MOLECULAR ONCOLOGY XXX (2011) 1–10

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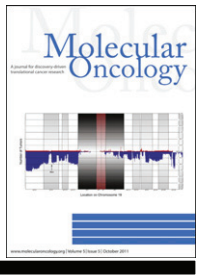

Genomic imbalances in endometrial adenocarcinomas – Comparison of DNA ploidy, karyotyping and comparative genomic hybridization

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ARTICLE INFO

Article history:
Received 6 September 2011
Received in revised form
12 October 2011
Accepted 12 October 2011
Available online ■

Keywords:
DNA ploidy
Comparative genomic hybridization
Karyotyping
Endometrial adenocarcinoma
Endometrioid
Aneuploidy

ABSTRACT

DNA ploidy analysis is useful for prognostication in cancer patients, but the genomic details underlying ploidy changes are not fully understood. To improve this understanding, we compared DNA ploidy status with karyotypic and comparative genomic hybridization data on 51 endometrial adenocarcinomas. Out of 34 DNA diploid tumors evaluated by CGH, 16 (47%) showed imbalances, though only two had more than four copy number changes. Ten (29%) had aberrations involving chromosome 1, seven (21%) involving chromosome 10, while one tumor had a chromosome 8 aberration. Four of the seven DNA tetraploid tumors (57%) had imbalances detected by CGH with two (29%) having more than four. Six out of eight DNA aneuploid tumors showed imbalances by CGH, with five (63%) having more than four. The aberrations were observed on chromosomes 1 and 8 in five/eight (63%) cases while four imbalances (50%) involved chromosomes 5, 7 and X. Not surprisingly, we observed a significant correlation between increasing DNA ploidy complexity and increasing number of copy alterations. Gains of material from chromosomes 8 and 7 might be specifically correlated to DNA aneuploidy in endometrial adenocarcinomas since 63% and 50% of the aneuploid compared to 3% of the diploid tumors showed imbalances involving these chromosomes.

1. Introduction

Assessment of DNA ploidy status has been shown to be clinically useful in the prognostic evaluation of patients with epithelial cancers, including gynecological cancers (Pisani et al., 1995; Kristensen et al., 2003; Terada et al., 2004). In general, patients with DNA diploid tumors have a more favorable outcome than do patients with DNA aneuploid tumors

Abbreviations: Endometrial adenocarcinoma, EAC; comparative genomic hybridization, CGH; average number of copy alterations, ANCA.

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doi:10.1016/j.molonc.2011.10.002

(Kristensen et al., 2003; Terada et al., 2004). Mostly, one does not know in any detail which genomic changes are behind the observed ploidy patterns, at least not on the same set of tumors, and we therefore undertook the present study to compare findings from DNA ploidy analyses with those obtained by comparative genomic hybridization (CGH) and karyotyping. Ideally, this might reveal patterns of genetic changes in diploid, tetraploid and aneuploid carcinomas that could shed some light on the mechanisms behind aneuploidization and polyploidization. So far the mechanisms are not fully understood, but a recent paper suggests that activation of oncogenes and inactivation of tumor suppressor genes could lead to aneuploidy (Solomon et al., 2011). Endometrial adenocarcinomas (EAC) are of two main subtypes (Bokhman, 1983). Type I carcinomas typically develop in peri-menopausal women, show mainly endometrioid differentiation and patients with these tumors generally have a favorable prognosis (Bokhman, 1983). The tumors frequently show mutations of DNA mismatch repair genes, *PTEN*, *KRAS* and *CTNNB1*. Type II carcinomas are typically characterized by DNA aneuploidy, *TP53* mutations and *ERBB2* amplifications (Lax et al., 2000), the tumors are of the serous, clear cell, and/or undifferentiated histological subtypes and the patients have a less favorable prognosis (Bokhman, 1983).

Contrary to non-endometrioid carcinomas where over 50% are non-diploid, most endometrioid EAC are DNA diploid (Prat, 2004; Pradhan et al., 2006). The DNA diploid tumors are often grade 1 or 2 carcinomas and the patients typically have longer survival than do patients with aneuploid carcinomas (Geisinger et al., 1986; Britton et al., 1989; van der Putten et al., 1989; Sorbe et al., 1990; Stendahl et al., 1991; Pisani et al., 1995; Terada et al., 2004; Pradhan et al., 2006; Susini et al., 2007). One report showed up to 91% 10-year disease free survival for patients with DNA diploid carcinomas, compared to 53% for DNA aneuploid carcinomas (Susini et al., 2007). For this study we specifically selected EAC of the endometrioid subtype because we primarily wanted to examine the pattern of acquired genomic aberrations in aneuploid but close to diploid tumor cell nuclei.

Cytogenetic studies of endometrioid EAC have shown many tumors to have hyperdiploid karyotypes with only few chromosomal aberrations, mostly partial or whole chromosome gains, although cases with complex karyotypes do exist (Sonoda et al., 1997; Suzuki et al., 1997; Pere et al., 1998; Suehiro et al., 2000; Mitelman et al., 2010). Often the aberrations involve chromosome 1 leading to gain of material from the long arm, followed by gains of or from chromosomes 2, 7, 10 and 12 (Mitelman et al., 2010). Also CGH analyses usually show only minor genomic imbalances in endometrioid EAC (Sonoda et al., 1997; Suehiro et al., 2000), the most common being gains from chromosomes 1, 3, 8, 10 and 20 and losses from chromosomes X, 4 and 13 (Sonoda et al., 1997; Suzuki et al., 1997; Pere et al., 1998; Suehiro et al., 2000). Our study is based on the karyotypic and CGH analyses performed by Micci et al. (2004) which showed that endometrioid EAC mostly harbor gains from chromosome arms 1q and 8q and losses from Xp, 9p, 9q, 17p, 19p and 19q. In that study, a gradually increasing number of aberrations from well to poorly differentiated type I carcinomas was seen (Micci et al., 2004).

2. Material and methods

The material consisted of paraffin embedded tissue samples from a consecutive series of 51 EAC of the endometrioid histological subtype surgically removed at The Norwegian Radium Hospital between 2000 and 2002. Eight of the 51 endometrioid tumors showed squamous differentiation. Tumors from six of the patients contained a component of another histological subtype (i.e., they were mixed type), and of these three had a component with mucinous differentiation, one had a clear cell component, one had serous papillary differentiation, and one had both a mucinous and a serous papillary component. There were 16 well, 20 moderately, and 15 poorly differentiated tumors, 27 were in FIGO Stage I, 10 in Stage II and 14 in Stage III.

DNA ploidy measurements were performed as previously described (Kristensen et al., 2003; Kildal et al., 2004). On average, 1087 (ranging from 259 to 1373) tumor cell nuclei were examined for each case. The mean coefficient of variation of the DNA diploid population was 2.92. Karyotyping and CGH had been performed previously, on fresh tissue from the same tumors, and the results of these analyses have been presented in Micci et al. (2004).

Concordance between CGH and DNA ploidy was defined as ≤four average number of copy alterations (ANCA), as measured by CGH in DNA diploid or tetraploid lesions, and above four ANCA in aneuploid lesions (Kildal et al., 2004).

Comparison of groups was performed by Fisher's exact test. P-values <0.05 were considered statistically significant.

Table 1 – Relationship between DNA ploidy classification and histological grade, FIGO stage and ANCA.[a]

	FIGO stage				Histological grade				ANCA		
	I	II	III	*p*-value[b]	Well	Moderate	Poor	*p*-value	<=4	>4	*p*-value
Diploid	19	6	11	0.730	14	16	6	0.021	32	2	<0.001
Tetraploid	4	1	2		0	2	5		5	2	
Aneuploid	4	3	1		2	2	4		3	5	

a Abbreviations: ANCA – average number of copy alterations, FIGO – International Federation for Obstetrics and Gynaecology.
b *p*-values from Fisher's exact test (2-sided).

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Table 2 – DNA ploidy, karyotypic data and genomic imbalances of 51 endometrial carcinomas of the uterine corpus.[a]

ID	Histological type	Grade	Stage	DNA Ploidy	CV	% diploid	DNA Index	*Karyotype* [number]	CGH imbalances	ANCA
1	Endometrioid/clear cell	L	IB	Tetraploid	2.6	62	1.99	46,XX [9]	No imbalances	0
2	Endometrioid	M	IB	Diploid	4.4	81		Failure	rev ish enh[b] 1(q)	1
3	Endometrioid	L	IIIC	Diploid	2.7	85		46,XX [7]	rev ish enh (1q23qter)	1
4	Endometrioid	W	IIA	Diploid	2.6	89		Failure	rev ish enh (1q), dim(13q14q34)	2
5	Endometrioid	W	IIA	Diploid	2.5	93		46,XX [11]	no imbalances	0
6	Endometrioid	W,M,L	IIIA	Tetraploid	2.5	68	1.93	Failure	rev ish enh (12q23q24)	1
7	Endometrioid	M	IC	Diploid	2.9	91		Failure	rev ish enh (1q32q42)	1
8	Endometrioid/squamous	W	IIA	Aneuploid	3.7	6	1.21	46,XX [5]	No imbalances	0
9	Endometrioid/mucinous	W	IIA	Diploid	1.9	83		46,XX [35]	No imbalances	0
10	Endometrioid	W	IIIA	Diploid	3.7	95		46,XX [2]	rev ish enh (1q,5q11q13,5q14q32)	3
11	Endometrioid	M	IC	Tetraploid	2.7	72	1.93	Failure	rev ish enh (20q13,22q13)	2
12	Endometrioid/squamous	W,L	IIIC	Diploid	3.6	87		Failure	No imbalances	0
13	Endometrioid	W	IIIA	Diploid	3.1	94		Failure	No imbalances	0
14	Endometrioid	W	IC	Diploid	2.8	86		Failure	No imbalances	0
15	Endometrioid	W,M	IC	Diploid	2.7	96		46,XX [7]	rev ish enh (10q24qter)	1
16	Endometrioid	M	IC	Diploid	3.0	93		48,XX,+del(1)(p13)x2[4]/46,XX[21]	No informative result	–
17	Endometrioid/squamous	W,M	IIIA	Diploid	3.0	93		47,XX,+i(1)(q10)[2]/46, XX [2]	rev ish enh (1q24q25)	1
18	Endometrioid	M	IIIC	Diploid	3.0	87		49~50,XX,+X,inc[5]	rev ish enh(7,10p11)	2
19	Endometrioid	W	IC	Diploid	2.7	85		46,XX,+1,der(1;15)(q10;q10)[2]/46,XX[46]	No DNA	–
20	Endometrioid	L	IC	Tetraploid	3.0	63	1.93	46,XX [12]	No imbalances	0
21	Endometrioid	W	IIA	Diploid	2.8	87		Failure	No imbalances	0
22	Endometrioid	M	IB	Diploid	2.5	79		46,XX [4]	No imbalances	0
23	Endometrioid/squamous	L	IIIC	Diploid	3.6	84		40~52,XX,-1,der(1;16)(q10;p10),+2, +6,t(11;18)(p15;q11), +12,+20,+r[4]/50~52, idem,add(4)(p13), +10,[3]/46,XX [1]	rev ish enh (1q24q31)	1
24	Endometrioid/mucinous and serous papillary	M	IB	Diploid	2.9	83		Failure	No imbalances	0
25	Endometrioid	L	IIIA	Tetraploid	2.2	71	1.96	46,XX [44]	No imbalances	0
26	Endometrioid	M	IIA	Diploid	3.0	83		46,XX [3]	No imbalances	0
27	Endometrioid	W	IC	Diploid	4.0	84		47,XX,+12[2]/46,XX[10]	rev ish enh(1q24q42,8p12p23, 8q12qter,10p13p15	4
28	Endometrioid	L	IIIA	Aneuploid	5.0	6	1.16 and 2.24	47~9,+2,add(7)(q22)x2,inc[10]/46,XX[4]	rev ish enh(Xp21pter, Xq13qter,1q,2p12pter, 2q14q21,2q22q37, 8p11p22,8q,12p)	9
29	Endometrioid/squamous	W	IIIA	Diploid	3.1	87		Failure	No imbalances	0
30	Endometrioid	M	IIB	Aneuploid	2.4	12	1.13 and 2.17	51,XX,add(3)(q26),+5,del(5)(q33)x2, +7,+7,+del(8)(q22),+12,add(22)q13)[14]/ 46,XX[4]	rev ish enh(5p14p15,5q11q32, 7p12pter,7q11qter,8q, 12p11p13,12q12,12q13q22), dim(3q27q29,5q35,17p13,22q13)	12

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Table 2 – (*continued*)

ID	Histological type	Grade	Stage	DNA Ploidy	CV	% diploid	DNA Index	*Karyotype* [number]	CGH imbalances	ANCA
31	Endometrioid	L	IIB	Tetraploid	4.2	68	1.96	Failure	rev ish enh(Xp21pter,Xq21q26, 1p22p31,1q21q41,2p16p25, 2q22q35,3p21pter,3q13qter, 4q22q31,5p,5q23,5q32q34, 6p12,8p11,8q,11p12p15, 11q13q25,12q21,13q22q31, 20q13,21q22),dim(1p36, 3p12p21,4p13pter,5q14, 6q16qter,7p14p21,7q31q33, 8p22pter,9,10q23qter,11p15, 12q23qter,13q12q21,13q34, 15q21q25,17p,17q11q12, 18q21qter,19p13,19q13), amp(3p23pter,8q12q24)	46
32	Endometrioid	M	IIIA	Diploid	4.5	85		46,XX [15]	rev ish enh(10q11qter), dim(19p13,19q13)	3
33	Endometrioid	M	IB	Diploid	2.4	85		46,XX [4]	No imbalances	0
34	Endometrioid	M,L	IIIA	Diploid	2.9	90		87~90,XXXX,+del(1)(p22p32)x2, inc[6]/46,XX[6]	rev ish enh (1q23q44)	1
35	Endometrioid/squamous	M,L	IC	Diploid	1.8	90		Failure	rev ish enh(10q24qter), dim(16p11,16q,19p13)	4
36	Endometrioid/serous papillary	L	IB	Aneuploid	1.9	3	1.62	45,XX,-2[6]/46,XX[39]	rev ish enh(1q25q41,2p,2q11q13,3p11p24, 3q,4p12p14,4q12q23,5p13p15, 5q23q31,6p11p25,6q12,7p14pter, 7q11q21,7q11q21,7q31qter, 8q21qter,10q21qter,13q12q21, 13q22qter,14q31q32,15q24qter, 20p11p13,20q11q12),dim(Xp11, Xq,2q37,4q31qter,5q12q13, 6q25qter,8p21p23,9p12p13, 9q34,16q23q24,17p11p13), amp(3q24qter)	35
37	Endometrioid/squamous	M	IB	Aneuploid	1.3	7	1.13 and 1.49	48,X,-X,+i(1)(q10), +7,+10[15]	rev ish enh(1q21qter, 7p13pter,7q11qter,8p12pter, 8q12q24,10p12pter,10q21q26), dim(Xp11)	8
38	Endometrioid	W	IC	Diploid	2.7	86		46,XX [24]	No imbalances	0
39	Endometrioid/mucinous	W	IC	Diploid	3.0	89		46,XX [26]	No imbalances	0
40	Endometrioid	W	IB	Diploid	3.9	92		46,XX [18]	rev ish enh(10)	1
41	Endometrioid	M	IIA	Diploid	2.6	80		46,XX [14]	rev ish enh(1q23qter,5q14q21,6q15q16), dim(4q34q35,9p12p13,9q,13q33p34)	7
42	Endometrioid	M,L	IC	Diploid	2.3	81		46,XX [3]	No imbalances	0

(*continued on next page*)

Table 2 – (*continued*)

ID	Histological type	Grade	Stage	DNA Ploidy	CV	% diploid	DNA Index	*Karyotype* [number]	CGH imbalances	ANCA
43	Endometrioid	L	IB	Aneuploid	3.6	3	1.61 and 3.23	62~75,i(8)(q10),inc[6]	rev ish enh(1p32p33,1q25q32, 2p13pter,2q23qter,3q,5p14pter, 6p,7q22q32,8p11p12,8q,10p13pter, 14q31qter,19p13,20p112,20q12q13), dim(Xp11p22,Xq12q13,Xq23q28, 4q32q34,5p12,5q11q22,9p12p21, 9q13q22,9q34,17p?,17q11q12, 17q21q24)	27
44	Endometrioid/squamous metaplasia	W	IC	Aneuploid	1.9	45	1.11	46,XX,add(5)(p11)[2]/46,XX[77]	No imbalances	0
45	Endometrioid	M	IB	Tetraploid	2.6	76	2	46,XX [29]	rev ish enh(7),dim(X,9,12,17)	5
46	Endometrioid	W	IB	Diploid	3.4	84		46,XX [21]	No imbalances	0
47	Endometrioid	M	IB	Diploid	3.1	83		46,XX [4]	No imbalances	0
48	Endometrioid	M	IB	Diploid	2.4	80		Failure	No imbalances	0
49	Endometrioid	M	IB	Diploid	3.6	85		46,XX [12]	rev ish enh(4p15,10p12p15, 10q11qter),dim(19p,19q13)	5
50	Endometrioid/mucinous	M	IIIC	Diploid	3.2	89		46,XX [34]	No imbalances	0
51	Endometrioid	M,L	IIB	Aneuploid	2.9	25	1.09	47,XX,+i(1)(q10)[4]	rev ish enh(1q25q32),dim(19q13)	2

a Abbreviations: ANCA – average number of copy alterations, L – low, M – middle, W – well, CV – coefficient of variation.
b rev ish auh (low level gains), rev ish amp (high level gains) and rev ish dim (losses).

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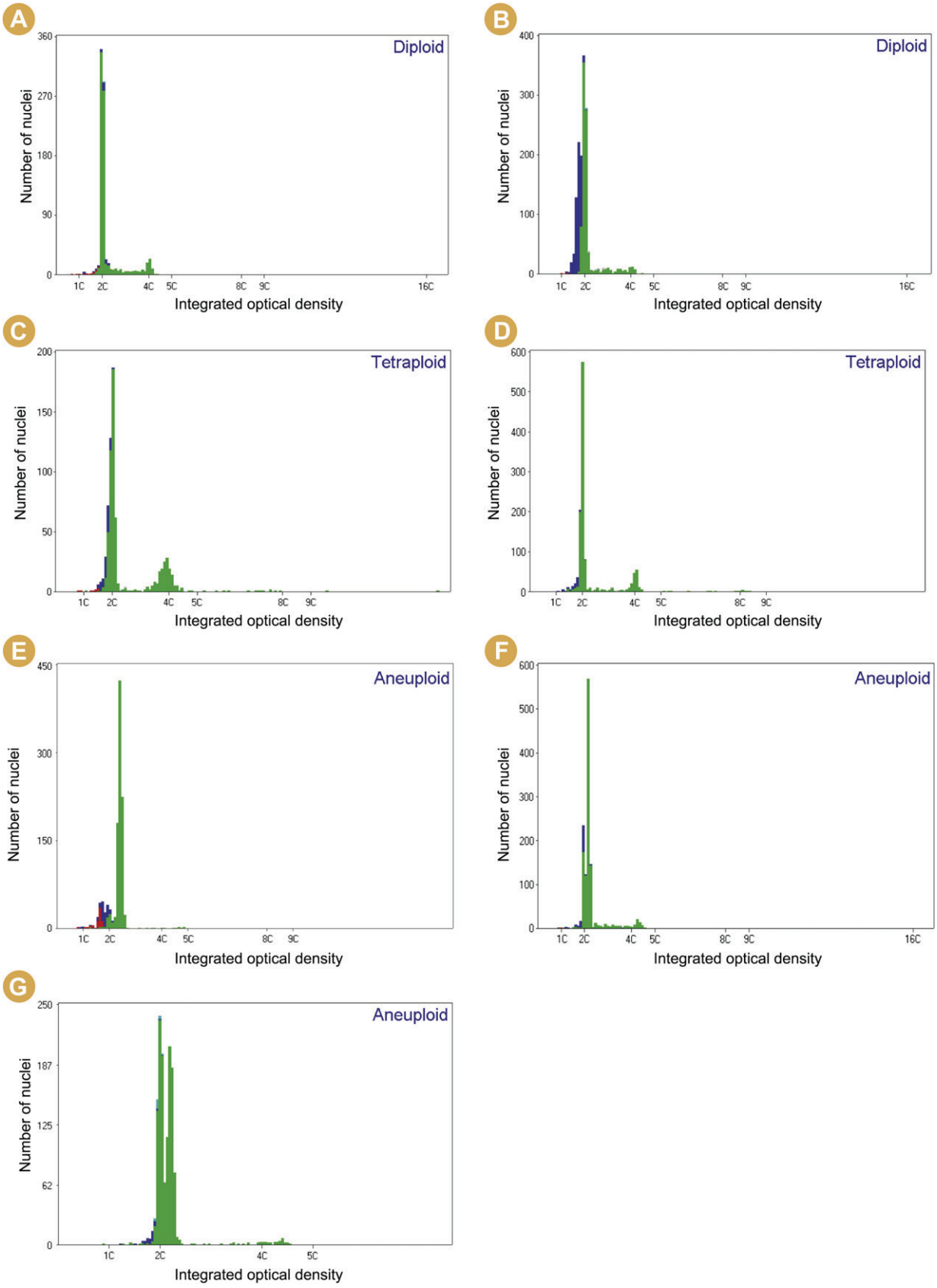

Figure 1 – Display of the DNA ploidy histograms that were discordant when comparing DNA ploidy and comparative genomic hybridization (CGH) data. A. Case 41: A DNA diploid histogram with a normal karyotype 46,XX[14], whereas the alterations rev ish enh (1q23qter, 5q14q21, 6q15q16), dim (4q34q35, 9p12p13,9q, 13q33p34) were detected by CGH. B. Case 49: A DNA diploid histogram, a normal karyotype 46,XX[12] and the CGH alterations rev ish enh (4p15, 10p12p15, 10q11qter), dim (19p, 19q13). C. Case 31: A DNA tetraploid histogram with the CGH

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Statistical software was used for all calculations (SPSS 15, SPSS, Chicago, IL).

The study has been approved by the Regional Ethics Committee.

3. Results

Thirty-six tumors (71%) were DNA diploid, seven (14%) were tetraploid and eight (16%) were aneuploid. There was a statistically significant correlation between DNA ploidy and histological grade ($p = 0.021$; Table 1), with increasing DNA ploidy complexity being associated with poorer differentiation, but not with increasing stage. Of the six tumors showing a mixed histology, the three with a mucinous component were all DNA diploid, the only tumor with a clear cell component was tetraploid, the tumor with a serous papillary component was aneuploid, and, finally, one DNA diploid tumor had components showing both mucinous and serous papillary differentiation.

Detailed results of the DNA ploidy classification, karyotyping and CGH analyses are presented in Table 2. There was good overall concordance between DNA ploidy and ANCA in 42 of 49 cases (86%). The seven discordant cases included three DNA aneuploid tumors with ≤four ANCA and two tetraploid tumors as well as two diploid tumors with ANCA above four (Figure 1). In the remaining two of the 51 cases, the CGH analyses were not informative and the samples showed a diploid profile. A significant correlation was observed between increasing DNA ploidy complexity and increasing ANCA ($p < 0.001$, Figure 2, $p = 0.010$; Table 1). DNA diploid cases had a median ANCA of 0 (range 0–7), while DNA tetraploid and DNA aneuploid cases had a median of 1 (0–46) and 8.5 (0–35) ANCA, respectively. Fisher's exact analyses showed a significant difference in ANCA between DNA diploid and aneuploid cases ($p = 0.005$), but not between DNA tetraploid and diploid ($p = 0.410$) or aneuploid ($p = 0.286$) cases.

Sixteen of the 34 (47%) diploid tumors analysed by CGH showed aberrations. Ten (29%) had aberrations on chromosome 1, seven (21%) on chromosome 10, while in only one case (3%) aberrations were observed on chromosome 8 (Figure 3). Four of seven tetraploid cases (57%) had one or more aberrations as shown by CGH; those were observed on nearly all chromosomes. CGH was successful on all eight DNA aneuploid cases showing aberrations on chromosome 1 and 8 in five cases each (63%), while four (50%) had aberrations on chromosomes 5, 7 and X. Five of the eight cases (63%) had more than four copy number changes.

The karyotypic data and DNA ploidy classification showed concordance in 25 of 37 cases (68%). In 14 cases (27%), culturing failed and thus no karyotype information was available. The concordant cases were either DNA diploid with a normal karyotype or aneuploid with an abnormal karyotype. The discordant cases included seven DNA diploid cases with an abnormal karyotype and four DNA tetraploid and one DNA aneuploid tumor with a normal karyotype. In three of the eight DNA aneuploid cases, there was approximate agreement between the karyotype and at least one of the populations detected by DNA ploidy. Finally, one discordant tetraploid case had an abnormal but not tetraploid karyotype. In five of seven discordant DNA diploid cases, the aberrations identified by karyotyping were small (involving 1–3 chromosomes).

In two cases, the results were discordant when comparing DNA ploidy analyses and both CGH and karyotyping. One case was DNA aneuploid (DNA index = 1.21) but with a normal karyotype and no CGH aberrations. The second case was DNA tetraploid, had above four ANCA, but had a normal karyotype.

4. Discussion

In the present study, three methods were used to identify the genomic changes in endometrioid EAC. Although there are large differences in resolution between DNA ploidy, CGH and karyotyping, we showed that these different measurements of genomic instability in EAC yield consistent results for the majority of the examined tumors. In the discordant cases, one could assume that at least some of the differences may have been due to tumor heterogeneity, since the same cells could never be subjected to the three different analyses. In fact, differences in DNA ploidy between curettage and hysterectomy specimens from the same patient have been shown by our group (Pradhan et al., 2010). We therefore performed DNA ploidy analyses on both curettage and hysterectomy specimens for 29 of the cases included in this study. In all these cases the DNA ploidy classifications were pairwise identical, indicating little tumor heterogeneity at least with respect to DNA ploidy in the present material (data not shown).

Of the seven cases that were discordant when comparing DNA ploidy analyses and CGH, the differences of three aneuploid cases with few copy number changes might in part be explained by the fact that they all had a near diploid DNA index (1.09–1.21), i.e., only a small deviation from the normal DNA content. CGH only detects major imbalances in the total DNA content. Accordingly, if a change does not appear in more than 50% of the test material or if there are many non-tumor parenchyma cells in the sample, imbalances may remain undetected. In one of these cases the selected area did in fact contain benign components. A previous study showed

alterations rev ish enh (Xp21pter, Xq21q26, 1p22p31, 1q21q41, 2p16p25, 2q22q35, 3p21pter, 3q13qter, 4q22q31, 5p, 5q23, 5q32q34, 6p12, 8p11, 8q, 11p12p15, 11q13q25, 12q21, 13q22q31, 20q13, 21q22), dim (1p36, 3p12p21, 4p13pter, 5q14, 6q16qter, 7p14p21, 7q31q33, 8p22pter, 9, 10q23qter, 11p15, 12q23qter, 13q12q21, 13q34, 15q21q25, 13q12q21, 13q34, 15q21q25, 17p, 17q11q12, 18q21qter, 19p13, 19q13), amp (3p23pter, 8q12q24). D. Case 45: A DNA tetraploid histogram with a normal karyotype 46,XX[29] and the CGH alterations rev ish enh (7), dim (X, 9, 12, 17). E. Case 8: A DNA aneuploid histogram with a DNA index of 1.20, whereas no changes were detected by either karyotyping or CGH. F. Case 51: A DNA aneuploid histogram with a DNA index of 1.09. The karotype was 47,XX,+i(1)(q10)[4]. The following imbalances were seen by CGH: rev ish enh (1q25q32) and dim (19q13). G. Case 44: A DNA aneuploid histogram with a DNA index = 1.1. The karyotype was 46, XX,add(5)(p11)[2]/46,XX[77]. No imbalances were detected by CGH.

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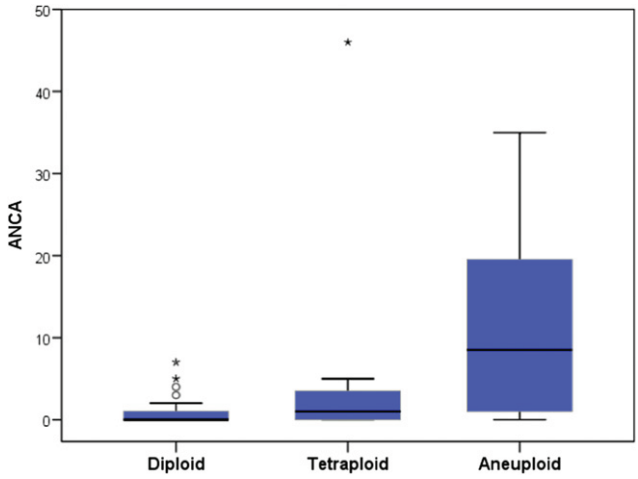

Figure 2 – Relationship between DNA ploidy status and average number of copy changes as measured by CGH. The filled circles represent outliers and the asterisk represents extreme outliers (Fisher's exact test $p = 0.010$).

a relationship between increasing proportion of aneuploid nuclei in a tumor and an increasing number of DNA copy number changes (Kildal et al., 2004), indicating that changes in small aneuploid populations might be overlooked by CGH. Finally, two of the discordant cases were DNA tetraploid with more than four ANCA (Figure 1). This observation indicates that these tumors were most likely aneuploid, but that the tumor populations had a DNA content near the double of the diploid and thus were not detectable by DNA ploidy. CGH does not detect balanced rearrangements, i.e., translocations, insertions, and inversions, as they do not lead to a relative difference in DNA content between tumor and normal DNA, nor will balanced DNA tetraploidy be detectable by CGH. On the other hand, our group previously observed significantly more DNA copy number changes in DNA tetraploid compared to DNA diploid lesions (Kildal et al., 2004), suggesting that some of these cases are actually aneuploid. Two cases showed diploid DNA ploidy classification and above four ANCA (Figure 1). We registered both gains and losses of DNA in these tumors, and this might explain why the changes were not detectable by DNA ploidy analyses.

Twelve of 37 cases (32%) were discordant when comparing DNA ploidy and karyotyping. In five of seven discordant DNA diploid cases the aberrations identified by karyotyping were small (involving 1–3 chromosomes only). These changes were not detected by DNA ploidy analyses because they were below the approximately 10% deviation from the diploid (2c) detection level of this method. In one case, only a translocation between chromosomes 1 and 15 was detected. In an aneuploid case with normal karyotype, the DNA index was near diploid and only a small aneuploid population was observed. Furthermore, the three DNA tetraploid cases were classified as such because of rather small tetraploid nuclei populations (4c) ranging from 13 to 20% and with a 5c exceeding rate (nuclei with DNA content above 5c) from 1.8 to 3.4%. Thus, a possible explanation for the discordances in these four cases might be that karyotyping is dependent on *in vitro* culturing and the dividing clone(s) may not be fully representative of

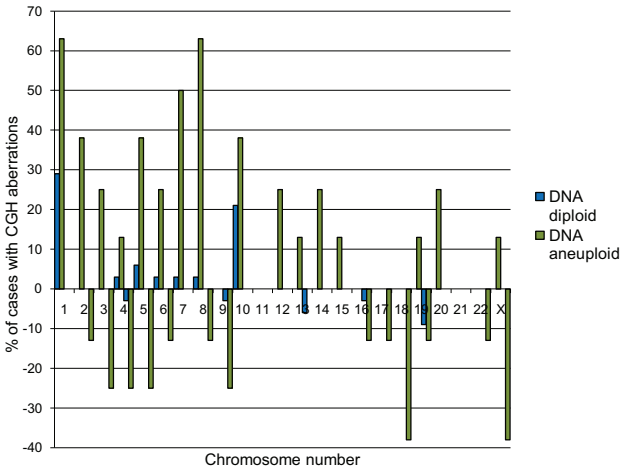

Figure 3 – Relationship between CGH imbalances and DNA ploidy. Blue bars show the percentage of CGH aberrations in DNA diploid cases, whereas CGH aberrations in DNA aneuploid cases are represented by green bars.

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the tumor cell population. Another disadvantage of karyotyping is the high rate of culture failure which we observed in 27% of the tumors.

The most frequent CGH findings in the DNA diploid tumors were gains from chromosome arm 1q and of parts of chromosome 10. Indeed, gains of 1q are already known as common, possibly even primary, changes in EAC (Milatovich et al., 1990; Micci et al., 2004). Our findings indicate that gains from chromosome 10 could be an early event in EAC as well. We observed that 1q and 10 gains were equally distributed among the DNA ploidy groups, in contrast to aberrations on chromosome 7 and 8 that were rare in DNA diploid tumors but frequent in DNA aneuploid tumors. There were also aberrations of chromosomes 5 and X, both gains and losses, in 50% of the DNA aneuploid cases. However, gains of chromosome 5 material were seen in two DNA diploid tumors as well.

Aberrations of chromosome arm 8q, especially leading to gain of material, are frequent in human cancers (Schulten et al., 2004), and for patients with EAC, lymph node metastasis is significantly associated with copy number gains at 8q (Suehiro et al., 2000). Studies have also shown that gains of 8q correlate with poor survival in patients with prostatic cancer (Ribeiro et al., 2006) and structural changes of chromosome 8 may be a predictor of shorter overall survival in patients with colorectal cancer (Bardi et al., 2004). In addition, the prognostic value of DNA ploidy in endometrial carcinoma has been extensively documented (Geisinger et al., 1986; Britton et al., 1989; van der Putten et al., 1989; Sorbe et al., 1990; Stendahl et al., 1991; Pisani et al., 1995; Nordstrom et al., 1996; Terada et al., 2004). These previous reports together with the findings presented here suggest that the association between DNA aneuploidy and aberrations on chromosome 8 and 7 might arise via an influence on the aggressiveness of the tumor. Unfortunately, we do not have follow up data on the patients in the present series.

None of the typical genes known to be elevated in EAC, like *PTEN*, *KRAS* and *CTNNB1*, are located on chromosomes 7 and 8. However, gain of 8q material could operate through gain of the oncogene *MYC* located at 8q24, a potential target gene for tumor progression. Needless to say, also copy number as well as regulatory changes at other chromosome 8 gene loci could be pathogenetically important in tumors displaying 8q alterations.

The present study confirms that DNA aneuploidy, as detected by image analysis, is linked to the chromosomal aberrations detected by CGH and karyotyping in a generally consistent manner. These three methods, which all assess genomic instability, should be viewed as complementary since they measure DNA changes at different levels of resolution. The use of all three methods together gives the most accurate results and therefore opens up for a better understanding of the genetic aberrations acquired by tumors in general, but for tetraploid tumors in particular.

Conflict of interest statement

None declared.

Acknowledgments

We are indebted to Monica Jenstad for valuable suggestions in the preparation of the manuscript and to Marian Seiergren for the preparation of Figure 1.

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