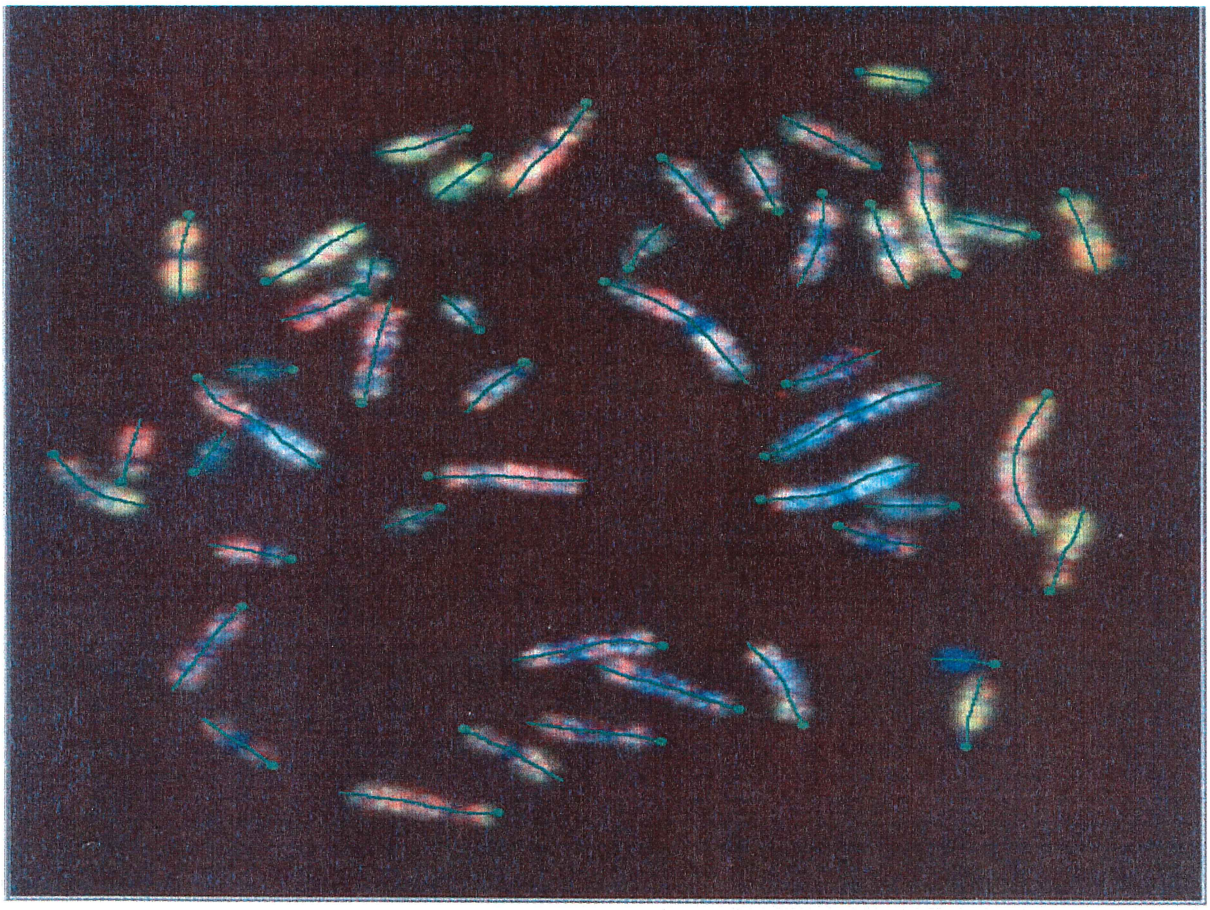
FORDYPNINGSOPPGAVE VED INSTITUTT FOR SMÅDYRSJUKDOMMER
NORGES VETERINÆRHØGSKOLE

Karakterisering av et oppregulert område på kromosom 19q13 i en cellelinje fra ovariekreft hos menneske ved hjelp av fluorescens *in situ* hybridisering (FISH).



av Marianne Gilhuus, Kull-96

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SAMMENDRAG

Målet ved dette forsøket var å minimalisere et påvist oppregulert område på kromosom 19 (19q13), og finne ut om det nylig oppdagede genet TRIP-Br1 var inkludert i dette området. Dette genet mistenkes å være involvert i reguleringen av cellesyklus, og på den måten kunne medvirke til utviklingen av kreft. Hypotesen er at TRIP-Br1 "kopler" regulerende signaler fra PHD zink finger og/el. bromodomain-inneholdende transkripsjons-faktorer og promotoren E2F/DP1.

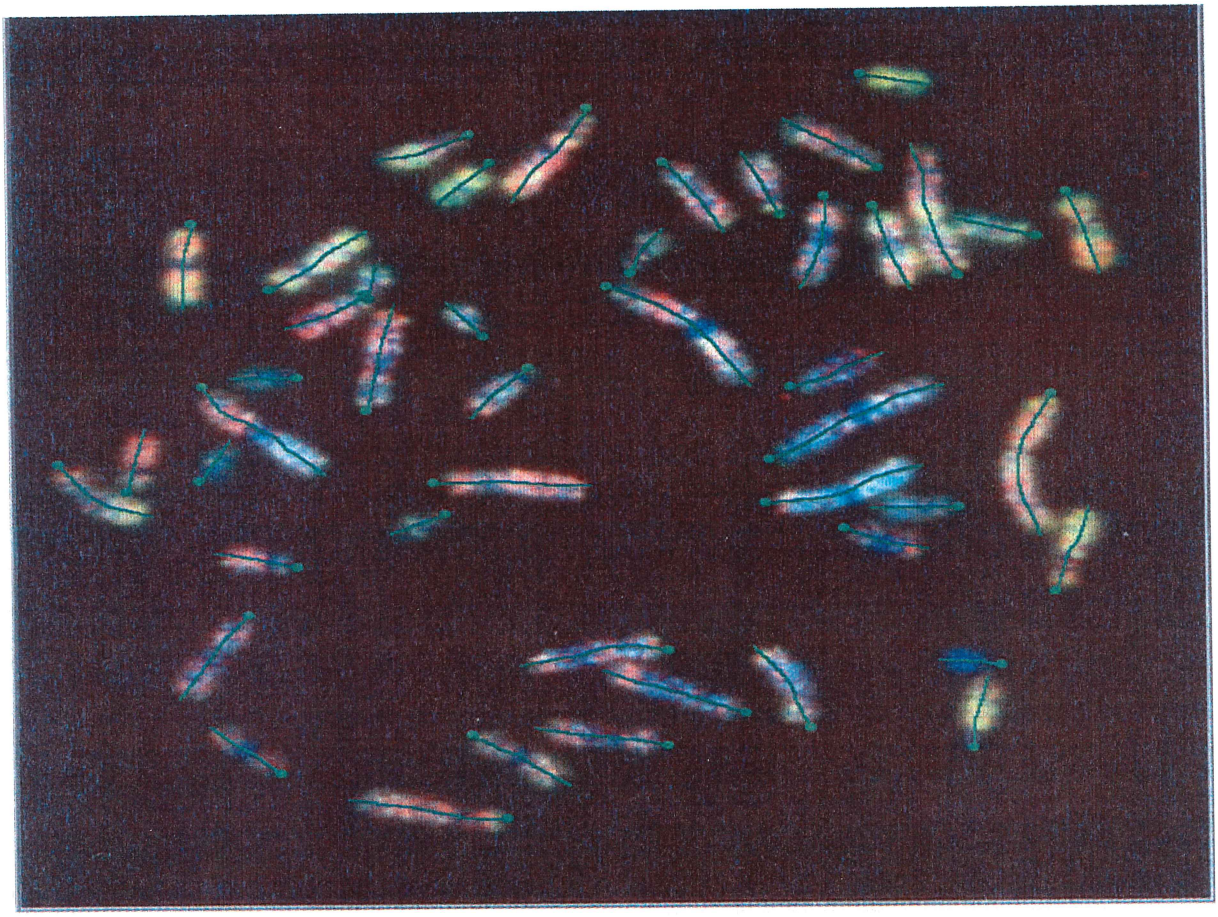
Det ble demonstrert tap av kromosomer og økt antall kopier av kromosomer i tre ulike cellelinjer fra ovariekreft (OVCAR-3, OVCAR-8 og CaOV3), ved hjelp av CGH (Comparative Genomic Hybridisation) . 19 BAC`s (Bacterial Artificial Chromosome), forventet å spenne over området 19q13.1 til 19q13.3 på kromosom 19, ble brukt som prober. Én BAC, RP11-452D9, var med gen-spesifikk PCR forventet å bære genet TRIP-Br1. Locus-spesifikk FISH ble utført på cellelinjen OVCAR-8.

Vi fant økt antall kopier av noen av BAC-ene, men ingen oppregulering ble påvist. Flere av BAC-ene, inkludert RP11-452D9, hybridiserte ikke med kromosomet av interesse (kromosom 19). Cellelinjen OVCAR-3, som man ved hjelp av Southern Blot fant hadde oppregulering av TRIP-Br1, var ikke tilgjengelig for FISH på grunn av for dårlig vekst.

Skal man med sikkerhet kunne avgrense oppgraderingen i området 19q3, bør det i fremtidige studier inkluderes flere cellelinjer. Videre bør man være sikker på at BAC-ene virkelig inneholder TRIP-Br1. Først når disse to kriteriene er oppfylt, kan man med mer sikkerhet avgjøre om oppgraderingen og/el. genet TRIP-Br1 er involvert i utviklingen av ovariekreft.

Innholdsfortegnelse

Characterising an amplicon on chromosome 19q13 in ovarian cancer cell lines, by fluorescence in situ hybridisation (FISH)



Summer School Project
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Summary

We demonstrated losses and gains of chromosomes in three ovarian cancer cell lines (OVCAR-3, OVCAR-8 and Caov-3) by CGH. With the use of 19 BACs, spanning the region 19q13.1-q13.3 on chromosome 19, we aimed to minimise the amplification found within this region. Locus specific FISH was done on the ovarian cancer cell line OVCAR-8. One BAC, RP11-452D9, was by gene specific PCR expected to carry the novel gene TRIP-Br1. This gene is expected to be involved in regulation of the cell cycle, by interaction with the coupling between PHD zinc fingers- and/or bromodomain-containing transcription factors and the promoter E2F/DP1. We found increased copy number of some of the BACs, but no amplification was detected. Several BACs, including RP11-452D9, did not hybridise to the chromosome of interest (chromosome 19). OVCAR-3, an ovarian cancer cell line found to have amplification of TRIP-Br1 by Southern blot, was not available for FISH due to growth difficulties. Continuing studies should include more ovarian cancer cell lines and BACs truly containing TRIP-Br1.

Introduction

Ovarian cancer represents the fifth most common form of fatal cancer in women (Bell and Scully, 2001). Epithelial neoplasm's are accounting for 75% of ovarian cancers (Fox, 1998). Karyotypic analysis of epithelial ovarian cancer has revealed frequent abnormalities in chromosomes 1, 3, 6, 11, 17 and 19, consisting of deletions, rearrangements and/or amplifications (Elbendary *et al.*, 1995). These structural abnormalities may result in the activation of oncogenes, deletion of normal alleles of tumour suppressor genes or may be of no functional significance. Chromosome 19 alterations appear to occur throughout all stages of ovarian cancer, and molecular genetic studies demonstrate increased gene copy numbers and/or gene expression from band region 19q13.1-q13.2 (Thompson *et al.*, 1996). AKT2, a gene located to this area, is found to be amplified and over expressed in some ovarian cancer cell lines and primary tumours. There has been suggested that AKT2 in co-operation with other proto-ocogenes and tumour suppressor genes, contribute to the pathogenesis of ovarian cancer (Cheng *et al.*, 1992; Thompson *et al.*, 1996). A novel gene, TRIP-Br1, has recently been localised very close to the map location of AKT2, and an ovarian cancer cell line (OVCAR-3) was found to have increased copy number of this gene by Southern blot (Hsu *et al.*, personal communication). It is proposed that TRIP-Br proteins function as transcriptional "integrators" that couple regulatory signals (both positive and negative) provided by PHD zinc finger – and/or bromodomain – containing transcription factors with the well defined transcriptional and cell cycle regulatory pathway mediated by E2F-1/DP-1 and RB (Hsu *et al.*, 2001).

It is widely accepted that cancer results from the accumulation of mutations in the genes that directly control cell growth or cell death. Gene amplification is an important mechanism for increased expression of genes involved in tumourigenesis, and is seen at the molecular level as multiple copies of an amplicon, containing 0.5 – 10 mega bases of DNA (Lengauer *et al.*, 1998).

Increased copies of TRIP-Br1 could, through their sequential effects on the transcriptional activity of E2F-responsive promoters during G1 and S phases, play a part in increased transcription in cancer cells.

We wanted to minimise the 19q13-amplification region, and to determine whether TRIP-Br1 is included in this region. Three ovarian cancer cell lines were chosen: OVCAR-3, OVCAR-8, which in previous studies has shown amplification of AKT2 (Thompson *et al.*, 1996; Cheng *et al.*, 1992) and Caov-3, which did not show increased TRIP-Br1 gene copy number by Southern blot (Hsu *et al.*, personal communication). A normal ovarian epithelial cell line (IOSE-80) immortalised with SV40 was used as control.

Initially the cell lines were characterised by comparative genomic hybridisation (CGH) to identify whole chromosomal gains and losses. On the basis of the established physical map of human chromosome 19, 19 BACs (obtained from The National University Hospital, Dept. of Medicine, Singapore), spanning the region 19q13.1 – 19q13.3 (39.5 Mb – 56.6 Mb according to ENSEMBLE) were used as probes (Table1).

Using IOSE-80, and normal male metaphases, the localisation of all 19 BACs were confirmed.

Using OVCAR-8, the copy number of each BAC was determined

Materials and methods

Cell lines

Three ovarian cancer cell lines, OVCAR-3, OVCAR-8 and CaOV3, were chosen based on known AKT2 copy number. As control, a normal ovarian epithelial cell line, IOSE-80, immortalised with SV40, was used. The OVCAR-3 cell line, in which AKT2 and TRIP-Br1 are co-amplified approximately 10-fold, together with Caov-3, an ovarian cancer cell line not amplified in the 19q13 region, were obtained from Dr. S. Hsu, The National University Hospital, dept. of Medicine, Singapore. Ovcar-8, a cell line showing amplification of AKT2 (Chen *et al.*, 1992; Thompson *et al.*, 1996), was obtained from Tony Kouzarides lab, Cambridge, while IOSE-80 was obtained from The Uiversity of British Colombia.

The cells were cultured as follows: OVCAR-3: 80% RPMI, supplemented with 2 mM glutamine, 1.0 mM sodium pyruvate, 0.01 mg/ml bovine insulin and penicillin/streptomycin + 20% fetal bovine serum (FBS). OVCAR-8: 90% DMEM supplemented with 2 mM glutamine and penicillin/streptomycin + 10% FBS. CaOV3: 90% DMEM supplemented with penicillin/streptomycin + 10% FBS.

Metaphase preparations: Cells were harvested when approximately 80% confluent, arrested in mitosis through the addition of Colcemid (100µl/ml) in the media for 20 minutes, before pelleting cells at 1250 rpm for 5 minutes at room temperature. Media was removed and cells were loosened by gently tapping the tube. Fifteen millilitres of 75 mM potassium chloride (KCl)was added drop wise, and cells were incubated at 37^0C for 20 minutes. Approximately one millilitre of fixative (methanol acetic acid 3:1) was added, and cells were pelleted by centrifugation at 1250 rpm for 5 minutes at room temperature, supernatant removed and cells loosened by gentle tapping. The last two steps were repeated using cold (-20^0C) methanol:acetic acid (3:1) (fixative),

transferred to 2ml eppendorf vials and topped up with fresh fixative for storage. Metaphase slides were prepared by making the slide humid before dropping fixed metaphase from a distance.

CGH

CGH was used to identify whole losses or gains of chromosomes in the ovarian cancer cell lines. Normal male metaphase slides were purchased from Vysis UK. Tumour and control DNA were labelled using DOP PCR with digoxigenin and biotin, respectively. One µg of control DNA, one µg of tumour DNA and 80 µg of human Cot-1 DNA were co-precipitated with ethanol, before resuspension in 15 µl hybridisation buffer (50% formamide, 10% dextran sulphate, 2xSSC, 40 mM sodium phosphate buffer, 1x Denhardt's solution). Probes were denatured at 70^0C for 10min, snap cooled on ice and then allowed to pre-anneal at 37^0C for 30 minutes. The slides were denatured in 70% formamide/2xSSC at 70^0C for 3 minutes, immediately snap-cooled in ice-cold ethanol series (70%, 90% and 100%, 5 minutes each). Finally the probes were hybridised to the slides at 37^0C for 72 hours. Slides were washed in 50% formamide/ 1xSSC, 0.1xSSC and 4xSSC + o.o5 Tween-20, at 45^0C, 2x5 minutes each, blocked in 5% non fat dry milk in 4xSSC+Tween-20, at 37^0C for 20 minutes, followed by washing in 4xSSC+Tween20 at 37^0C for 3x3 minutes. Tumour and control DNA were detected with FITC and CY3, respectively, using indirect immuno fluorescence labelling. Three layers of antibodies were used: 1^0: α-dig-FITC (1:200) and avidin-CY3 (1:400); 2^0: Rb-α-FITC (1:400) and bio-α-avidin (1:200); 3^0: GARb-FITC (1:400) and avidin-CY3 (1:400). Chromosomes were mounted with vectorshield containing 4,6-diamidino-2-phenylindole (DAPI).

Chromosome 19 paint

Flow sorted chromosome 19 was amplified and labelled with biotin-16-dUTP by DOP-PCR, and detected with avidin pacific blue.

BACs/Probe production

19 stab cultures of BAC clones covering the interval 19q13.11 – 19q13.31 was sent from Dr. Stephen I. Hsu, Dept. of Medicine, National University Hospital, Singapore (Table1). They got the BACs from Research Genetics, and by gene specific PCR they determined which BAC was expected to carry TRIP-Br1 (Table 1).

Bacteria were streaked on 2xTY agar plates containing 25 µg/ml chloramphenicol at 37^0C over night. A single colony was picked and cultured in 5 ml of media (2xTY + chloramphenicol) by shaking vigorously at 37^0C for 7 hours. 95 ml of media was inoculated with the 5 ml of culture and grown over night at 37 C. DNA from 9 of the BACs was prepared using the alkaline lysis method: bacteria were resuspended in 4 ml of GTE (5 mM Glucose, 25 mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0), added 60 µl of 100mg/ml lysozyme in TE, and incubated at room temperature for 5 minutes, eight ml of 0.2 M NaOH/ 1% SDS was added, tubes inverted and placed on ice for 5 minutes. 6 ml of 3M KAC was added and following 5 minutes on ice, the lysates were precipitated by centrifugation (4000 rpm for 30 minutes). 180 µl of RNAse A (50µg/ml) was then added to the solution, followed by incubation at 37^0C for 1 hour. We added an equal volume of phenol/chloroform (1:1), mixed it by inversion and centrifuged at 4000 rpm for 30 minutes. Aqueous phase was removed, equal volume of isopropyl alcohol added, mixed and centrifuged at 4000 rpm for 30 minutes. After removing the supernatant and adding 2.5 ml of 70% ethanol, the suspension was transferred to 15 ml falcon tubes, centrifuged at 15,000 rpm for

20 minutes, supernatant removed and pellets air-dried. The pellets were then resuspended in 500 µl of water, added 500µl RNAse One-solution and incubated at 37^{0}C for 1 hour. The phenol/chloroform – step was repeated and suspension spun down at 4000 rpm for 15 minutes. The supernatant was transferred to 2 ml tubes (2 x each BAC), added 4.5 µl 3M NaOH + 1.3 ml absolute ethanol and stored at –20^{0}C.

DNA from the remaining 10 BACs was extracted using the vector isolation kit Nucleo Bond BAC 100 (Macherey – Nagel), following manufacturer's instructions.

Locus specific FISH

Probes were labelled, using nick translation, with either digoxigenin or estradiol. Each probe (1µg) were then ethanol precipitated with 50 µg of human Cot-1 DNA, resuspended in 45µl of hybridisation buffer and incubated at 37^{0}C for 30 min. A probe mixture containing 50 µg of each labelled probe and chromosome 19 paint was prepared per hybridisation. The probes were denatured at 70^{0}C for 10 minutes, snap cooled on ice and pre-annealed at 37^{0}C for 30 minutes. Slides were denatured in 70% formamide/2xSSC at 70^{0}C for 1 minute, immediately transferred into ice cold ethanol series (70%, 90% and 100% ethanol; 3 minutes each). Slides were incubated at 42^{0}C in a moist chamber for 72 hours. Slides were then washed in 50% formamide/ 1xSSC, 0.1xSSC and 4xSSC + o.o5% Tween-20, at 45^{0}C, 2x5 minutes each, blocked in 5% non fat dry milk in 4xSSC+Tween-20, at 37^{0}C for 20 minutes, followed by washing in 4xSSC+Tween20 at 37^{0}C for 3x3 minutes. Probe signals were detected with one layer of antibodies : avidin-pacific blue 1:100, anti-digoxigenin-FITC 1:200 and anti-estradiol-rhodamine 1:200 in 1 ml of filtered 4xSSC+Tween-20. 200ul of antibody-solution was pipetted onto each slide, and incubated at 42^{0}C for 30 minutes, followed by a new wash in 4xSSC+Tween-20 (preheated to 37°C) for 3x3

minutes. Chromosomes were mounted with vector shield containing 4,6-diamidino-2-phenylindole (DAPI).

Digital image analysis

Images were collected under an automated fluorescence microscope (Zeiss Axioplan II), captured using SmartCapture VP software.

CGH:

Separate digitised images of FITC and CY3 fluorescence were obtained with a cooled charged coupled device (CCD) camera (Photometrics, Tucson, AZ) connected to an epi-fluorescence microscope equipped with selective filter sets (Fig.2). Chromosomes were identified interactively on inverted DAPI images, and the ratio of FITC/ CY3 fluorescence intensities was calculated along each individual chromosome with dedicated software. Ratios obtained from 10 to 20 metaphases were averaged, and the resulting profile was plotted next to chromosomal ideograms (Fig.3). The threshold for over- and underrepresentation correspond to the values expected for monosomy or trisomy in 50% of diploid cells

Locus specific FISH:

Different filters were used to visualise the various fluorochromes, and the individual probes (BAC) and chromosome 19 were detected by their colour (dig-FITC = green; est-rhodamine = orange; bio-pacific blue = blue), and the average copy number per metaphase and/or interphase nuclei was calculated. To detect possible copy number change of BACs, at least 10 metaphases and/or interphase nuclei per BAC-combination and cell line were combined. IOSE80 and the normal male cell line were used to control if the BACs (probes) attached to the chromosome of interest (chromosome 19).

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Results

CGH

The evaluation of chromosomal imbalances was based on a quantification of the fluorescence intensities. *Blue*, regions not affected by copy number changes in the cell line; *red*, loss of genetic material; *green*, regions over represented in the cell line genome. (Figure1)

Chromosomes that, due to overlap or for other reasons, were not complete, were excluded from the analysis. Based on at least ten analysed metaphases, an average ratio profile along the axis was computed for all chromosomes. Ratios of 1 indicate equal copy umbers of the respective chromosomes in the cancer cell line and reference genome, ratios of 0.80 a deletion of one homologous chromosome, and ratios of 1.2 a trisomy in a diploid tumor.

The CGH studies revealed chromosomal imbalances, including amplification of chromosome 19, in all three ovarian cancer cell lines (Table 2). Other imbalances observed were gains of chromosome 22, the distal part of the 1p and 9q arms, and the proximal 11q arm (all three ovarian cancer cell lines); gains of chromosome 17 and the distal part of the16p arm (OVCAR-8, Caov-3); gain of the distal part of the17q arm, and loss of the distal part of the 13q arm (OVCAR-3); gains of the proximal part of the 1q and 9q arms (Caov-3); gain of the 20q (OVCAR-3, OVCAR-8); gains of the distal part of the 6p, 8q and 12q arms, and loss of the distal part of the 18q arm (OVCAR-8).

IOSE-80 also exhibited imbalances with gains mapped to chromosome 22 and distal part of the 9q and 1p arms, and loss of chromosome 13. There was no time to combine the results from our three cell lines (Fig.2)

Cell line	Gains	Losses
IOSE-80	1p, 9q, 22	13
OVCAR-3	1, 9q, 11q, 17q, 19, 20q, 22	13q
OVCAR-8	1p, 6p, 8q, 9q, 11q, 12q, 16p, 17, 19, 20q, 22,	18q
Caov3	1, 9q, 11q, 16p, 17, 19, 22,	

Table2. Gains and losses in the normal cell line IOSE-80, and the ovarian cancer cell lines OVCAR-3, OVCAR-8 and Caov-3, detected by CGH. Gains and losses are written as parts of chromosomes.

FISH

Initially every BAC was checked for its correct mapping to chromosome 19, on IOSE-80 and nomal male metaphases. Five BACs were located to chromosomes other than chromosome 19 (Fig.4): RP11-430N3 had two copies located on chromosome 6p, RP11-452D9 two copies located on chromosome 10p, RP11-541B22 two copies located on chromosome 10p12, and RP11-551E10 and RP11-RP11-363M21 both with three copies located to chromosome 7q36. RP11-422M7 was found to be hybridised to all acrocentric tips. The remaining 13 BACs were all located to chromosome 19 in IOSE-80 and the normal male metaphase slides (Table 1).

Locus specific FISH was used to reveal possible amplification of the BACs, spanning the region 19q13.1 – q13.3, based on copy numbers of each fluorescence colour (green and orange) per interphase or metaphase (Fig.5). The BACs that localised to 19q13 were hybridised on to OVCAR-8, revealing three copies of RP11-566G7, RP11-307P23, RP11-316D19, RP11-343J4, RP11-363M21 and RP11-47023 (Table 1). We were not able to grow OVCAR-3, and therefore no metaphases could be made from this cell line. Our intesion was to test the BACs on ceveral ovarin cancer cell lines, but unfortunately there was no time for this.

BAC	Map Position (Mb)	IOSE-80 + normal male metaphase	OVCAR-8 Copy Numbers
RP11-434E22	--	19q	2
RP11-534C23		19q	2
RP11-303J9	39.5-40.2	19q	2
RP11-329F17	41.1-41.3	19q	2
RP11-430N3	47.0(AKT2)	6p	2
RP11-328E9	--	19q	2
RP11-426L3	45.3-46.1	19q	2
RP11-447A19	46.4	19q	2
RP11-541B22	--	10p12	2
RP11-452D9	48.9(TRIP-Br1)	10p	2
RP11-566G7	--	19q	3
RP11-316D19	47.2-47.6	19q	3
RP11-307P23	--	19q	3
RP11-427D14	--	19q	3
RP11-343J4	--	19q	3
RP11-363M21	49.5-49.9	7q36	3
RP11-551E10	49.8-49.9	7q36	3
RP11-470C23	53.2-53.6	19q	3
RP11-422M7	56.6-56.8	Acrocentric tips	--

Table 1. BACs presented by their RPCI Clone number, and their mapping position according to ENSEMBLE. We describe which chromosome the different BACs hybridised to, followed by copy number.

Discussion

Chromosome imbalances detected by CGH

.CGH is an ideal tool for analysing chromosomal imbalances in cancer cell lines (tumour material) and for examining possible correlation between these findings and gene amplifications (Ried *et al.*, 1997). To date, few analysis of ovarian cancer cell lines and tumours using CGH (Iwabuchi *et al.*, 1995; Arnold *et al.*, 1996; Pejovic *et al.*, 1999; Watanabe *et al.*, 2001) have been reported. All studies showed that ovarian carcinomas reveal consistent chromosomal abnormalities. However, in none of these studies CGH was combined with double locus specific FISH.

The three ovarian cancer cell lines (OVCAR-3, OVCAR-8 and Caov-3) and the normal ovarian cell line (IOSE-80) characterised by CGH all revealed chromosomal imbalances. Gain of chromosome 19 seemed to be one of the most frequent chromosomal changes, confirming the frequent involvement of chromosome 19 in ovarian cancer (Thompson *et al.*, 1996), although this sample is too small to make any definite conclusion. Our findings are quite comparable with Arnold *et al*, (1996), many recurrent aberrations, including gain of chromosome 19q, being found in common. Iwabuchi *et al.* (1995), however, reported fewer chromosomal changes, and Watanabe *et al.* (2001) changes located to a number of other chromosomes.

A couple of surprising results was the gain of chromosome 19 in Caov-3, expected to have no amplification in the 19q13 region, and a loss of chromosome 13 in IOSE-80, expected to be a normal ovarian cell line. Other frequent gains or losses emerging from this study may also harbour oncogenes or tumour suppressor genes relevant to the development or progression of these tumours that remain to be identified. The gain of chromosome 19, may include an amplicon containing the novel gene TRIP-Br1, which we aimed to detect using double locus specific FISH.

Increased BAC copy number detected by locus specific FISH

We checked the possible amplification of 19q13 sequences by FISH using a chromosome 19 paint and probes specific for the 19q13.1 – q13.4 region. Our test of BAC status on a normal male cell line and IOSE-80, revealed five BACs not hybridising to the chromosome of interest (chromosome 19). This is either because of mislabelling at origin or incorrect mapping of the different BACs.

We describe increased copy number of six BACs located to chromosome 19q13 in the ovarian cancer cell line OVCAR-8. The BACs are thought to span the interval from 46 Mb to 53.6 Mb, according to ENSEMBLE (Table 1). Mechanisms underlying gene copy number change may include polyploidy or regional amplification, which was confirmed by comparison with the CGH-findings. We conclude that no amplifications were found.

As mentioned before, this sample is too small to make any definite conclusion. Continuation of these studies may reveal TRIP-Br1 being included in the amplicon on chromosome 19q13. These studies should include several more ovarian cancer cell lines, particularly OVCAR-3. BACs should be collected from other commercial or laboratory sources, and should be sequenced as part of the human genome project. There are several possible explenations to why some of our BACs, included the one expected to carry TRIP-Br1, were hybridised to other chromosomes than chromosome 19: the BACs could be mislabelled at origin or incorrectly mapped to the 19q13.1-q13.3 region. A third possibility is the existence of other chromosomes carrying genes homologous to TRIP-Br1, which PCR is not specific enough to reveal.

It is now commonly accepted that cancer development is due to accumulation of genetic alterations in somatic cells. Mutations result either in the activation of oncogenes, which promote cellular proliferation or inhibit cell death, or in the inactivation of tumour suppressor genes, which inhibit proliferation or promote cell death. To become a cancer cell, a normal cell needs to

accumulate at least five or six of these mutations (Caldas, 1998). If identified as an oncogene, the correlation of the specific profile of this alteration (e.g. amplification of TRIP-Br1) with clinical outcome might help define prognosis of ovarian cancer more accurately and enable clinicians to target treatment more effectively.

In summary, gene amplification is an important mechanism for increased expression of genes involved in tumour development. Studies on the possible amplification of TRIP-Br1 should therefore be continued, because:

- TRIP-Br1 is a possible "integrator" of the actions of PHD zinc finger- and/or bromodomain-containing proteins on E2F-1/DP1, which mediates transcription (Fig.1). TRIP-Br1 is overexpressed during the G1 and S phases of the cell cycle, suggesting that it is a putative cell cycle regulator.
- OVCAR-3, an ovarian cancer cell line, was by Southern blot found to have an amplification of TRIP-Br1.

These results are indirect evidence of TRIP-Br1 being involved in cancer, especially ovarian cancer. Further research in this area should include more ovarian cancer cell lines, particularly OVCAR-3, which we were not able to grow. In addition, it is critical that the BACs being used are in fact carrying the gene TRIP-Br1.

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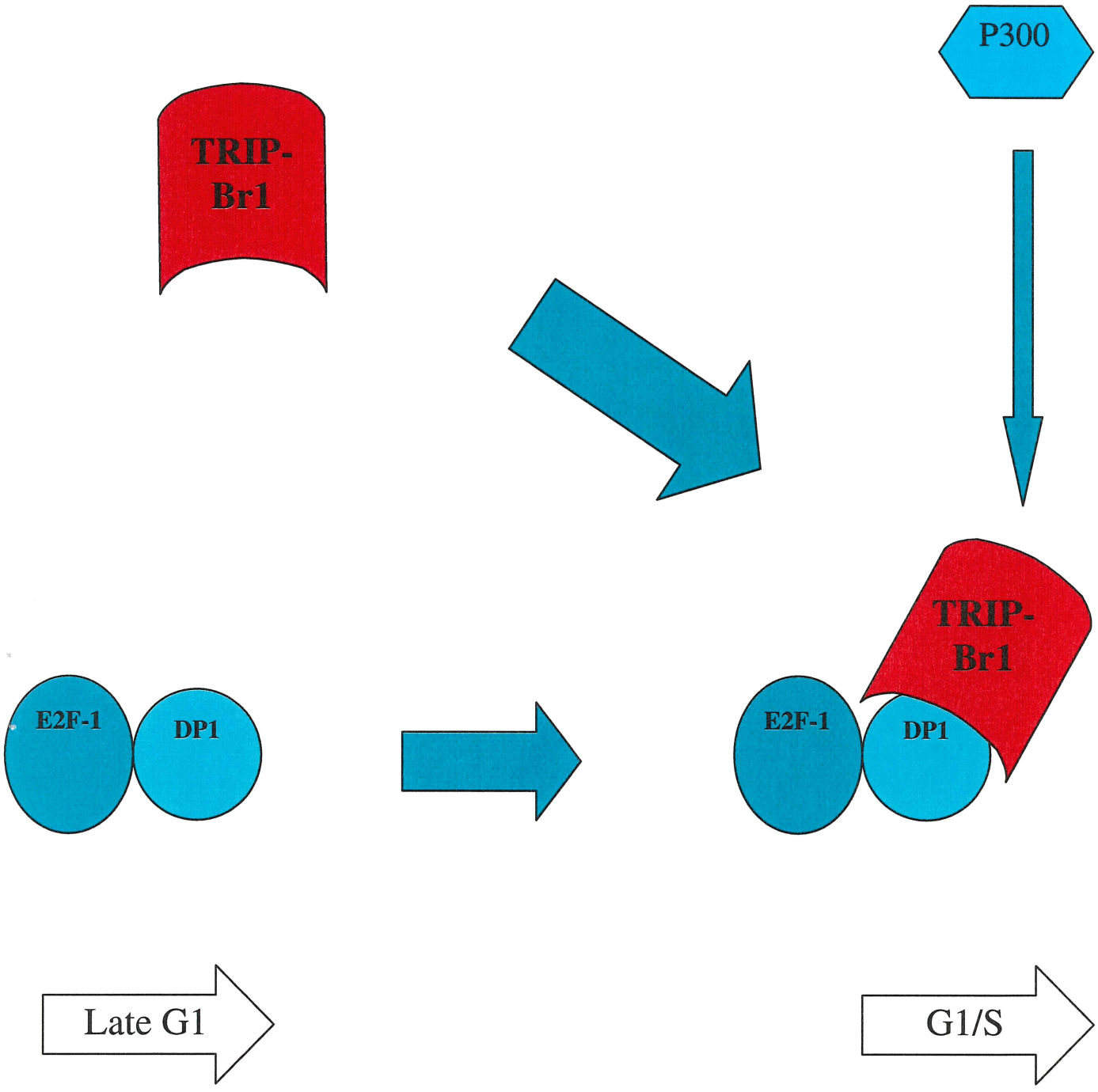


Figure 1. TRIP-Br1 as a possible regulator of transcription: RIP-Br1 proteins function as transcriptional"integrators" to couple regulatory signals provided by PHD zinc fingers and/or bromodomain-containing transcription factors (here represented by P300), with E2F-1/P1. E2F-1/DP1 mediating transcription and cell cycle regulation. TRIP-Br1 is found to be overexpressed during G1 and S phases of the cell cycle.

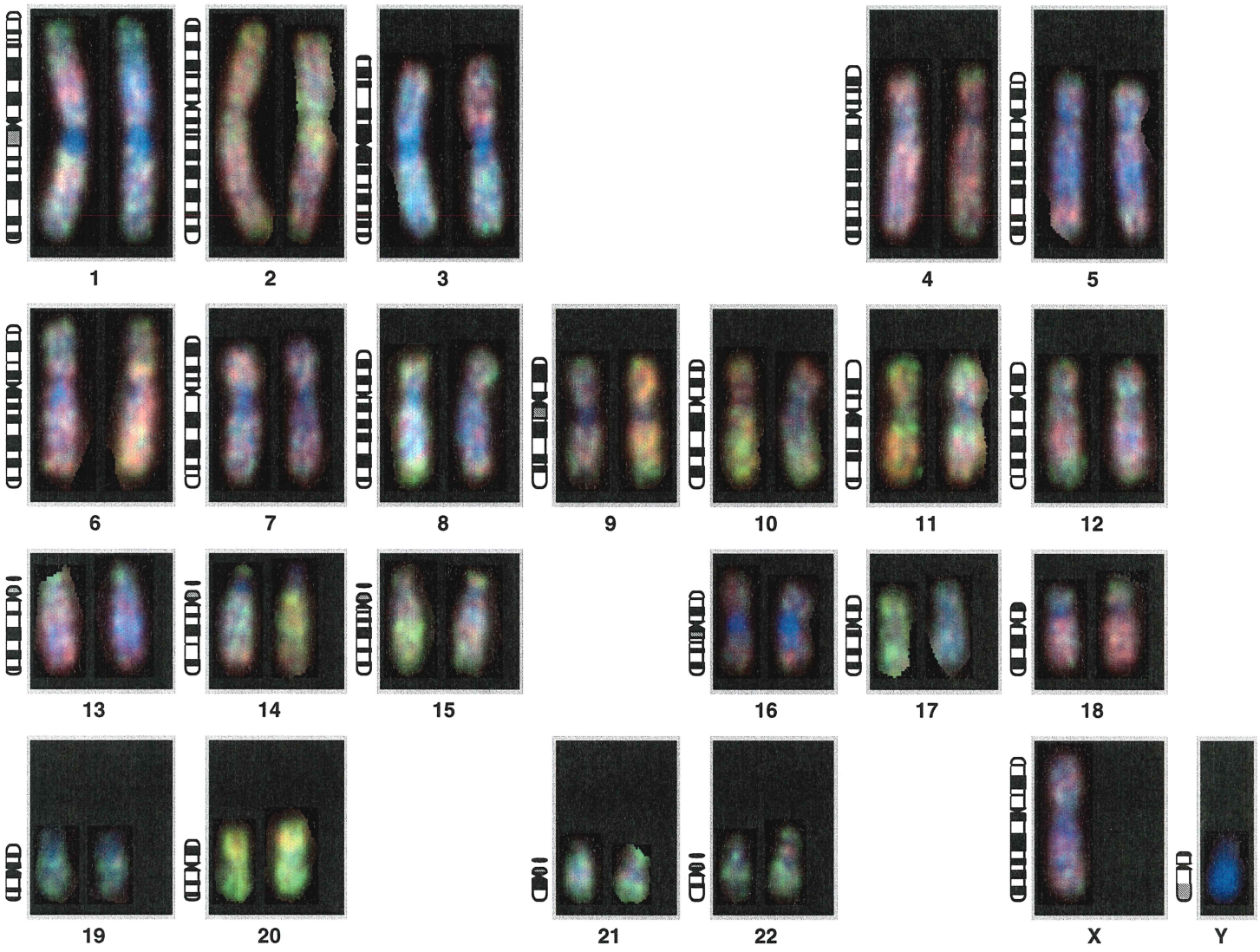
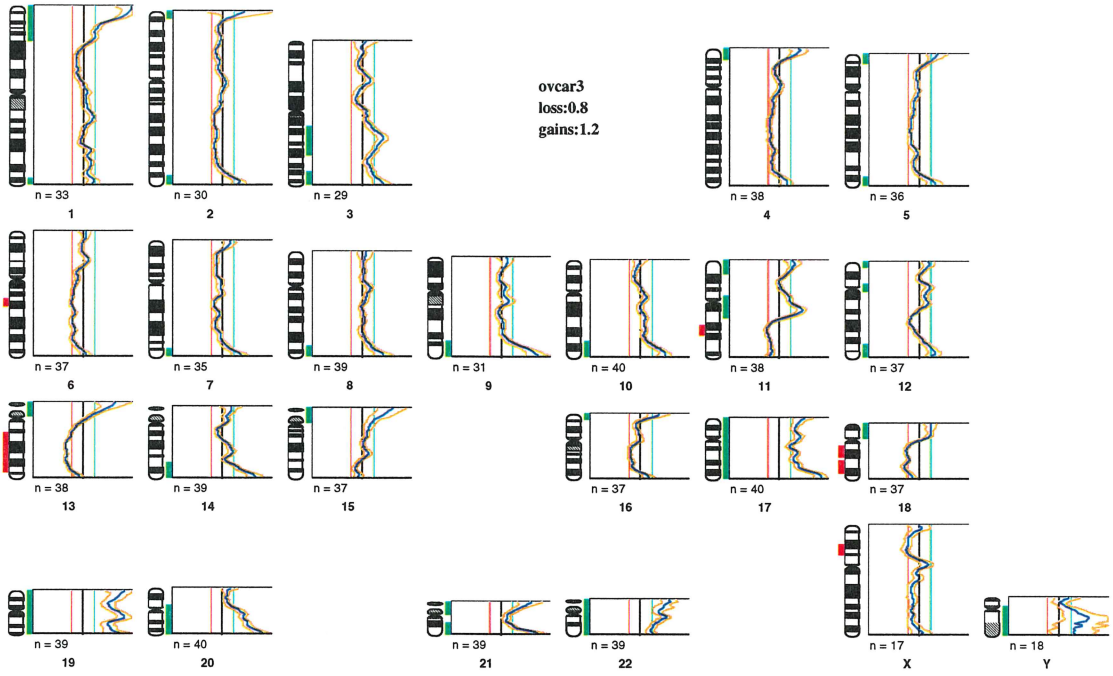


Figure 2. CGH of OVCAR8. Metaphase images were captured and karyotyped. The relative amount of fluorescence intensities along each chromosome is determined by digital image analysis: *Blue,* regions not affected by copy number changes in the cell line; *red,* loss of genetic material; *green;* regions over represented in the cell line genome.



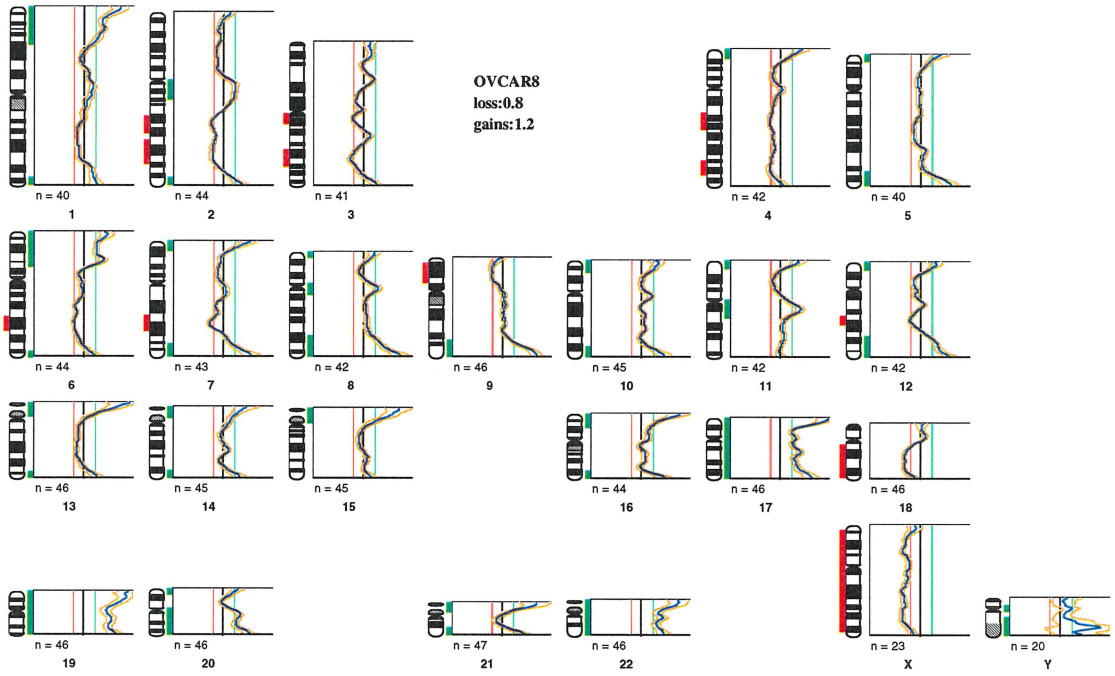
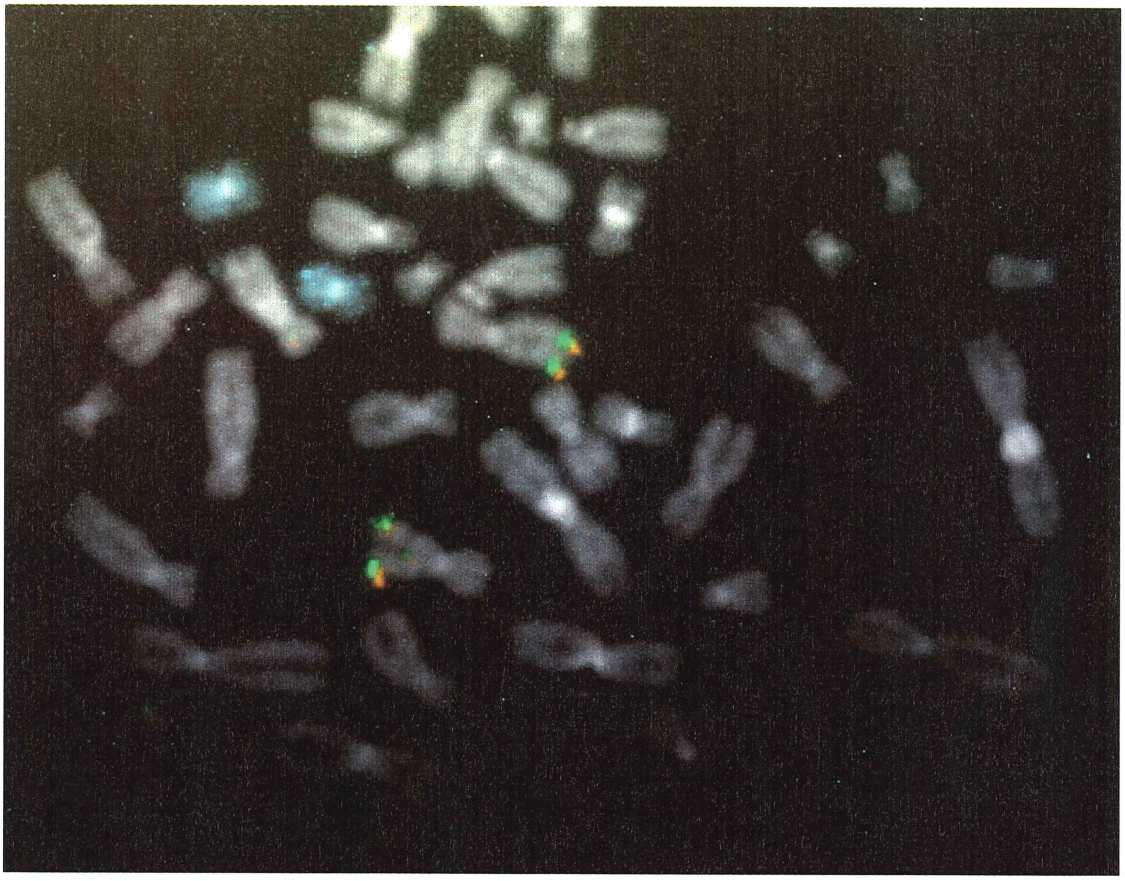


Figure 3. Summary of chromosome imbalances detected in the OVCAR-3 and OVCAR8 by CGH. Bars on the left and right of each chromosome ideogram indicate losses and gains, respectively.
OVCAR-3: DNA gains were observed on chromosomes and chromosome arms 1p, 9q, 11q, 17q, 19, 20q and 22. Loss was mapped to the distal part of the 13q arm.
OVCAR-8: DNA gains were observed on chromosomes and chromosome arms 1p, 6p, 8q, 11q, 12q, 16p, 17, 19, 20q and 22. Loss was mapped to the distal part of the 18q arm.

Figure 4. Locus spesific FISH using the two BACs labelled with different colours (*RP11363M2*, estradiol – detected with rhodamine; *RP11-551E10*, digoxigenin – detected with FITC) and chromosome 19 paint (biotin – detected with pacific blue), on to a normal male metaphase. Both BACs (orange=estradiol; green=FITC) hybridising to the same chromosome, not identified as chromosome 19 (blue).



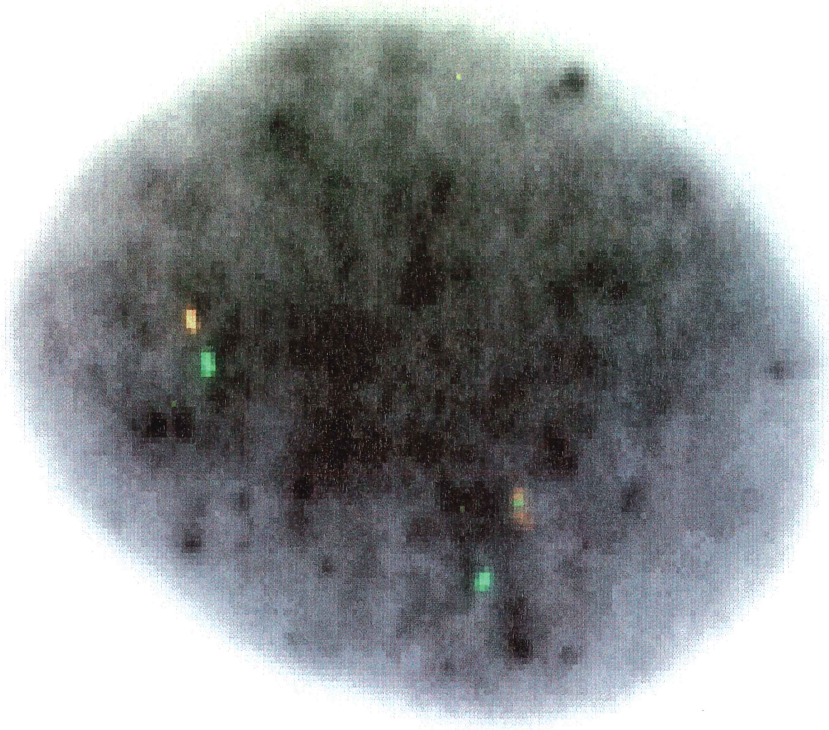


Figure 5. BAC copy number were counted per interphase, after ensuring they hybridised to the chromosome of interest (chromosome 19). This image shows an interphase of OVCAR-8, with two copies of each BAC (RP11-434E22 and RP11-534C23).