; 22%). We also found three additional fusion transcripts involving genes belonging to the cyclin family: ANXA5-CCNA2 and PDE4D-CCNB1 were detected in two endometrioid carcinomas, whereas CCNY-NRG4 was identified in a clear cell carcinoma. The recurrent involvement of CCNL2 in four fusions and of three other genes of the cyclin family in three additional transcripts hints that deregulation of cyclin genes is important in the pathogenesis of ovarian carcinomas in general but of endometrioid carcinomas particularly.
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obtained from the patients. Helseforskning.etikkom.no/) and written informed consent was

paired-end RNA-sequencing at the Norwegian Sequencing

bioinformatics analyses

High-throughput paired-end RNA-sequencing and

bioinformatics analyses

Three micrograms of total RNA were sent for high-throughput

paired-end RNA-sequencing at the Norwegian Sequencing

Center, Ullevål University Hospital (https://www.sequencing.

uio.no/) as described previously.9 The software used for
detection of fusion transcripts included Fusioncatcher v0.99.4e
(https://github.com/ndaniel/fusioncatcher),10 ChimeraScan v0.4.3
(https://github.com/ndaniel/chimera-vrl),9 FusionMap 31.03.15
(https://omictools.com/fusionmap-tool),12 and TopHat 2.0.9
(https://ccb.jhu.edu/software/tophat/index.shtml).13 The candidate
fusion transcripts obtained by bioinformatic analysis were
checked using BLAST (http://blast.ncbi.nlm.nih.gov/blast.cgi)
and BLAT (https://genome.ucsc.edu/cgi-bin/hgBlat?command=start).

Reverse transcriptase-polymerase chain reaction (RT-PCR)
and Sanger sequencing

One microgram of RNA was reverse transcribed using iScript
Advance cDNA synthesis kit (Bio-Rad). To validate the
fusion genes detected as part of the bioinformatic analyses,
cDNA equivalent to 10 ng RNA was amplified using the
TAKARA Premix Ex Taq (TaKaRa-Bio, Europe/SAS, Saint-
Germain-en-Laye, France). The primers are listed in Support-
ing Information I. The PCR cycling program for all reactions
was as follows: 30 sec at 94°C followed by 35 cycles of 7 sec
at 98°C, 30 sec at 55°C, 60 sec at 72°C and a final extension
for 2 min at 72°C. Expression of the housekeeping gene
ABL1 was monitored as cDNA quality control. We also tested
our series of tumors for presence of a fusion gene
CDKN2D-WDFY2. The primers and PCR conditions were as
reported.14 Three microliters of the PCR products were
stained with GelRed (Biotium, Hayward, CA) and analyzed
by electrophoresis through 1.0% agarose gel. The gel was
scanned with G-Box (Syngene, Hayward, CA) and the
images were acquired using GeneSnap (Syngene). The remaining 22 µl of the amplified fragments were purified
using the QIAquick PCR purification Kit (Qiagen). Direct
sequencing was performed using the light run sequencing
service of GATC Biotech (http://www.gatc-biotech.com/en/
sanger-services/lightrun-sequencing.html) or the ABI3500
Genetic Analyzer (ThermoFisher Scientific, Waltham, MA)
using BigDye Terminator V1.1 cycle sequencing kit. The
BLAST and BLAT programs were used for computer analysis
of sequence data.

Results

RNA-sequencing gave informative results for all 34 samples.
The subsequent bioinformatic analysis was also informative

What’s new?

Chimeric genes formed by fusion of previously separate genes are associated with many malignant tumors, but rare in ovarian
cancer. Here the authors performed transcriptome sequencing of different types of ovarian tumors and identify novel fusion
genes, involving cyclin genes, the master regulators of the cell cycle. As most of these fusions were found in ovarian cancer of
the endometrioid type, which represent about 10% of all ovarian cancers, the data point to a novel role of cyclin deregulation
in this specific cancer subtype.

KANSL1 to be the most frequent fusion transcript in their series (2.7% of all tumors). Our group recently reported the
involvement of DPP9 in two out of 18 samples of HGSC kar-
yotypically characterized by rearrangements of chromosome 19.8 Taken together, these results suggest that ovarian cancer
is not characterized by highly recurrent fusion transcripts. It should be taken into account, however, that the majority of
studies referred to above focused exclusively on HGSC meaning that the other histotypes have not yet been extensively
analyzed. We therefore screened a series of 34 tumors repres-
enting the whole spectrum of ovarian malignant epithelial
tumors to look for new recurrent fusion transcripts arising in
non-HGSC tumors.

Material and Methods

Tumor material

The material consisted of fresh frozen samples from ovarian
tumors surgically removed at The Norwegian Radium Hospital
between 1999 and 2010. Samples from 34 ovarian carcinomas
(including borderline tumors) were sequenced (two borderline,
two low-grade serous, three mucinous, four clear cell, nine EC,
and 14 HGSC). A second cohort of 113 samples was subse-
quently used to validate the results and test how frequent were
the novel fusion transcripts that had been detected. The latter
series consisted of 10 fibromas, 10 thecobiromas, 10 borderline
epithelial tumors and 83 carcinomas of which 35 were HGSC,
16 mucinous, 18 EC, 10 clear cell and 4 low-grade serous. The
study was approved by the regional ethics committee (Regional
komité for medisinsk forskningsetikk Sør-Ost, Norge, https://helseforskning.etikkom.no/) and written informed consent was
obtained from the patients.

RNA extraction

Total RNA was extracted using miRNeasy Kit (Qiagen, Hilden,
Germany) and QIAcube (Qiagen). The concentration and
purity of the RNA was measured with the Nanovue Spectrophotometer (GE Healthcare, Pittsburgh, PA). The RNA
quality of the 34 samples sequenced was checked with Experion Automated Electrophoresis System using the RNA
StdSens analysis kit (Bio-Rad Laboratories, Oslo, Norway).

High-throughput paired-end RNA-sequencing and
bioinformatics analyses

Three micrograms of total RNA were sent for high-throughput
paired-end RNA-sequencing at the Norwegian Sequencing
Table 1. List of the candidate fusion transcripts

| Sample | Diagnosis | Candidate fusion genes | Location | Breakpoint (exons) | Fusion outcome |
|--------|-----------|------------------------|----------|-------------------|---------------|
| I      | CCC       | CCNY NRG4              | 10p11.21 | 15q24.2           | In-frame      |
| I      | CCC       | MRPL21 TADA2A          | 11q13.3  | 17q12             | In-frame      |
| I      | CCC       | MICALL1 GGA1           | 22q13.3  | 22q3              | In-frame      |
| II     | E         | PCMTD1 CCNL2           | 8q11.23  | 1p36.33           | In-frame      |
| III    | E         | ANXA5 CCNA2            | 4q27     | 4q27              | In-frame      |
| IV     | E         | PKA1 GYLT1B            | 11q13.3  | 15p11.2           | In-frame      |
| IV     | E         | AP1M2 HIP1             | 19p13.2  | 7q11.23           | Out-of-frame  |
| IV     | E         | CTBP2 DENND3           | 10q26.13 | 8q24.3            | In-frame      |
| V      | E         | NAIP OLCN              | 5q13.2   | 5q13.2            | In-frame      |
| V      | E         | PDE4D CCNB1            | 5q12.1   | 5q12.1            | In-frame      |
| V      | E         | MELK TMEM88            | 9p13.2   | 9p13.3            | In-frame      |
| VI     | E         | PCMTD1 CCNL2           | 8q11.23  | 1p36.33           | In-frame      |
| VII    | E         | PCMTD1 CCNL2           | 8q11.23  | 1p36.33           | In-frame      |
| VIII   | HGSC      | SCNN1A CHD4            | 12p13.31 | 12p13.31          | In-frame      |
| IX     | HGSC      | TSPAN3 NRG4            | 15q24.3  | 15q24.2           | In-frame      |
| X      | HGSC      | TRIM68 NRG4            | 11p15.4  | 15q24.2           | In-frame      |
| XI     | HGSC      | NCAPG2 RBPM5           | 7q36.3   | 8q12              | In-frame      |
| XI     | HGSC      | MBD2 PERP              | 18q21    | 6q23              | Out-of-frame  |
| XI     | HGSC      | DHX30 ABHD14B          | 3p21     | 3p21.2            | In-frame      |
| XI     | HGSC      | SNTB1 ZNF250           | 8q24.1   | 8q24.3            | In-frame      |
| XI     | HGSC      | MAP3K10 C19orf47       | 19q13.2  | 19q13.2           | In-frame      |
| XI     | HGSC      | FUT8 FNTB              | 14q23.3  | 14q23.3           | In-frame      |
| XII    | HGSC      | AP2B1 ZNF512           | 17q12    | 2p23.3            | In-frame      |
| XII    | HGSC      | FARP2 PPP1R7           | 2q37.3   | 2q37.3            | In-frame      |
| XII    | HGSC      | FAM160B1 NHLC2         | 10q25.3  | 10q25.3           | Out-of-frame  |
| XII    | HGSC      | CTIF MOB2              | 18q21.1  | 11p15.5           | In-frame      |
| XII    | HGSC      | MGEA5 KCNIP2           | 10q24.32 | 10q24.32          | In-frame      |
| XII    | HGSC      | MAF20C SUGCT           | 7p22.3   | 7p14.1            | Out-of-frame  |
| XIII   | HGSC      | ARHGAP35 UNC13A        | 19q13.32 | 19p13.11          | In-frame      |
| XIII   | HGSC      | FGF2R2 FAM24B          | 10q26.13 | 10q26.13          | In-frame      |
| XIII   | HGSC      | KDM5A NIN2             | 12p13.3  | 12p13.3           | In-frame      |
| XIII   | HGSC      | PDZD8 ABLIM1           | 10q26.11 | 10q25.3           | In-frame      |
| XIV    | HGSC      | VRK1 TDP1              | 14q32.2  | 14q32.11          | In-frame      |
| XIV    | HGSC      | NSD1 ZNF346            | 5q35.3   | 3q35.2            | In-frame      |
| XIV    | HGSC      | NFIX RAD23A            | 19p13.3  | 19p13.3           | In-frame      |
| XIV    | HGSC      | PRKD1 CNH1             | 14q12    | 14q22.2           | In-frame      |
| XIV    | HGSC      | TMEM123 MMP27          | 11q22.2  | 11q22.2           | In-frame      |
| XIV    | HGSC      | KLC1 ZFAT              | 14q32.33 | 8q24.22           | In-frame      |

1The fusion transcripts that were validated with RT-PCR and Sanger sequencing are written in bold.

Abbreviations: CCC: clear cell carcinoma; E: endometrioid; HGSC: high-grade serous carcinoma.
giving a mean of four fusion transcripts per tumor. The list of fusion candidates was shortened by checking every transcript with the BLAST and BLAT programs. All fusion sequences that did not involve the coding regions of both genes (3’UTR-coding DNA sequence (CDS), intronic–CDS and/or intronic–intronic), were discarded as were sequences identified as readthroughs. We focused on transcripts involving genes known to be relevant in cancer and transcripts that were identified by more than one program, the only exception being PCMTD1-CCNL2 which was identified only by TopHat. Using these criteria, we came up with a list of 42 candidate fusion transcripts (Table 1) present in 11 samples out of 34 sequenced. More specifically, we found seven fusions in 14 HGSC, three in nine EC and one in the four clear cell carcinomas analyzed.

Figures 1 and 2. Schematic illustration of the putative chimeric proteins resulting from the detected fusions of cyclin genes. (a) Illustration of CCNL2 protein and the putative chimeric protein resulting from the PCMTD1-CCNL2 fusion gene with a chromatogram showing the fusion junction identified by Sanger sequencing. (b) Wild-type CCNA2 and putative fusion protein translated from ANXA5-CCNA2 with a chromatogram showing the fusion junction. (c) CCNB1 protein illustration and the putative chimeric protein encoded by the fusion gene PDE4D-CCNB1 with a chromatogram of the fusion junction. [Color figure can be viewed at wileyonlinelibrary.com]
and Gene Fusions in Cancer (https://cgap.nci.nih.gov/Chromosomes/Mitelman). Only the fusion transcripts KDMAS-NINJ2 and NSD1-ZNF346 were previously identified, by Yoshihara et al.5 in HGSC. All other fusion genes were novel.

We found recurrent involvement of genes belonging to the cyclin family in endometrioid carcinomas in the form of fusion transcripts PCMTDI-CCNL2, ANXA5-CCNA2 and PDE4D-CCNB1 (Figure 1). Furthermore, CCNY-NRG4, another fusion involving a cyclin gene, was found in a tumor showing mixed endometrioid/clear cell histotype in its primary location (uterus) but only a clear cell pattern in the ovarian recurrence (see below). In addition, we found two transcripts involving the neuregulin 4 (NRG4) gene, the already mentioned CCNY-NRG4 and TSPAN3-NRG4; the latter was found in an HGSC. In both cases, the fusion involved exon 4 of NRG4 (Figure 2). The bioinformatic analyses identified an additional fusion transcript involving exon 4 of NRG4 and exon 7 of the Tripartite Motif Containing 68 gene (TPRMS68) in another sample of HGSC; however, we could not confirm the presence of this transcript by means of PCR and sequencing analysis.

As all these fusion transcripts were non-recurrent in the original series of 34 tumors subjected to NGS, we also tested a larger cohort of 113 ovarian tumors for their possible presence. PCMTD1-CCNL2 was found in three additional cases of EC (thus it was found present in four out of 18 carcinomas of the endometrioid histotype in total; 22%). The PCMTD1-CCNL2 in-frame fusion juxtaposes exon 3 of the Protein-L-Isoaspartate (D-Aspartate) O-Methyltransferase Domain Containing 1 gene (PCMTD1; accession number: NM_052937.3) from 8q11.23 with exon 6 of the Cyclin L2

Table 2. Overview of the expression status, at RNA and protein level, of the genes found involved in fusion events

| Gene     | RNA expression | Protein expression | Protein expression |
|----------|---------------|--------------------|--------------------|
|          | Illumina      | (normal tissue)    | (cancer samples)   |
| PCMTD1   | 48            | Low                | Medium             |
| CCNL2    | 59            | Medium             | Medium             |
| ANXA5    | 156           | Medium             | Low                |
| CCNA2    | 10            | Not detected       | Low                |
| PDE4D    | 21            | Medium             | Medium             |
| CCNB1    | 15            | Not detected       | Medium/low         |
| CCNY     | 70            | Low                | Medium/low         |
| TSPAN3   | 143           | Not detected       | Medium/low         |
| NRG4     | 13            | Not detected       | Low                |

1Ovarian tissue RNA expression from Illumina Human BodyMap 2.0 dataset in reads per kilobase million (RPKM) assessed on 70 samples of ovarian normal tissue. Source: GeneCards (http://www.genecards.org).
2Protein expression of normal ovarian tissue assessed by immunohistochemistry. Source: the Human Protein Atlas (http://www.proteinatlas.org).
3Protein expression in 12 samples of ovarian cancer assessed by immunohistochemistry. Source: the Human Protein Atlas.

Figure 3. Histological appearance of the tumor of Case I. Hematoxylin–eosin staining at (a) 50× and (b) 200× magnification and (c) Napsin A immunostaining of the primary uterine carcinoma with a mixed clear cell-endometrioid morphology. Hematoxylin eosin staining at (d) 50× and (e) 200× magnification and (f) Napsin A immunostaining of the secondary clear cell carcinoma in the ovary. [Color figure can be viewed at wileyonlinelibrary.com]
were initially described as recurrent in HGSC at rates of 15%, 20% and 7%, respectively, the findings have not been validated by other groups or in different series. More than 700 samples were screened in other studies and fusion transcripts were found at frequencies ranging from 0.5% (KAT6B-ADK) to 2.7% (CRHR1-KANSL1). It is worthy of note in the context that most studies focused on HGSC, whereas the other four types of ovarian carcinoma were less often investigated; only 63 endometrioid carcinomas, 36 clear cell carcinomas, and six mucinous carcinomas had been analyzed for fusion genes prior to this study. 

In a recent study, Earp et al. identified UBA1-TGMT in clear cell carcinomas exclusively, finding it in two out of 20 tumors of this histotype.

We identified PCMTD1-CCNL2 as a novel and recurrent fusion in endometrioid carcinomas, finding the transcript in four out of 18 (22%) EC. The CCNL2 gene encodes three cyclin L2 isoforms. The main isoform (Cyclin L2α) contains two cyclin domains, spanning amino acids 76–150 and 192–281, and a C-terminal RS site (arginine-serine dipeptide) (385–423) that plays a role in protein–protein interactions with the SR family of splicing factors. The three splicing variants L2β/B/1/2/3 have exon 6 as the last coding exon and code for the 226 aa Cyclin L2βA isoform. The variant L2β/3 terminates in exon 7 and codes for the 236 aa Cyclin L2βB. Cyclin L2, which is different from most other cyclins, is expressed during the entire cell cycle and was detected in many tissues, ovary included (Table 2). Cyclin L2 participates together with Cyclin L1 and CDK11 in pre-mRNA splicing processes. Lack of a functional Cyclin L2 may impair normal splicing mechanisms as all three Cyclin L2 isoforms have been shown to be fundamental components of the splicing complex.

The role of Cyclin L2 in cancer has not been investigated extensively; however, Li et al. showed that it acts as a tumor suppressor protein in gastric cancer enhancing both apoptosis and chemosensitivity. Yang et al. showed a similar tumor suppressor activity of Cyclin L2 in hepatocarcinoma and that both cyclin domains were fundamental for the protein’s proper functioning. The fusion PCMTD1-CCNL2 leads to a chimeric Cyclin L2α lacking the first cyclin domain (76–150) and containing only 27 aa of the second cyclin domain (Figure 1). Due to the fusion, the chimeric protein is no longer able to bind CDK11 and suppress tumor growth. The fusion leads to loss of the other two isoforms since the splicing variants coding for isoforms L2β/2A/3 are lost.

Besides the aforementioned fusion, we found also another two fusion transcripts, ANXA5-CCNA2 and PDE4D-CCNB1, involving other cyclin genes in two different samples of EC. The in-frame fusion ANXA5-CCNA2 juxtaposes exon 3 of the Annexin A5 gene (ANXA5; NM_001154.3, from 4q27) with exon 3 of the gene coding for Cyclin A2 (CCNA2; NM_001237.3, mapping in the same genomic region only 160 kb more distal). The fusion results in a 2250 bp (259 bp from ANXA5 and 1991 bp from CCNA2) transcript which codes for a functional chimeric cyclin composed of 31 aa

Table 3. Overview of genes found involved in chimeric transcripts in this series and in previous studies

| Gene       | Type of Cancer |
|------------|----------------|
| ABDH14B    | Breast         |
| AP2B1      | Lung, breast   |
| ARHGAP35   | Breast         |
| CCNB1      | Osteosarcoma, breast |
| CCNY       | Prostate, breast, kidney, bladder, thyroid |
| CTIF       | Lung, breast   |
| DHX30      | Breast         |
| FARPS      | Prostate, lung |
| KDM5A      | Acute myeloid leukemia |
| MELK       | Breast         |
| MGEA5      | Soft tissue tumor |
| NINJ2      | Lung, brain, ovary, breast |
| NRG4       | Breast         |
| NSD1       | Acute myeloid leukemia, ovary, lung, kidney |
| PAK1       | Ovary, breast |
| PCMTD1     | Acute lymphocytic leukemia |
| RBPMS      | Lung, breast, kidney |
| SNTB1      | Ovary, breast  |
| TSPAN3     | Breast         |
| UNC13A     | Brain, lung    |
| VRK1       | Lung, oral cavity |
| ZNFS12     | Ovary, breast  |

Gene (CCNL2; NR_135154.1) from 1p36.33 (Figure 1). The putative chimeric transcript is 3209 bp long and consists of a sequence of 762 bp from PCMTD1 fused with a sequence of 2447 bp from CCNL2. It codes for a chimeric protein of 409 aa containing the first 136 aa (1–136 out of 357) of PCMTD1 (NP_443169) and 273 aa (253–526 out of 526) of Cyclin L2 (NP_1121992).

No other fusion was found to be recurrent. We also tested the cohort for presence of the CDKN2D-WDFY2 transcript that was previously reported with a recurrence rate of 20% in HGSC, finding no such fusion.

Discussion

Studies over the past decades have uncovered the oncogenic role of fusion genes in hematological malignancies and mesenchymal tumors, and have highlighted the diagnostic and therapeutic advantages provided by the detection of these chimeric transcripts and their tumor-specific expression. A similar search for fusion transcripts in ovarian cancer has shown that they are not common. Though the fusions ESSRA-C11orf20, CDKN2D-WDFY214 and BCAM-AKT217...
(1–31 out of 320) from ANXA5 (AAH01429.1) and 274 aa (158–432 out of 432) from Cyclin A2 (AAI04784.1) (Figure 1). The functional cyclin domains of Cyclin A2 are located in regions 181–307 and 309–427 (Figure 1) and are therefore conserved in the chimeric cyclin encoded by ANXA5-CCNA2.

The fusion gene PDE4D-CCNB1 brings together exon 1 of the Phosphodiesterase 4 D gene (PDE4D; NM_001197223.1) from 5q11.2 and exon 2 of a gene coding for Cyclin B1 (CCNB1, NM_031966.3) from 5q13.2. The fusion results in a chimeric transcript of 1886 bp (627 bp from PDE4D and 1886 bp from CCNB1) which codes for a putative protein of 584 aa containing 151 aa (1–151 out of 809) from phosphodiesterase 4 D (NP_001098101) and the whole Cyclin B1 (433aa) (AAP88038) (Figure 1). The consequences of these two fusions should be similar despite the fact that they affect different genes.

Cyclin genes act in concert.22 The expression levels of Cyclin A2 are tightly synchronized with cell cycle progression. CCNA2 transcription begins in late G1, peaks and plateaus in mid-S, and then declines in G2.23 The transcription is mostly regulated by the transcription factor E2F that derepresses the promoter.24 Cyclin B1 appears in S phase and accumulates in G2 and mitosis before disappearing at transition from metaphase to anaphase. Synthesis of Cyclin B1 during the cell cycle is mainly regulated at the transcriptional level by p5325 and is enhanced by the P300 coactivator,26 USF1,27 and Myc.28 The fusions ANXA5-CCNA2 and PDE4D-CCNB1 bring the cyclins under the control of the
promoter of their 5' partners that are normally expressed in ovarian cells (Table 2). This promoter swapping overcomes the normal regulation of the cyclin genes resulting in deregulation, that is, overexpression/permanent expression of chimeric Cyclins A2 and B1. This may profoundly affect cell cycle regulation since these two cyclins cooperate in both early and late mitosis.29

The CCNY-NRG4 fusion transcript was found in a clear cell ovarian carcinoma. In this transcript, exon 4 of NRG4 is fused with exon 1 of CCNY, that is, another cyclin gene. The fusion causes the complete loss, at the genomic level, of the entire cyclin gene (CCNY; NM_181698.3), replacing it by NRG4 (exons 4–6; NM_138573.3). Despite the fact that the metastatic tumor sample (the one examined) showed clear cell histology, it is interesting to note that its primary in the uterus had mixed morphology with clear cell and endometrioid morphology (Figure 3). Presence of the latter phenotype again seems consistent with involvement of cyclin genes in fusion transcripts in tumors showing endometrioid features.

The NRG4 gene was found rearranged also with another partner, Tetraspanin-3 (TSPAN3). The transcript consisted of exon 6 from TSPAN3 fused with exon 4 from NRG4 (similar to CCNY-NRG4). The gene NRG4 is located on chromosomal band 15q24 and codes for Nereugulin 4 (CAL35829.1), a ligand of the EGF receptor family. Whereas the Neuregulin 4 gene is not expressed at high levels in the normal ovary, it is a ligand of the EGF receptor family and has the EGF-like domain fundamental in the activation since these two cyclins cooperate in both early and late mitosis.29

Although the results obtained are limited and our preliminary conclusion should be borne out in larger studies, the presented evidence clearly hints that cyclin fusion transcripts play a role in the pathogenesis of a subset of EC. All other fusion transcripts validated using PCR were nonrecurrent in our series. However, taking into account information from the Mitelman database (http://cgap.nci.nih.gov/Chromosomes/Mitelman) we found that some of the fusion genes identified in this study have indeed been previously reported, both in ovarian cancer and other tumors (Table 3 and Figure 4), albeit in some cases with a different partner. The fusions KDMAS-NINJ2 and NSD1-ZNF346 were previously identified in HGSC as was seen in our case (Figure 4). The genes NINJ2, PAK1, PCDT1I, SNTB1, and ZNF512 were also found fused with different partners in HGSC (Figure 4).5 Taking all these results together, we see that all the mentioned genes were recurrently rearranged in ovarian cancer, admittedly at very low frequencies. Furthermore, 21 of the genes we found involved in fusions were previously reported in 35 different fusion transcripts in studies of breast cancer and 14 fusion transcripts were identified in lung cancer.25,31,32 (Table 3), hinting that they may be generally relevant in carcinogenesis of different types.

As we did not find any sample carrying the CDKN2D-WDFY2 fusion and no evidence for the presence of ESSRA-C11orf20 and/or BCAM-AKT2 in our series, we conclude that the frequency of these fusions must be much lower than was initially reported.14,16,17 Indeed, it seems evident that the occurrence of pathogenetically essential fusion events in ovarian carcinomas is well below what is the case in hematological malignancies and/or mesenchymal tumors.42 The fusion genes found in this and other studies may also be a reflection of massive pathogenetic heterogeneity in ovarian cancer, thus identifying tumor subsets within, but on other occasions transcending, the accepted phenotypic subgroups of ovarian cancer. One may hope that further elucidation of this tumorigenic variability will contribute to a more meaningful classification of these malignancies and eventually to the finding of medicines directed at the molecular genetic changes that are central to the disease process.

References

1. Prat J. Ovarian carcinomas: five distinct diseases with different origins, genetic alterations, and clinicopathological features. Virchows Arch 2012; 460:237–49.

2. Kurman RJ, Carcangiu ML, Herrington CS, et al. WHO classification of tumours of female reproductive organs, vol. 6. IARC, 2014.

3. Prat J. New insights into ovarian cancer pathology. Ann Oncol 2012;23 Suppl 11:x111–7.

4. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature 2011;474:609–15.

5. Yoshihara K, Wang Q, Torres-Garcia W, et al. The landscape and therapeutic relevance of cancer-associated transcript fusions. Oncogene 2015;34:4845–54.

6. Patch AM, Christie EL, Etemadmoghadam D, et al. Whole-genome characterization of chemoresistant ovarian cancer. Nature 2015;521:489–94.

7. Earp MA, Raghavan R, Li Q, et al. Characterization of fusion genes in common and rare epithelial ovarian cancer histologic subtypes. Oncotarget 2017.

8. Smeye ML, Agostini A, Johannessen B, et al. Involvement of DPP9 in gene fusions in serous ovarian carcinoma. BMC Cancer 2017;17:642.

9. Heim S, Mandal N, Mitelman F. Genetic convergence and divergence in tumor progression. Cancer Res 1988;48:5911–6.

10. Nicorici D, Satalan M, Edgren H, et al. Fusion-Catcher - a tool for finding somatic fusion genes in paired-end RNA-sequencing data. bioRxiv 2014.

11. Iyer MK, Chinnaiyan AM, Maher CA. ChimeraScan: a tool for identifying chimeric transcription in sequencing data. Bioinformatics 2011;27:2903–4.

12. Ge H, Liu K, Fau - Juan T, et al. FusionMap: detecting fusion genes from next-generation sequencing data at base-pair resolution. Bioinformatics 2011;27:1922–8.

13. Kim D, Selberg SL. TopHatFusion: an algorithm for discovery of novel fusion transcripts. Genome Biol 2011;12:R72–15.
14. Kannan K, Coarfa C, Rajapakshe K, et al. CDKN2D-WDFY2 is a cancer-specific fusion gene recurrent in high-grade serous ovarian carcinoma. PLoS Genet 2014;10:e1004216.

15. Parker BC, Zhang W. Fusion genes in solid tumors: an emerging target for cancer diagnosis and treatment. Chin J Cancer 2013;32:594–603.

16. Salzman J, Marinelli RJ, Wang PL, et al. ESRRA-C11orf20 is a recurrent gene fusion in serous ovarian carcinoma. PLoS Biol 2011;9:e1001156.

17. Kannan K, Coarfa C, Chao PW, et al. Recurrent BCAM-AKT2 fusion gene leads to a constitutively activated AKT2 fusion kinase in high-grade serous ovarian carcinoma. Proc Natl Acad Sci USA 2015;112:E1272–7.

18. Micci F, Panagopoulos I, Thorsen J, et al. Low frequency of ESRRB-C11orf20 fusion gene in ovarian carcinomas. PLoS Biol 2014;12:e1001784.

19. Loyer P, Trembley JH, Grenet JA, et al. Characterization of cyclin L1 and L2 interactions with CDK11 and splicing factors: influence of cyclin L isoforms on splice site selection. J Biol Chem 2008;283:7721–32.

20. Yang L, Li N, Wang C, et al. Overexpression of cyclin L2 inhibits growth and enhances chemosensitivity in human prostate cancer cells. Asian Pac J Cancer Prev 2012;13:1425–30.

21. Vermeulen K, Van Bockstaele DR, Berneman ZN. The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. Cell Prolif 2003;36:131–49.

22. Vermeulen K, Van Bockstaele DR, Berneman ZN. The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. Cell Prolif 2003;36:131–49.

23. Henglein B, Chenwivesse X, Wang J, et al. Structure and cell cycle-regulated transcription of the human cyclin A gene. Proc Natl Acad Sci USA 1994;91:5490–4.

24. Yam CH, Fung TK, Poon RY. Cyclin A in cell cycle control and cancer. Cell Mol Life Sci 2002;59:1317–26.

25. Krause K, Wasmier M, Reinhard W, et al. The tumour suppressor protein p53 can repress transcription of cyclin B. Nucleic Acids Res 2000;28:4410–8.

26. Wasmier M, Tischop K, Spiesbach K, et al. Cyclin B1 transcription is enhanced by the p300 coactivator and regulated during the cell cycle by a CHR-dependent repression mechanism. FEBS Lett 2003;536:66–70.

27. Cogswell JP, Godlevski MM, Bonham M, et al. Upstream stimulatory factor regulates expression of the cell cycle-dependent cyclin B1 gene promoter. Mol Cell Biol 1995;15:2782–90.

28. Yin XY, Grove L, Datta NS, et al. Inverse regulation of cyclin B1 by c-Myc and p53 and induction of tetraploidy by cyclin B1 overexpression. Cancer Res 2001;61:6487–93.

29. Gong D, Ferrell JE. The roles of cyclin A2, B1, and B2 in early and late mitotic events. Mol Biol Cell 2010;21:3149–61.

30. Hitschler M, Voss D, Xu Y, et al. Upstream stimulatory factor regulates expression of the cell cycle-dependent cyclin B1 gene promoter. Mol Cell Biol 1995;15:2782–90.

31. The Cancer Genome Atlas Research N. Comprehensive molecular portraits of human breast tumors. Nature 2012;490:61–70.

32. Antonescu CR, Zhang L, Nielsen GP, et al. Consistent t(1;10) with rearrangements of TGFBR3 and MGEA5 in both myoinflammatory fibroblastic sarcoma and hemoiidesoid fibrolipomatous tumor. Genes Chromosomes Cancer 2011;50:757–64.

33. Hallor KH, Sciot R, Staat J, et al. Two genetic pathways, t(1;10) and amplification of 3p11–12, in myoinflammatory fibroblastic sarcoma, hemoiidesoid fibrolipomatous tumour, and morphologically similar lesions. J Pathol 2009;217:716–27.

34. Agrawal N, Akbani R, Aksay BA, et al. Integrated genomic characterization of papillary thyroid carcinoma. Cell 159:676–90.

35. van Zutven LJ, Onen E, Velthuizen SC, et al. Identification of NUP98 abnormalities in acute leukemia: JARID1A (12p13) as a new partner gene. Genes Chromosomes Cancer 2006;45:437–46.

36. Hallor KH, Sciot R, Staat J, et al. Two genetic pathways, t(1;10) and amplification of 3p11–12, in myoinflammatory fibroblastic sarcoma, hemoiidesoid fibrolipomatous tumour, and morphologically similar lesions. J Pathol 2009;217:716–27.

37. Atak 2K, Gianfelici V, Hulselman G, et al. Comprehensive analysis of transcriptome variation uncovers known and novel driver events in T-cell acute lymphoblastic leukemia. PLoS Genet 2013;9:e1003997.

38. Semple C, Meldman F. Cancer cytogenetics: Chromosomal and molecular genetic aberrations of tumor cells, 4th ed. Elsevier, 2015.