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DNA ploidy and *MYC* DNA amplification in ovarian carcinomas

Correlation with p53 and bcl-2 expression, proliferative activity and prognosis

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Abstract There is increasing evidence that DNA ploidy is a prognostic factor in ovarian carcinomas, but it is uncertain whether *MYC* DNA amplification is an epiphenomenon of DNA nondiploidy or a distinct biological change with an impact on the clinical course of the disease. To clarify these issues we analysed DNA ploidy by flow and image cytometry and *MYC* copy number by polymerase chain reaction in archival material from ovarian carcinomas with known follow up. The results were compared with proliferative activity (Ki67 index) and *p53* and *bcl-2* expression. DNA cytometry revealed nondiploidy in 84 of 144 cases (58.3%). Nondiploidy was statistically significantly correlated with histological tumour type, histological grade, Ki67 index >10%, FIGO stage, presence of residual tumour after debulking surgery and adverse postoperative outcome. Furthermore, DNA nondiploidy was associated with p53 accumulation. We found that 84.9% of the p53-positive cases were nondiploid. This points to the paramount importance of wild type p53 for the maintenance of genome integrity in this tumour type. *MYC* DNA amplification was seen in 33.8% (26/77 cases) of ovarian carcinoma. There was no correlation between *MYC* DNA amplification and histological tumour type, histological grade, FIGO stage, DNA ploidy, proliferative activity or prognosis. However, when p53 and bcl-2 expression was taken into account, a statistically significant correlation between gene alteration or expression patterns and histological tumour type was revealed. The group of mucinous carcinomas demonstrated both *MYC* DNA amplification and strong bcl-2 expression in 50% and contained the largest fraction of cases without aberration (37.5%). Endometrioid carcinomas were characterized by strong bcl-2 expression in 85%, whereas serous and undifferentiated carcinomas predominantly exhibited p53 alterations, frequently accompanied by bcl-2 overexpression or *MYC* DNA amplification. Thus, in interaction with other genes *MYC* DNA amplification may play a role in the determination of the varying differentiation patterns of ovarian carcinomas.

Key words Ovarian carcinoma · DNA ploidy · *MYC* amplification · Multiplex PCR · Prognosis

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Introduction

DNA nondiploidy (aneuploidy or tetraploidy) is a frequent finding in ovarian carcinoma, particularly in advanced stages [4, 8]. Some studies have provided evidence that nondiploidy is an independent prognostic factor in this tumour type [4, 31, 41]. Since progression is accompanied by oncogene amplification and DNA nondiploidy in various human and experimental tumours [10, 16], the two phenomena may be interrelated. In ovarian carcinomas *MYC* DNA amplification seems to be particularly frequent, as repeatedly demonstrated [1, 3, 6, 21, 27, 37, 38, 43]. However, the studies cited were mostly limited to relatively small number of cases and often provided little information on histological tumour type and clinical follow up. It is unclear whether *MYC* DNA amplification has a prognostic relevance and whether this alteration is relevant to all types of ovarian carcinomas.

For maintenance of the integrity of the genome the tumour suppressor gene *p53* is thought to be of pivotal importance [25, 26]. The *p53* gene is frequently mutated in ovarian carcinomas [7, 22, 24, 29, 30], but is not clear whether *p53* alterations are correlated with DNA nondiploidy and *MYC* DNA amplification in these neoplasms.

In physiological situations *MYC* acts as a transcription factor that is mainly involved in the regulation of genes controlling cell proliferation [23]. In addition, it has become evident that *MYC* plays a part in the regulation of apoptosis (programmed cell death), [39, 42], cooperating with other regulatory factors, such as *p53* or *bcl-2* (summarized in [39]). Although it can be assumed that the interaction of these genes or gene products differs considerably in neoplastic conditions, little is known about the altered interplay of these growth regulatory factors in ovarian carcinomas.

By applying DNA flow and image cytometry as well as the polymerase chain reaction (PCR) and immunohistochemistry to archival material we were able to analyse a series of ovarian carcinomas comprising all tumour stages and all major histological types retrospectively. Clinical follow up was available for the majority of patients. The purpose of our study was to achieve a better understanding of the relevance and the possible relations between DNA ploidy, *MYC* DNA amplification, *p53* alteration, and *bcl-2* overexpression in this tumour type. This study is the largest combined analysis of DNA ploidy and *MYC* DNA amplification in ovarian carcinomas that has been carried out in paraffin-embedded material.

Materials and methods

The total study material comprised archival specimens of 148 sequential ovarian carcinomas and ovarian tumours of low malignant potential: 69 serous (mean age ± SD: 64 ± 10 years), 18 mucinous (53 ± 16 years), 15 endometrioid (60 ± 12 years), 5 clear cell (52 ± 7 years) and 11 undifferentiated carcinomas (62 ± 9), as well as 23 serous (52 ± 15 years) and 7 mucinous tumours of low malignant potential (57 ± 18 years). All patients underwent surgery between 1985 and 1991 at the same institution (Department of Gynaecology of the University of Munich, Grosshadern). All cases with invasive carcinomas (except FIGO stage Ia, grade 1 and a few FIGO stage IV cases) were treated with platinum-based chemotherapy. Carcinomas were graded as highly, moderately or poorly differentiated (grades 1–3). Tumours of low malignant potential were assigned grade 0. All FIGO (=International Federation of Gynecology and Obstetrics) stages were represented [32]: FIGO I 50 cases (Ia 16 cases, Ib 5 cases, Ic 29 cases), FIGO II 21 cases (IIa 2 cases, IIb 14 cases, IIc 5 cases), FIGO III 65 cases (IIIa 5 cases, IIIb 35 cases, IIIc 25 cases), FIGO IV 10 cases. With regard to the small numbers in some subgroups and the uneven distribution of cases only the four major groups, FIGO I–IV, were used for analysis. Survival data with follow up between 2 months and 11 years (average 3 years) were available for 130 patients. Benign ovarian cysts and cystadenomas were included for comparison.

In all cases a DNA analysis was performed by means of flow cytometry (FCM) and image cytometry (ICM) using Feulgen-stained nuclear suspensions as well as tissue sections. For FCM analysis the tumour tissue was microdissected from paraffin blocks under microscopical control and processed by a modification of the method of Hedley et al. [20] as described previously [2]. Briefly, 5–15 sections (30 µm thick) were dewaxed, rehydrated and washed. Tissue disintegration was performed mechanically with scissors followed by enzymatic digestion with 5 ml, 0.5% pepsin (Sigma, St. Louis, Mo.) in 0.9% NaCl/pH 1.1 at 37°C for 60 min on a rocking table. After filtration and centrifugation the pellets were stained with 4,6-diamidino-2-phenylindol (DAPI). The samples were adjusted to a concentration of $2–4\times10^6$ nuclei/ml and analysed using an Ahrens FCM (Ahrens, Bargteheide, Germany) with mercury arc lamp as light source; from each probe 20,000 impulses were measured. Histograms were classified as either diploid or nondiploid, the latter comprising aneuploid and tetraploid cases. DNA nondiploidy was diagnosed when a histogram revealed more than one identifiable cell population (more than one G0/1 peak). The coefficient of variation (CV) was determined as previously described [2] and served as a parameter for the quality of the measurements. Only histograms with a CV value of the diploid peak ≤7.5% were accepted.

For ICM analysis the sections (6 µm thick) were dewaxed in xylol and rehydrated. Afterwards sections or nuclear pellets from disintegrated tumour tissue were stained according to the Feulgen method (5 N HCl/22°/55 min, 120 min Schiff's reagent, 3×10 min SO_2 water). For the measurements, a PC based image analyser ACAS (Ahrens) with the special DNA software ACAS 4 (Ahrens) and Laborlux S microscope (Leitz, Wetzlar, Germany; magnification of the lens×40) equipped with a high-resolution CCD/RGB colour camera (Sony, Japan) were used. For assessment of the diploid (2c) range, the integrated absorbance of the nuclei of 25–30 granulocytes within the sections was measured. The CV value of these reference cells did not exceed 6%. Subsequently, an average of 150 (100–400) nonoverlapping carcinoma cells were measured selectively. Nondiploidy was assumed when more than 45% of the nuclei exhibited a DNA content above 2.5c and/or more than 5% nuclei were seen in the range above 5c [28].

Cases were classified as nondiploid when an abnormal DNA content was revealed either by FCM or by ICM or by both methods.

PCR analysis was performed on 88 cases. Tissue samples were taken from 8 µm slides of formalin-fixed and paraffin-embedded tumour specimens. PCR with oligonucleotide primers for human *MYC* exon 2 in the presence of α-32P-dATP resulted in a labelled 139 bp *MYC* PCR product. As an internal standard a 110 bp β-*GLOBIN* PCR product was co-amplified. The following primers were used for this multiplex PCR: myc1 AGG AAC TAT GAC CTC GAC TA; myc2 AGC AGC TCG AAT TTC TTC CA; β-globin1 ACA CAA CTG TGT TCA CTA GC; β-globin2 CAA CTT CAT CCA CGT TCA CC [36]. The PCR was carried out in a 100-µl reaction using Taq polymerase and Taq buffer (Boehringer, Mannheim, Germany). The temperature profile for amplification in a thermal cycler (DNA thermal cycler 480, Perkin Elmer, Norwalk, USA) was 1 min 94°C, 2 min 55°C and 3 min 72°C. Owing to the formalin fixation 40 cycles of PCR were used. Every reaction was accompanied by negative controls, including samples of normal müllerian epithelium of the fallopian tube. Amplification yield, product length and purity were determined by electrophoresis in a 10% acrylamide gel. The bands were visualized by autoradiography or by UV transillumination using ethidium bromide staining prior to photography. In each sample the ratio between the two co-amplification products was determined by laser densitometry (Shimadzu, Japan). This enabled an assessment of the ratio of copy numbers of the two genes in the samples. *MYC* DNA amplification was assumed if the *MYC*/β-*GLOBIN* ratio was higher at least by a factor of 2 in tumour tissue than in normal tissue. The reproducibility of the ratio was ascertained by repeating the analysis with altered concentrations and by running replicate samples.

Bcl-2 expression, *p53* accumulation and the proliferative activity (Ki67 index) were evaluated by immunohistochemistry. The technique and the results have been reported previously [13]. Briefly, tissue sections were deparaffinized, rehydrated and heated in a microwave oven at 750 W for 3×5 min in 10 mM citrate buffer (pH 6.0). Subsequently the plastic jar was allowed to cool for about 1 h at room temperature. The monoclonal antibodies bcl-2 "clone 124", p53 "DO-1" and mib-1 (all purchased from Dianova, Hamburg, Germany) were applied at a dilution of 1:60 (4°C, overnight), 1:35 (room temperature, 60 min), and 1:20 (30 min, RT), respectively. Afterwards the primary antibodies were detected by use of the alkaline phosphatase- anti- alkaline phosphatase (APAAP) method.

The data were compared by Chi-square tests and two-tailed Fisher's exact tests. For survival analyses Kaplan-Meier curves were calculated and log-rank tests performed using the SAS/STAT statistical software (SAS Institute, Cary N.C.). P values <0.05 were regarded as statistically significant.

Table 1 Comparison[a] of the results of DNA flow cytometry and DNA image cytometry in 144 ovarian carcinomas

DNA image cytometry			
DNA flow cytometry	DNA diploid	DNA nondiploid	Total
DNA diploid	60 (41.7%)	8 (5.5%)	68 (47.2%)
DNA nondiploid	1 (0.7%)	75 (52.1%)	76 (52.8%)
Total	61 (42.4%)	83 (57.6%)	144 (100%)

[a] χ^2-test for comparison of DNA flow and image cytometry reveals a significance of $P<0.001$

Table 2 DNA ploidy and *MYC* DNA amplification in relation to histological type of ovarian carcinomas (*Ca* carcinoma, *LMP* tumor of low malignant potential)

Histological type	DNA nondiploidy[a]		*MYC* DNA amplification[b]	
	$n_{nondiploid}/n_{total}$ cases	%	$n_{MYC\ ampl.}/n_{total}$ cases	%
Serous LMP	2/22	9.1	2/6	33.3
Mucinous LMP	0/6	0.0	0/1	0.0
Serous Ca	54/68	79.4	13/39	33.3
Mucinous Ca	7/17	41.2	4/8	50.0
Endometrioid Ca	7/15	46.7	4/13	30.8
Clear cell Ca	4/5	80.0	0/3	0.0
Undifferentiated Ca	10/11	90.9	3/7	42.9
Total	84/144	58.3	26/77	33.8

[a, b] χ^2-tests for comparison of DNA ploidy and histological type and for comparison of *MYC* copy number and histological type reveal [a] $P<0.001$ and [b] $P=0.771$ for significance of differences

Results

DNA ploidy was assessed by FCM and/or ICM in 144 of 148 cases (97.3%). The results of FCM and ICM agreed in 93.4% ($P<0.001$, Table 1). A total of 84 cases (58.3%) were DNA nondiploid (Table 2). An example of DNA histograms of a nondiploid ovarian carcinoma is given in Fig. 1. Nondiploidy was statistically significantly correlated with histological tumour type ($P<0.001$), histological grade ($P<0.001$), Ki67 index >10% ($P<0.001$), FIGO stage ($P = 0.01$), and presence of residual tumour after debulking surgery ($P=0.001$; Tables 2, 3). Survival analysis on 109 cases (FIGO IV and cases with additional extraovarian carcinomas excluded) showed a statistically significantly worse outcome for patients with DNA nondiploid carcinomas ($P = 0.026$; Fig. 2A).

Quantitative PCR on paraffin-embedded tissue was successfully performed in 88% of the samples. Benign ovarian cystadenomas and normal müllerian epithelium (from the fallopian tube) did not show any changes in *MYC* copy number. *MYC* DNA amplification was seen in 33.8% (26/77 cases) of the ovarian carcinomas. Examples are depicted in Figs. 3 and 4. All cases with evidence of amplification were validated in replicate PCRs. Furthermore the reproducibility of the *MYC/GLOBIN* ratio was ascertained in serial dilution experiments (Fig. 5).

The number of cases with *MYC* DNA amplification varied between the different histological types of ovarian carcinomas under investigation. The group of mucinous carcinomas contained the largest fraction of positive cases (4/8 cases). However, overall there was no statisti-

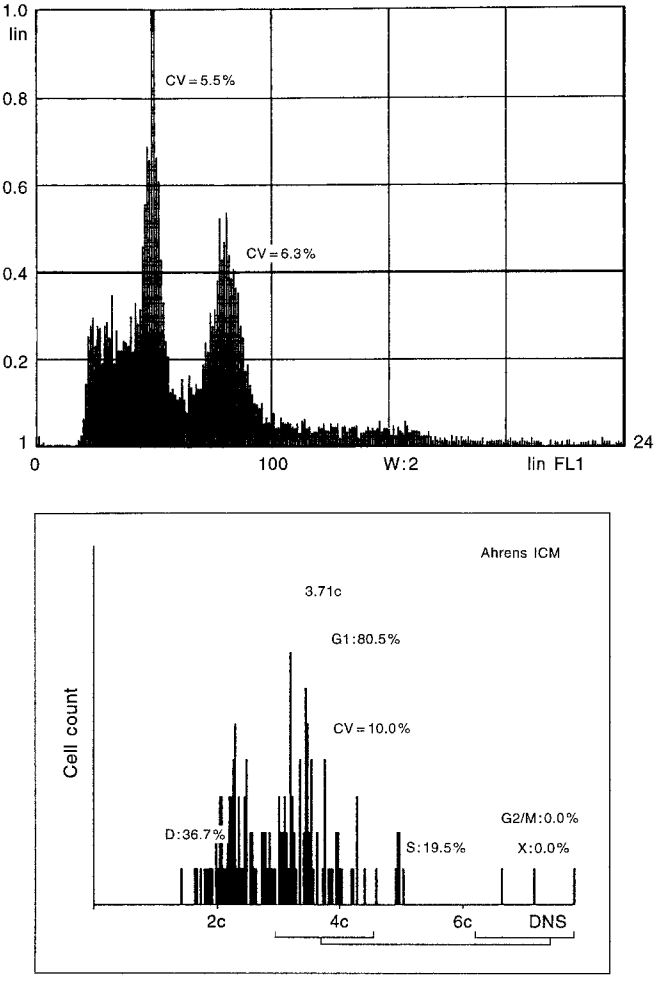

Fig. 1 DNA flow cytometrical histogram (*upper half*) and DNA image cytometrical histogram (*lower half*) of a nondiploid ovarian carcinoma

Table 3 DNA ploidy and *MYC* DNA amplification in relation to FIGO stage of ovarian carcinomas

FIGO stage	DNA nondiploidy[a]		*MYC* DNA amplification[b]	
	$n_{nondiploid}/n_{total}$ cases	%	$n_{MYC\ ampl.}/n_{total}$ cases	%
I	19/48	39.6	7/24	29.2
II	15/21	71.4	5/15	33.3
III	44/64	68.6	9/31	29.0
IV	6/10	60.0	5/7	71.4
Total	84/143	58.7	26/77	33.8

[a, b] χ^2-tests for comparison of DNA ploidy and FIGO stage and for comparison of *MYC* copy number and FIGO stage reveal [a] $P<0.01$ and [b] $P=0.173$ for significance of differences

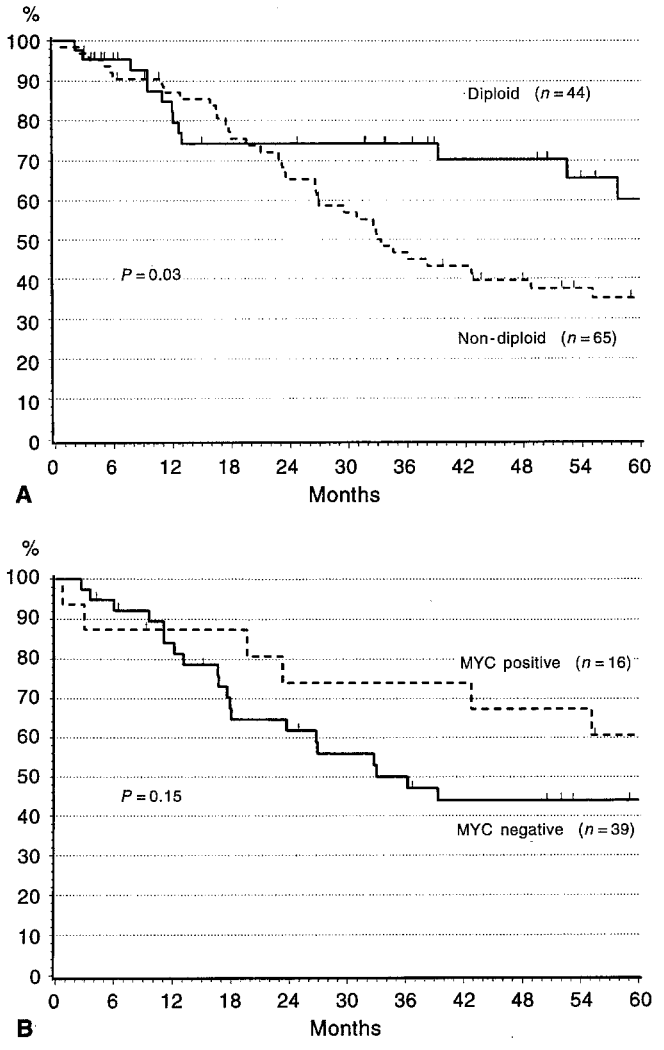

Fig. 2 **A** Survival analysis by Kaplan-Meier curves for diploid and nondiploid cases. *P*-value from log-rank test. **B** Survival analysis by Kaplan-Meier curves for cases with (=*MYC positive*) and without (=*MYC negative*) *MYC* amplification. *P*-value from log-rank test

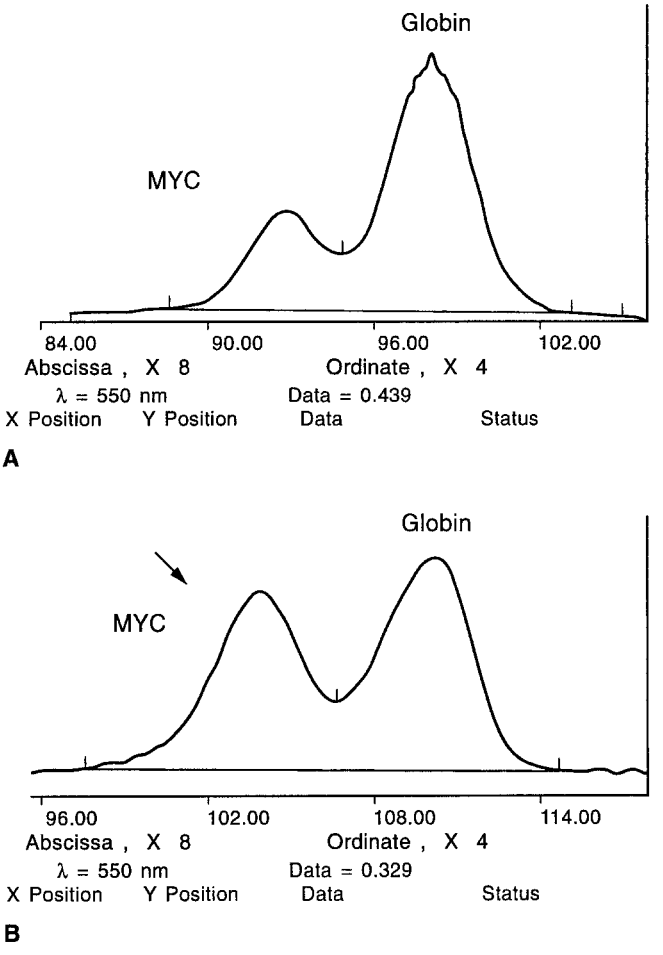

Fig. 4 **A** Laser densitometer print showing a normal ratio of *MYC* and *GLOBIN* PCR amplification products in normal müllerian epithelium. **B** Laser densitometer print showing an increased ratio of *MYC* (*arrow*) and *GLOBIN* PCR amplification products in an ovarian carcinoma

Fig. 3 Examples of electrophoresis gels showing multiplex-PCR products after simultaneous amplification of *MYC* and *GLOBIN* sequences from paraffin-embedded tissue samples of ovarian carcinomas. *GLOBIN* shows a constant band intensity in normal and tumour DNA. For *MYC* an increased band intensity indicates gene amplification. At the *top* of *lanes n* indicates normal *MYC* copy number; *a*, amplified *MYC*. At the *bottom* of the *lanes* the case numbers are given (*t* tumour tissue, *N* normal tissue)

cally significant correlation between *MYC* DNA amplification and histological tumour type ($P = 0.771$), histological grade ($P = 0.290$), Ki67 index ($P = 0.441$) and FIGO stage ($P = 0.173$; Tables 2, 3). Similar numbers of cases with *MYC* amplification were seen in the DNA-diploid (9/25 cases, 36.0%) and the DNA-nondiploid group (17/52 cases, 32.7%; $P = 0.774$).

After exclusion of cases with additional extra-ovarian carcinomas and those with FIGO stage IV 55 cases remained for survival analysis. A trend for better postoperative outcome was observed in cases with *MYC* DNA amplification, but without reaching statistical significance ($P = 0.15$; Fig. 2B).

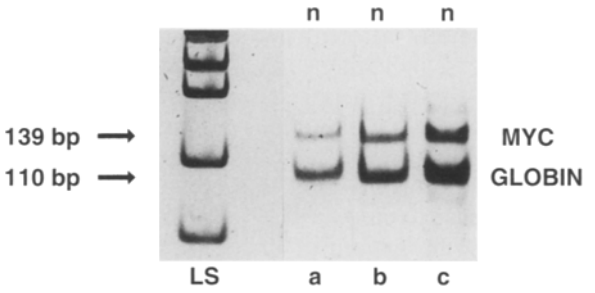

Fig. 5 Serial dilution of normal DNA to show the constant ratio of *MYC* and *GLOBIN* in spite of different amounts of amplified DNA in the gel. The *lanes* contain 1-fold (*a*), 2-fold (*b*) and 3-fold (*c*) amounts of DNA. At the *top* of the *lanes n* indicates normal *MYC* copy number. At the *bottom* of the *lanes LS* denotes DNA fragment length standard

Table 4 DNA ploidy and *MYC* DNA amplification in relation to p53 immunohistology of ovarian carcinomas

p53 immunohistochemistry	DNA nondiploidy[a]		*MYC* DNA amplification[b]	
	$n_{nondiploid}/n_{total}$ cases	%	$n_{MYC\ ampl.}/n_{total}$ cases	%
p53-positive cases	45/53	84.9	13/31	41.9
p53-negative cases	39/91	42.9	13/46	28.3
Total	84/144	58.3	26/77	33.8

[a, b] χ^2-tests for comparison of DNA ploidy and p53 immunohistochemistry and for comparison of *MYC* copy number and p53 immunohistochemistry reveal [a] $P<0.001$ and [b] $P=0.213$ for significance of differences

DNA nondiploidy was statistically highly significantly associated with *p53* accumulation. It was found in 84.9% of *p53*-positive cases and 42.9% of *p53*-negative cases (Table 4); only 13.3% of diploid cases were p53 positive ($P<0.001$).

MYC DNA amplification was seen more often in carcinomas with nuclear accumulation of *p53* than in *p53*-negative cases, but the difference did not reach statistical significance ($P = 0.213$; Table 4). 13 of 26 (50%) cases with *MYC* DNA amplification expressed nuclear *p53*. No correlation was found between *MYC* DNA amplification and *bcl-2* expression. (The detailed results of *bcl-2* and *p53* immunohistochemistry have been published elsewhere [13].)

In 67 cases data on all three genes or gene products under study were available. Regarding the different tumour types the pattern of oncogene alterations demonstrated characteristic variations ($P = 0.045$; Table 5). Mucinous carcinomas showed both *MYC* DNA amplification and strong *bcl-2* expression in 50%, but in 37.5% of mucinous carcinomas no abnormality of the analysed genes or gene products could be found. Endometrioid carcinomas were characterized by strong *bcl-2* expression in 85%. *p53* Accumulation was rare in mucinous and endometrioid carcinomas. In contrast, serous and undifferentiated carcinomas exhibited *p53* alterations predominantly, which were frequently accompanied by *bcl-2* overexpression or *MYC* DNA amplification. Furthermore, undifferentiated carcinomas comprised the largest fraction of cases with aberrations in all three factors examined (44%).

Discussion

The present investigation of ovarian carcinomas sought correlations between DNA nondiploidy and alterations of oncogenes and tumour suppressor genes known to be relevant in this type of cancer. Amplification of the *MYC* gene is a frequent finding in these neoplasms, however, it is not clear whether this represents only an epiphenomenon of DNA nondiploidy or whether it constitutes a biologically important change with an impact on the clinical course of the disease. Similarly, alterations of the tumour suppressor gene, *p53,* which has been called the "guardian of the genome" [25], are frequent in ovarian carcinomas [5, 7, 13, 19], but little is known about the relation of *p53* and DNA nondiploidy in these neoplasms. To clarify these issues we applied DNA flow and image cytometry as well as PCR and immunohistochemistry to archival material of a series of sequential ovarian carcinomas with known postoperative outcome.

Our study, which is one of the largest DNA cytometrical analyses of ovarian carcinomas based on paraffin-embedded material, reveals a good correlation between flow and image DNA cytometry and confirms that DNA nondiploidy is of prognostic significance in ovarian carcinomas, as described repeatedly in the past [8, 9, 31, 33]. In

Table 5 Gene alteration and expression patterns of the four major histological types of ovarian carcinomas (see Fig. 3)[a]

	p53 accumulation	*MYC* amplification or *bcl-2* expression, no *p53* accumulation	No aberration	Total
Mucinous Ca (*n/n* cases)	12.5% (1/8)	50% (4/8)	37.5% (3/8)	100% (8/8)
Endometrioid Ca (*n/n* cases)	23.1% (3/13)	69.2% (9/13)	7.7% (1/13)	100% (13/13)
Serous Ca (*n/n* cases)	53.9% (21/39)	33.3% (13/39)	12.8% (5/39)	100% (39/39)
Undifferentiated Ca (*n/n* cases)	71.4% (5/7)	28.6% (2/7)	0% (0/7)	100% (7/7)

[a] $P=0.045$ by Fisher's exact test (two-tailed)

advanced tumour stages (FIGO II–IV) around two-thirds of ovarian carcinomas are nondiploid, as in our series [18, 34]. Presumably due to the smaller size of previous studies, an association of DNA nondiploidy with serous and undifferentiated tumour type and with high histological grade as demonstrated in the present study, has not been observed by all investigators (reviewed in [4]). In agreement with our observations, several studies have described a correlation between DNA nondiploidy and presence of residual disease after debulking surgery [8, 14 ,15]. Thus, in ovarian carcinomas DNA nondiploidy is apparently a good indicator of more aggressive, highly proliferative neoplasms containing multiple DNA alterations.

Our immunohistochemical data concerning the protein product of the tumour suppressor gene *p53* provide an explanation for the high frequency of DNA nondiploidy in this type of cancer. Positive results of *p53* immunohistochemistry reflect *p53* gene mutations and loss of *p53* function in the vast majority of ovarian carcinomas [22, 24]. Elimination of functional *p53* leads to loss of control at the G1-phase check point in the cell cycle and thus allows the proliferation of cells with DNA damage [25, 26]. By this mechanism increasing DNA instability is allowed and is evidenced in the development of nondiploid stem lines seen in DNA cytometry. We recently showed that p53 is a prognostic variable in ovarian carcinomas, providing additional information on FIGO stage both in stages I/II and in stages III/IV [13]. The relationship between *p53* and DNA ploidy will need to be explored in prospective studies of prognostic factors in ovarian carcinomas, to clarify whether DNA cytometry can be partially or totally substituted for by *p53* immunohistochemistry, which is much easier to perform.

Our study shows that quantitative multiplex PCR, which has also been used to detect deletions and duplications of the dystrophin gene [12] or allelic loss in colorectal cancer [11], can be used on paraffin-embedded tissue samples. Gilbert et al. [17] reported that a differential PCR assay (multiplex PCR) for *N-MYC* gene amplification provided data entirely consistent with Southern analysis, but is easy to perform, fast and sensitive. These authors have demonstrated that it is possible to quantitate the number of gene copies by PCR in an undiluted sample in a manner similar to quantitation of gene copy number by serial dilution with Southern analysis.

We found *MYC* copy number changes in 33.8% of cases, in good agreement with previous investigations showing *MYC* DNA amplification in 17–37.5% of ovarian carcinomas [1, 3, 6, 21, 27, 37, 38, 43]. Since *MYC* DNA amplification was seen with almost the same frequency in DNA-diploid and DNA-nondiploid cases, amplification of this oncogene apparently does not simply reflect DNA nondiploidy, but is an independent phenomenon. Furthermore, our data show that *MYC* DNA amplification is not related to a specific phase of tumour progression. This view is in agreement with the observations by Tashiro et al. [40], who also saw *myc* overexpression in all tumour stages as well as in one "borderline" case (tumour of low malignant potential).

In physiological situations there is a tight control of cell proliferation, differentiation and cell death by numerous factors, including *myc*, *p53* and *bcl-2* [35, 42]. With regard to such neoplasms as ovarian carcinomas, our data indicate that alterations of these factors are associated with a loss of correlation between *MYC*, *p53* and *bcl-2* and probably lead to a complex situation differing from case to case.

Obviously the pattern of oncogene and tumour suppressor gene alterations observed in the present study does not easily fit into a multistep model of ovarian carcinogenesis. However, our observations show that these patterns, as well as DNA ploidy, can be significantly correlated with the phenotypic variability of ovarian carcinomas. With regard to the main histological subtypes, two major groups apparently exist: a less malignant group (mucinous and endometrioid carcinomas) with rare *p53* alterations and a relatively large number of diploid cases, and a more aggressive and mostly nondiploid group (serous and undifferentiated carcinomas) with frequent *p53* alterations. Within the first group endometrioid carcinomas differ from mucinous carcinomas in their *bcl-2* predominance, whereas for mucinous carcinomas the more frequent *MYC* DNA amplification may be particularly characteristic. Furthermore, mucinous carcinomas are the least well characterized subgroup. In the genes under study we were unable to find any abnormality in 37.5% of cases in this group. The relatively large percentage of cases with *MYC* DNA amplification among these prognostically favourable subtypes might explain the trend towards a better prognosis for cases with *MYC* DNA amplification in survival analysis. In the second, more aggressive, group the undifferentiated carcinomas are further characterized by their high frequency of multiple oncogene abnormalities. This finding agrees with the observations by Katsaros et al. [21], who reported a particularly aggressive behaviour of those ovarian carcinomas that simultaneously demonstrate *MYC* DNA amplification and overexpression of the *erbB-2* and the *ras* genes.

In conclusion, although *MYC* DNA amplification is frequent in ovarian carcinomas, its biological and prognostic impact is apparently small compared with that of *p53* alterations or DNA nondiploidy; however, through interaction with other oncogenes and tumour suppressor genes, *MYC* DNA amplification may play a part in determination of the varying differentiation patterns of ovarian carcinomas.

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