Histol Histopathol (2013) 28: 133-144
DOI: 10.14670/HH-28.133

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Phenotypic characterization of hereditary epithelial ovarian cancer based on a tissue microarray study

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Summary. The pathologic and immunohistochemical features of familial epithelial ovarian cancers are not well understood. We have carried out a comprehensive immunohistochemical study of familial ovarian carcinomas from women with and without *BRCA1* or *BRCA2* mutations, in order to identify specific and/or common features among these different familial case groups (BRCA1, BRCA2 and non-BRCA1/2) and to identify markers of diagnostic value that might help to select more specific treatments. 73 familial primary ovarian carcinomas were analyzed for the expression of 40 antibodies involved in different genetic pathways using a tissue microarray. Serous carcinomas comprised the majority of all three familial case groups. On the other hand, BRCA1 and BRCA2 carcinomas have similar histopathologic features; i.e. they are often high-grade and are usually diagnosed at a more advanced FIGO stage than non-BRCA1/2 carcinomas. In our series, BRCA1 carcinomas had better clinical evolution and they also more frequently over-expressed PR and P53 than BRCA2 and non-BRCA1/2carcinomas. Unsupervised cluster analysis and survival analysis identified ERCC1 as a potential marker of better clinical outcome for hereditary epithelial ovarian cancer.

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Key words: Hereditary, Epithelial ovarian cancer, Immunohistochemistry

Introduction

In Western countries, ovarian cancer (OC) is the leading cause of death from gynecological malignancy and is the fourth cancer-related cause of death among women, with an estimated worldwide prevalence of 192,000 cases per year (Hanna and Adams, 2006). Malignant epithelial tumors (carcinomas) are the most common ovarian cancers, accounting for 90% of cases (Auersperg et al., 2001). Histologically, epithelial ovarian tumors are classified into four major subtypes: serous, mucinous, endometrioid and clear cell tumors. Each subtype is further divided into benign, borderline and malignant depending upon the degree of cell proliferation (Cannistra, 2004). Due to the absence of early symptoms and the inadequacy of available screening methods, OC is often diagnosed at an advanced stage, resulting in a low survival rate under current treatments. Age at diagnosis, extent of disease, amount of residual disease after initial surgery, tumor grade, and tumor histological subtype are important clinical prognostic factors (Cannistra, 2004; Bristow et al., 2002) but the major risk factor is a family history of OC. In fact, the risk of developing the disease rises from 1.6% in the general population to 4% in women with a

first-degree relative with OC, and to 7% when two relatives are affected (Stratton et al., 1998; Werness and Eltabbakh, 2001). About 5-10% of OCs are hereditary and due to mutations in the *BRCA1* or *BRCA2* genes. In the Spanish population, the estimated average risk of developing OC is 22% for *BRCA1* mutation carriers and 18% for *BRCA2* mutation carriers (Milne et al., 2008).

Until now, only a few studies have analyzed the pathological and immunohistochemical characteristics of epithelial ovarian tumors in *BRCA1* and *BRCA2* mutation carriers and the results were not conclusive. In fact, the histopathologic features of OC associated with germline *BRCA1* and *BRCA2* mutations have not yet been well defined. Since germline *BRCA1* mutations are found four times more often than *BRCA2* mutations in patients with hereditary OC, most publications reporting correlations between clinical behavior and histopathologic features in familial OC include only *BRCA1* mutation carriers (Berchuck et al., 1998; Lakhani et al., 2004; Piver, 2002; Rubin et al., 1996). On the other hand, as far as we know, there are no publications about immunohistochemical characterization of epithelial ovarian tumors in familial cases without mutations in *BRCA1* or *BRCA2* (named non-*BRCA1/2* or *BRCAX*). High-grade serous carcinoma (HGSC) is the most common histological type occurring in patients with *BRCA1* mutations; it accounts for more than 90% of all cases, while it is found in about 60% of sporadic OCs (Berchuck et al., 1998). Mucinous tumors are uncommon in *BRCA1* mutation carriers (Piver, 2002), and other histopathologic subtypes, such as endometrioid and clear-cell carcinomas, are less frequent in *BRCA1* mutation carriers than in sporadic cases. OCs associated with germline *BRCA1* and *BRCA2* mutations are of high-grade and are diagnosed at a more advanced stage compared to sporadic ovarian tumors. In addition, they over-express p53 and show low HER2 expression (Lakhani et al., 2004). Although high grade and over-expression of p53 are generally recognized as unfavorable prognostic factors in sporadic cases, two publications have reported a better prognosis for BRCA1 mutation carriers with advanced OC compared with sporadic cases of the same stage (Rubin et al., 1996; Aida et al., 1998). It has been hypothesized that OCs associated with *BRCA1* and *BRCA2* mutations might have longer overall survival (OS) and progression-free survival (PFS) after chemotherapy because of the higher sensitivity of BRCA-deficient neoplastic cells to some cytotoxic agents. In fact, the BRCA1 and BRCA2 proteins are involved in the recognition and repair of DNA double-strand breaks produced by agents such as platinum compounds (Tagliaferri et al., 2009).

In the present study, we carried out a comprehensive immunohistochemical (IHC) study of familial OCs with and without mutations in *BRCA1* or *BRCA2* and correlated the results with survival data. We used a tissue microarray (TMA) to analyze the expression of 40 markers involved in different genetic pathways, with the aim of identifying specific and/or common features for each of the familial case groups (BRCA1, BRCA2, BRCAX) as well as diagnostic markers that might help to select more individualized and effective treatments.

Material and methods

Patients and samples

Patients were selected from high-risk families with breast and ovarian cancer (FBOC) fulfilling at least one of the following criteria: (i) at least three cases of breast or ovarian cancer in the same family line; (ii) at least two first-degree relatives diagnosed with breast cancer before age 50; (iii) at least one case of breast cancer and one case of ovarian or bilateral breast cancer in the same family line; (iv) at least one case of male breast cancer (Garcia et al., 2009). The index case of each family was screened for mutations in the *BRCA1* and *BRCA2* genes by a combination of denaturing high performance liquid chromatography (DHPLC) and sequencing. After genetic screening, we selected primary tumor samples from 44 index patients with mutations in *BRCA1* (28 tumors) or *BRCA2* (16 tumors), and from 34 patients without mutations in *BRCA1* or *BRCA2*. These were considered to represent three familial case groups, BRCA1, BRCA2 and BRCAX, respectively. Tumor samples from these patients were obtained from several centers in Spain. The required ethics committee approval was obtained, as well as informed consent from all participants in the study.

Morphological evaluation

Two pathologists (I.M-R. and J.P.), who had no knowledge of the germline mutation status or family history of participants, reviewed one representative histological slide from each ovarian tumor. All tumors were classified histopathologically and graded according to World Health Organization and FIGO criteria, respectively (Histological typing of ovarian tumours, 2004; Silverberg, 2000).

Tissue microarray construction

Representative areas of each tumor were selected on H&E-stained sections and marked on individual paraffin blocks. Two tissue cores (1-mm diameter) were obtained from each specimen. The tissue cores were arrayed into a new paraffin block using a TMA workstation (Beecher Instruments, Silver Spring, MD), as previously described (Palacios et al., 2003). Two TMAs were built, both with a spacing of 0.8 mm between cores. Samples included in duplicate in the first TMA were 22 BRCA1, 13 BRCA2 and 25 BRCAX carcinomas, and 5 BRCAX borderline tumors. In order to increase the statistical power of our study, we extended the set of samples to include 13 additional primary carcinomas (6 BRCA1, 3 BRCA2

and 4 BRCAX) collected while the IHC analysis was being conducted for the first step, and with these 13 tumors we constructed the second TMA. An H&E-stained section was reviewed to confirm the presence of morphologically representative areas of the original lesions.

Immunohistochemistry

We reviewed the scientific literature reporting IHC studies on the expression of different markers in sporadic epithelial ovarian tumors and we selected 36 markers involved in different genetic pathways:

Table 1. List of antibodies.

Antibody	Clone	Dilution	Supplier	Visualization System & Immunostainer	Threshold
ER-ALPHA FLEX	SP1	1:1	DAKO	En Vision FLEX/ DAKO Autostainer	>10%
PR FLEX	636 Mouse	1:1	DAKO	En Vision FLEX/ DAKO Autostainer	>10%
AR	AR441	1:100	DAKO	En Vision FLEX/ DAKO Autostainer	>10%
Ki-67 FLEX	MIB-1	1:1	DAKO	En Vision FLEX/ DAKO Autostainer	≥36% (median)
Topoisomerase IIα	Ki-S1	1:250	DAKO	En Vision FLEX/ DAKO Autostainer	IRS 4 (median)
P53 FLEX	DO-7	1:1	DAKO	En Vision FLEX/ DAKO Autostainer	>50%
E-Cadherin FLEX	NCH-38	1:1	DAKO	En Vision FLEX/ DAKO Autostainer	> 5% with strong staining
ß-Catenin	14	1:100	BD Transduction Lab	En Vision FLEX/ DAKO Autostainer	>5% with strong staining
G-Catenin	15	1:200	BD Transduction Lab	En Vision FLEX/ DAKO Autostainer	>5% with strong staining
IQGAP1	24/IQGAP1	1:75	BD Transduction Lab	En Vision FLEX/ DAKO Autostainer	Strong staining
Cyclin E	13AE	1:10	Novocastra	En Vision FLEX/ DAKO Autostainer	10%
Cyclin D1 FLEX	SP4 Rabbit	1:1	DAKO	En Vision FLEX/ DAKO Autostainer	10%
P27	57	1:1000	BD Transduction Lab	En Vision FLEX/ DAKO Autostainer	50%
P21 (WAF1)	EA10	1:10	Calbiochem	En Vision FLEX/ DAKO Autostainer	10%
P16	E6H4	1:1	MTM	En Vision FLEX/ DAKO Autostainer	IRS 6
RB	63-245	1:100	BD Pharmigen	En Vision FLEX/ DAKO Autostainer	>10%
Survivin	Polyclonal rabbit	1:1000	R&D Systems	En Vision FLEX/ DAKO Autostainer	10%
BCLXL	2H12	1:10	Zymed	En Vision FLEX/ DAKO Autostainer	IRS 6
BCL-2	124	1:1	DAKO	En Vision FLEX/ DAKO Autostainer	IRS ≥8
XPF	SPM228	1:2	Abcam	Vision Bio System/ Leica BOND MAX	80%
XPG	8H7	1:100	Neomarkers	Vision Bio System/ Leica BOND MAX	13% (median)
RAD50	13B3/2C6	1:300	Abcam	Vision Bio System/ Leica BOND MAX	80% with strong staining
RAD51	51RAD01	1:25	Neomarkers	En Vision FLEX/ DAKO Autostainer	5,5% (nuclear) and strong staining (cytoplasmic)
Chk2	DCS 270.1	1:25	Novocastra	Vision Bio System/ Leica BOND MAX	76,5% (mean)
ERCC1	D-10	1:50	Santa Cruz	Vision Bio System/ Leica BOND MAX	10-49% with strong staining or ≥50% with moderate/strong staining
CD105	4G11	1:50	Novocastra	Vision Bio System/ Leica BOND MAX	Any positive cell
VEGF	SP28	1:2	Abcam	Vision Bio System/ Leica BOND MAX	Strong staining
COX-2	SP21 Rabbit Monoclonal	1:1	Neomarkers	En Vision FLEX/ DAKO Autostainer	10% with strong staining
HER-2	Polyclonal	1:2000	DAKO	En Vision FLEX/ DAKO Autostainer	Complete strong membranous staining
EGFR	EGFR.113	1:10	Novocastra	En Vision FLEX/ DAKO Autostainer	Any positive cell
C-kit	Polyclonal rabbit	1:200	DAKO	En Vision FLEX/ DAKO Autostainer	10% with moderate/strong staining
TUBB3	TUJ1	1:500	Santa Cruz	En Vision FLEX/ DAKO Autostainer	10% with moderate/strong staining
KLK6	Polyclonal goat	1:25	R&D Systems	Vision Bio System/ Leica BOND MAX	Score mean ± standard deviation*
KLK7	Polyclonal goat	1:25	R&D Systems	Vision Bio System/ Leica BOND MAX	Score mean ± standard deviation*
EMA FLEX	E29	1:1	DAKO	En Vision FLEX /DAKO Autostainer	Moderate/strong staining
MMP7	SMP294	1:100	Abcam	Vision Bio System/ Leica BOND MAX	Any positive cell (nuclear) and Score mean ± standard deviation*
NM23	Polyclonal rabbit	1:8000	Santa Cruz	Vision Bio System/ Leica BOND MAX	Any positive cell (nuclear) and strong staining (cytoplasmic)
PIK3CA	C73F8	1:100	Cell Signaling	En Vision FLEX /DAKO Autostainer	Any positive cell (nuclear) and ≥50% (cytoplasmic)
CUL4A	Polyclonal rabbit	1:25	Cell Signaling	Vision Bio System/ Leica BOND MAX	≥82% (median)
PARK2	EP6684	1:50	Lifespan Biosciences	Vision Bio System/ Leica BOND MAX	Moderate/strong staining

ER: estrogen receptor; PR: progesterone receptor; AR: androgen receptor; RB: retinoblastoma 1; XPF: xeroderma pigmentosum complementation group F; XPG: xeroderma pigmentosum complementation group G; ERCC1: excision repair cross-complementing rodent repair deficiency, complementation group 1; VEGF: vascular endothelial growth factor; COX-2: cyclooxygenase 2; HER-2: human epidermal growth factor receptor-2; EGFR: epidermal growth factor receptor; TUBB3: ß-tubullin III; KLK6: kallikrein 6; KLK7: kallikrein 7; EMA: epithelial membrane antigen; MMP7: matrix metalloproteinase 7; PARK2: Parkinson protein 2. IRS: immunoreactive score (intensity of the staining - 0: no reaction; 1: weak; 2: moderate and 3: strong - and percentage of positive cells - 0: no positive cells; 1: <10% positive cells; 2: 10-50% positive cells; 3: 51-80% positive cells and 4: >80% positive cells - was scored. The final score was derived by multiplying the percentage of positive cells with staining intensity and ranged between 0 and 12). *T-test.

hormone receptors (ER, PR and AR), proliferation (p53, topoisomerase IIα and ki-67), cell cycle (cyclin D1, cyclin E, p21, p27, p16 and Rb), apoptosis (BCL-XL, Bcl-2 and survivin), cell adhesion (e-cadherin, ß-catenin, gamma-catenin and IQGAP1), tumor progression (KLK7, KLK6, EMA, MMP7, PIK3CA, cul4a and nm23), angiogenesis (CD105 and VEGF), drug target (HER2, c-kit, EGFR, COX-2, and ß-tubulin III), DNA repair (ERCC1, XPG, XPF, rad50, rad51, and CHEK2) and tumor suppression (PARK2) (Table 3). We also selected 4 additional markers (PIK3CA, IQGAP1, PARK2 and Rb) corresponding to genes located in regions with recurrent alterations, as determined by aCGH in a study carried out by our group using the same samples (data not published). The antibodies, dilutions, suppliers, visualization systems and immunostainers used are shown in Table 1. Between 100 and 150 cells per core were scored to determine the percentage of cells with positive nuclei, cytoplasm, or membrane, depending on the marker. Two pathologists (I.M-R. and J.P.) evaluated nuclear staining for estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), p53, ki-67, cyclins D1 and E, p27, p21, Rb, topoisomerase II·, survivin, rad50, rad51, XPF, XPG, CHEK2, ERCC1, EGFR, metalloproteinase 7 (MMP7), kallikrein 7 (KLK7), e-cadherin, ß-catenin, gamma-catenin, cul4a, PIK3CA and nm23; cytoplasmic staining for p16, BCL-XL, Bcl-2, survivin, kallikrein 6 (KLK6) and KLK7, EMA, VEGF, CD105, COX-2, ß-tubulin III, c-kit, MMP7, EGFR, e-cadherin, ß-catenin, gamma-catenin, rad51, PARK2, IQGAP1, PIK3CA and nm23; and membrane staining for HER2, EGFR, e-cadherin, ß-catenin and gamma-catenin. The thresholds used to determine over-expression of each marker (Lin et al., 2001; Schmandt et al., 2003; Ni et al., 2004; Raspollini et al., 2004; Honrado et al., 2005; Brun et al., 2008; Tangjitgamol et al., 2009; Xia et al., 2009) are listed in Table 1. The percentage of stained nuclei, independent of the intensity, was scored for ER, PR, AR, ki-67, p53, cyclin D1, cyclin E, p27, p21, Rb, rad51, XPF, XPG, CHEK2, ERCC1, EGFR, MMP7, KLK7 and cul4a. To evaluate EGFR, e-cadherin, ß-catenin and gamma-catenin expression, the percentage of cells with membrane staining and staining intensity were

Table 2. Comparative analysis of clinicopathological features of BRCA1, BRCA2 and BRCAX epithelial ovarian carcinomas.

CLINICOPATHOLOGICAL FEATURES	BRCA1=28 (38.4%) n (%)	p†	BRCA2=16 (21.9%) n (%)	p‡	BRCAX=29 (39.7%) n (%)	p§
MEAN AGE	50.27±8.57	0.029**	58.23±10.36	0.026**	49.78±10.13	NS**
HISTOLOGICAL CLASSIFICATION						
Serous carcinoma	24 (84%)	NS*	13 (82%)	NS*	20 (69%)	NS*
Endometrioid carcinoma	2 (8%)	NS*	0	NS*	3 (10%)	NS*
Clear cell carcinoma	1 (4%)	NS*	1 (6%)	NS*	1 (3%)	NS*
Mucinous carcinoma	0	NS*	0	NS*	2 (7%)	NS*
Mixed carcinoma	0	NS*	0	NS*	1 (3%)	NS*
Undifferentiated carcinoma	1 (4%)	NS*	2 (12%)	NS*	2 (7%)	NS*
GRADE						
1-2	10 (36%)	NS*	6 (36%)	0.0419*	20 (69%)	0.0007*
3	27 (60%)	NS*	10 (64%)	0.0419*	9 (31%)	0.0007*
NA	1 (4%)		0		0	
FIGO STAGE						
I-II	8 (29%)	NS*	4 (24%)	0.0495*	16 (55%)	0.0382*
III-IV	20 (71%)	NS*	12 (76%)	0.0495*	13 (45%)	0.0382*
NA	0		0			
BILATERAL						
Yes	16 (55%)	NS*	5 (32%)	NS*	15 (52%)	NS*
No	8 (29%)	NS*	6 (36%)	NS*	7 (24%)	NS*
NA	4 (16%)		5 (32%)		7 (24%)	
ASCITES						
Positive	5 (18%)	NS*	3 (19%)	NS*	8 (27%)	NS*
Negative	4 (16%)		2 (12%)		4 (14%)	
NA	19 (66%)		11 (69%)		17 (59%)	
COMPLETE REMISSION						
Yes	11 (39%)	NS*	4 (25%)	NS*	10 (34.5%)	NS*
No	3 (11%)	NS*	2 (12.5%)	NS*	4 (13.8%)	NS*
NA	14 (50%)		10 (62.5%)		15 (51.7%)	
PFS (months)	38.73±11.42	0.048**	16.67±4.25	0.711**	17.33±5.48	0.048**
OS (months)	102.05±13.5	0.51**	69.92±6.36	0.376**	99.26±15.92	0.857**

*: Fisher´s exact test; **: Student's t-test; †: P value for comparison between BRCA1 and BRCA2 carcinomas; ‡: P value for comparison between BRCA2 and BRCAX carcinomas; §: P value for comparison between BRCA1 and BRCAX carcinomas; NS: not statistically significant; NA: not available.

evaluated. HER2 was scored according to current ASCO/CAP criteria. A tumor was considered to have preserved expression of e-cadherin, ß-catenin and gamma-catenin when >5% of the cells showed strong continuous membrane staining (Voutilainen et al., 2006). Other cases were considered to have reduced e-cadherin, ß-catenin or gamma-catenin expression. Cases were also evaluated for aberrant cytoplasmic or nuclear expression of these markers, as reported for sporadic cases.

Statistical methods

Hierarchical unsupervised cluster analysis was performed using the "Cluster" software, adjusting by the mean center of genes and non-centered correlation distance of genes and arrays (http://bonsai.hgc.jp/~mdehoon/software/cluster/software.htm). The clustering was visualized using Java TreeView (http://jtreeview.sourceforge.net/). IHC results for each

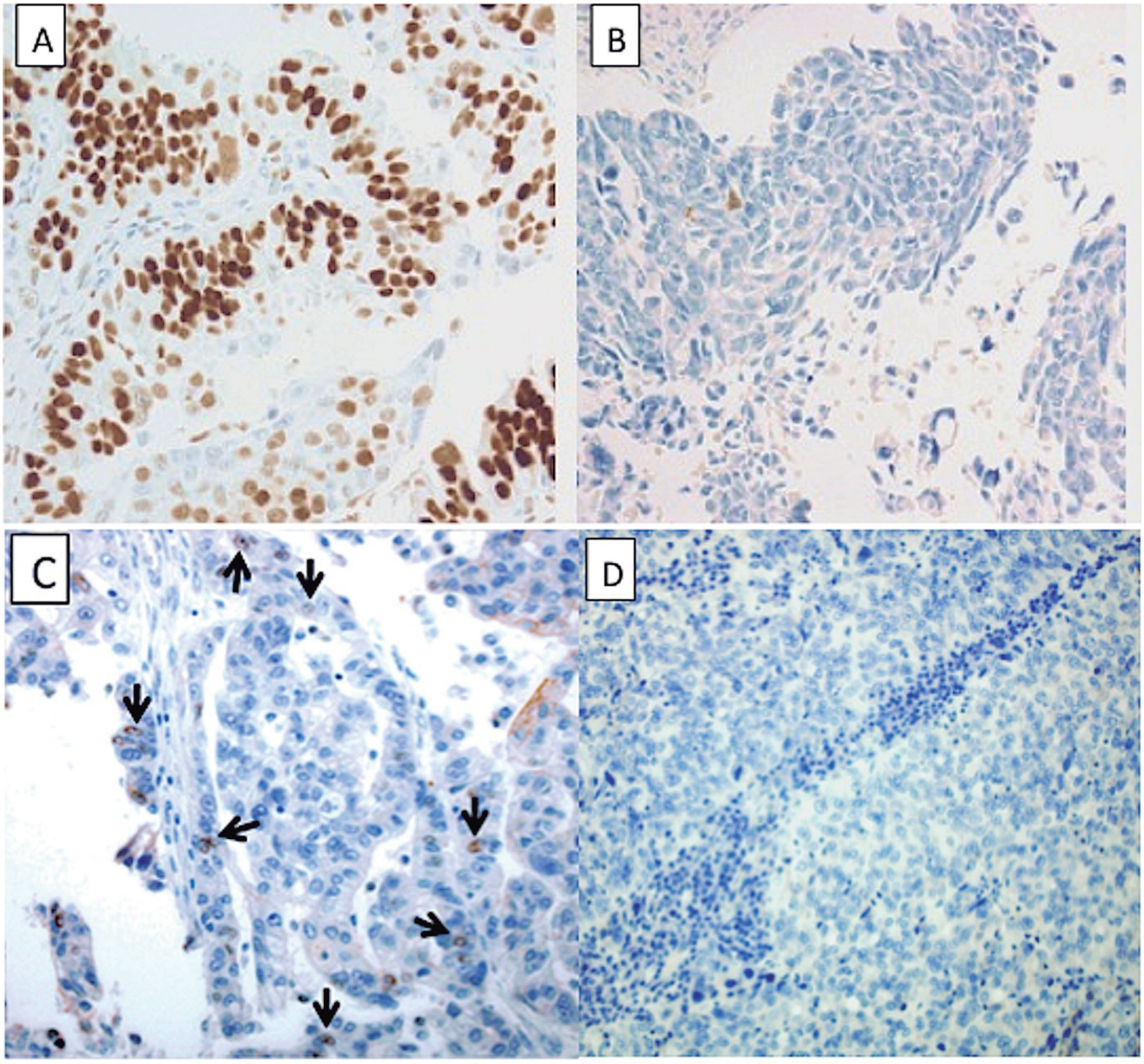

Fig. 1. Immunohistochemical staining for PR and nuclear EGFR in BRCA1 and BRCA2 carcinomas. Over-expression of PR is observed in the BRCA1 tumor **(A)** and there is low/null expression in the BRCA2 case **(B)**. Positive nuclear EGFR staining* in a BRCA1 carcinoma (**C** – black arrows) and negative in a BRCA2 carcinoma **(D)**. PR: progesterone receptor; EGFR: epidermal growth factor receptor. *Tumor is considered positive for nuclear expression of EGFR if there is any positive cell (Lin et al., 2001). x 200

marker were represented by a color scale from green to red, representing the lowest to highest scoring, respectively; tumours with no defined score were represented in white. This score was the percentage of positive cells for all markers, except the following: p16, KLK6, KLK7, VEGF and PARK2, which were scored as 0% for negative tumors, 33% for low intensity, 66% for moderate intensity, and 100% for strong intensity staining tumors; AR, EGFR, CD105, COX-2, ß-tubulin III, ERCC1, PIK3CA, IQGAP1 and nm23 which were scored as 0% for negative and 100% for positive tumors; e-cadherin, ß-catenin and gamma-catenin for tumors that showed loss of expression on the cytoplasmic membrane which were scored as 0% and those with preserved expression were scored as 100%.

Differential expression of markers between branches was determined by fitting linear models with the limma package (Smyth, 2004) using the Pomelo II web tool (Morrissey and Diaz-Uriarte, 2009). To account for multiple hypothesis testing, the estimated significance level (p value) was adjusted using the Benjamini & Hochberg False Discovery Rate (FDR) method. Markers with an FDR <0.05 were considered differentially expressed between branches. To compare the different histopathologic and IHC characteristics among the three familial ovarian cancer groups, student's t-test was used for continuous variables and Fisher´s exact test for categorical variables.

Survival analysis with respect to PFS and OS was performed using a log-rank test. PFS was calculated from the date of primary surgery to the date of disease progression as specified by a rise in CA125 or radiological or surgical evidence of relapse. The length of OS was defined from the date of primary laparotomy to the date of patient death. For both analyses, analysis time was censored at the date of last follow-up. Response to chemotherapy was evaluated retrospectively according to the World Health Organization evaluation criteria (Miller et al., 1981). This evaluation was based on data from medical records describing patients´ clinical condition and CA125 levels at 3–4 week intervals. Complete remission (CR) was defined as the disappearance of all clinical and biochemical symptoms of ovarian cancer evaluated after completion of first-line chemotherapy and confirmed at 4 weeks.

SPSS 17.0 for Windows (SPSS Inc., Chicago, IL) was used to conduct these statistical analyses, unless otherwise stated. Statistical tests were two-sided and nominal p values less than 0.05 were considered statistically significant.

Results

Morphological and clinicopathological features

The mean age at diagnosis for the BRCA1, BRCA2, and BRCAX familial case groups was 50.3, 58.2, and 49.8 years, respectively (Table 2). Familial cases with BRCA2 mutations were diagnosed at a later age than BRCA1 and BRCAX mutation carriers (p=0.029 and p=0.026, respectively). SC was the most prevalent histological subtype in each group comprising 84%, 82%, and 69% of BRCA1, BRCA2 and BRCAX cases respectively. We identified 5 (17%) borderline tumors among our BRCAX series (4 serous and 1 mucinous tumor; data not shown), which were excluded from further analyses.

We did not observe any differences in the morphological and clinicopathological features between BRCA1 and BRCA2 carcinomas (Table 2). When BRCA1 and BRCAX carcinomas were compared, statistically significant differences were seen for tumor grade and FIGO stage: grade 3 carcinomas were more frequent in the BRCA1 group (60% versus 31%, p=0.0007) as were advanced stage neoplasms (71% versus 45%, p=0.038). When BRCA2 and BRCAX carcinomas were compared, we also found statistically significant differences for tumor grade and FIGO stage: the BRCA2 group more often had grade 3 (64% versus 31%, p=0.042) and advanced stage neoplasms (76% versus 45%, p=0.049).The frequency of bilateral tumors was similar in the three groups and no differences were found regarding the presence or absence of ascites.

Immunohistochemistry

We analyzed IHC expression of 40 markers belonging to several genetic pathways. As a first step, the TMA containing 22 BRCA1, 13 BRCA2 and 25 BRCAX carcinomas was analyzed for all 40 markers (Table 3). When BRCA1 and BRCA2 carcinomas were compared, 3 markers showed statistically significant differences in expression: PR and cyclin D1 were more often over-expressed in BRCA1 than in BRCA2 carcinomas, and nuclear EGFR expression was also more frequent in the first group of carcinomas (p=0.033, p=0.043 and p=0.035, respectively) (Fig. 1). In addition, BRCA1 carcinomas tended to over-express p53 and BCL-XL more often than BRCA2 carcinomas (p=0.12, p=0.054, and p=0.078, respectively) while BRCA2 carcinomas tended to have more frequent cytoplasmic over-expression of MMP7 (p=0.096). We also compared BRCA1 and BRCAX carcinomas and 3 markers showed statistically significant differences in their expression: PR, p53 and IQGAP1 were more often over-expressed in BRCA1 than in BRCAX (p=0.009, p=0.007 and p=0.003, respectively). There was weaker evidence of more frequent cytoplasmic over-expression of e-cadherin in BRCAX carcinomas than in the BRCA1 group (p=0.07). When we compared BRCA2 and BRCAX carcinomas, 3 out of the 40 markers showed differences in their level of expression. BCL-XL over-expression and positive nuclear EGFR expression were more frequent in BRCAX than in BRCA2 carcinomas (p=0.043 and p=0.038, respectively). In contrast, cytoplasmic MMP7 was more highly expressed in BRCA2 carcinomas (p=0.024). In addition, BRCA2 carcinomas tended to over-express ki-67 more often than BRCAX carcinomas (p=0.086).

As a second step, and in order to increase the

statistical power, we evaluated in a second TMA with 13 additional primary carcinomas (6 BRCA1, 3 BRCA2 and 4 BRCAX) the expression of the reduced set of the IHC markers with significant p values (PR, p53, ki-67, cyclin D1, BCL-XL, nuclear EGFR and cytoplasmic MMP7). The complete set of 73 carcinomas (first and second steps) showed that several of the previously observed associations remained statistically significant (Table 4): for example, PR over-expression remained more frequent in BRCA1 carcinomas than in both BRCA2 and BRCAX carcinomas, although this difference was only statistically significant between BRCA1 and BRCAX carcinomas (p=0.015) and marginal between BRCA1 and BRCA2 carcinomas (p=0.054). We also confirmed a higher frequency of p53 over-expression in BRCA1 than in BRCAX carcinomas (p=0.013) and a higher frequency of ki-67 over-expression in BRCA2 than in BRCAX carcinomas (p=0.025). Finally, positive nuclear EGFR expression was more frequent in BRCA1 and BRCAX than BRCA2

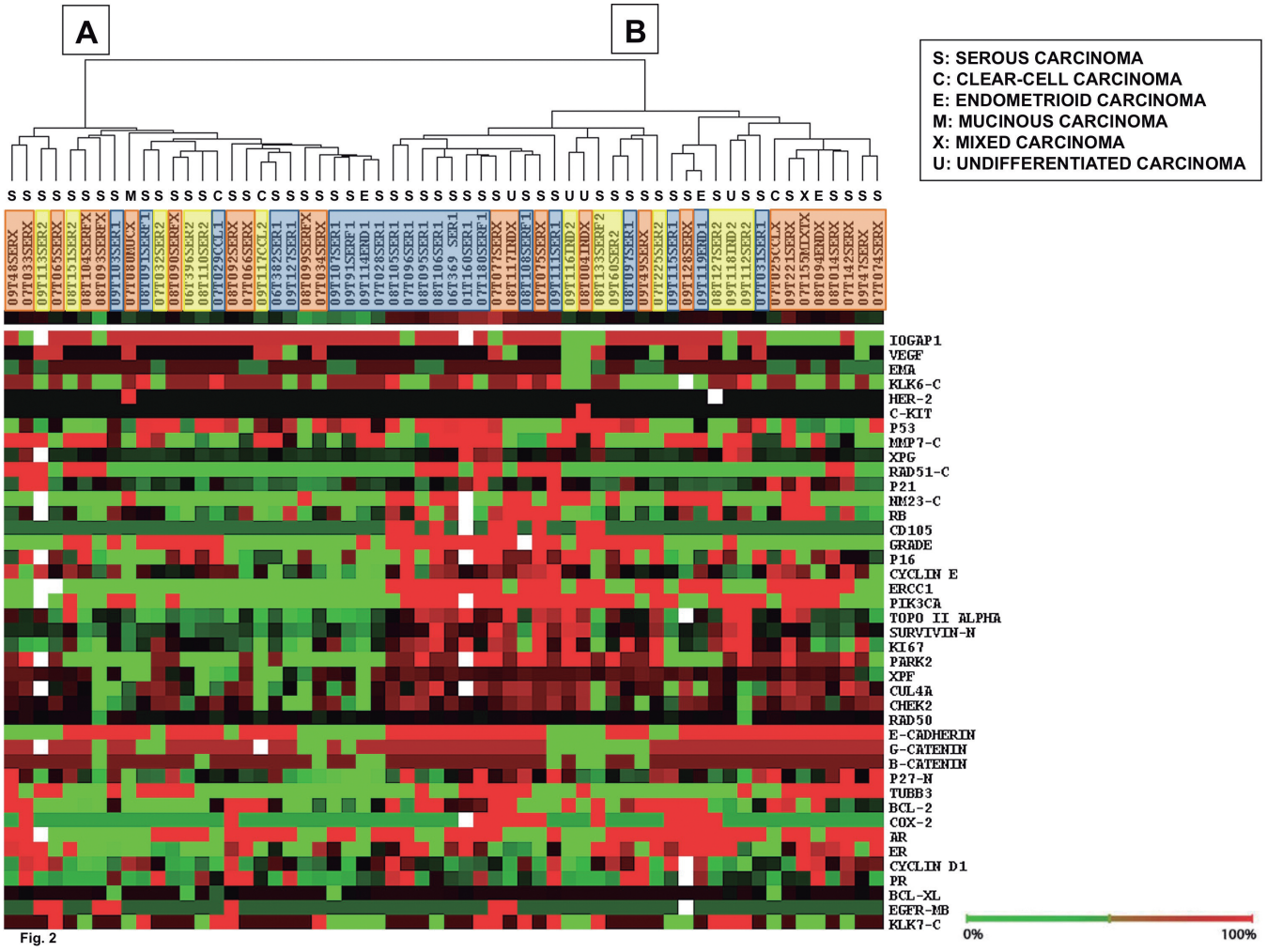

Fig. 2

Fig. 2. Unsupervised hierarchical clustering of familial carcinomas. Two main branches **(A and B)** are mainly clustered by ERCC1 expression. Branch A (left) shows negative expression for ERCC1 and branch B (right) positive expression. Heterogeneous distribution of phenotypes (BRCA1 - blue rectangles -; BRCA2 - yellow rectangles - and BRCAX - orange rectangles -), histological subtypes and histological grade are observed. Carcinomas grouped in branch B, also mainly show a significantly higher expression of PIK3CA, PARK2, nuclear survivin, topoisomerase II·, and XPF, whereas branch A carcinomas show a significantly higher expression of IQGAP1 and loss of expression of e-cadherin. Red indicates positive expression, green indicates negative expression, and the intensity of the color is a function of the immunohistochemical expression level. White indicates undetectable expression. VEGF: vascular endothelial growth factor; EMA: epithelial membrane antigen; KLK6-C: cytoplasmic expression of kallikrein 6; HER-2:human epidermal growth factor receptor-2; MMP7-C: cytoplasmic expression of matrix metalloproteinase 7; XPG: xeroderma pigmentosum complementation group G; RB: retinoblastoma 1; ERCC1: excision repair cross-complementing rodent repair deficiency, complementation group 1; TOPO II ALPHA: topoisomerase II alpha; SURVIVIN-N: nuclear expression of survivin; PARK2: Parkinson protein 2; XPF: xeroderma pigmentosum complementation group F; P27-N: nuclear expression of p27; TUBB3: ,-tubullin III; COX-2: cyclooxygenase 2; AR: androgen receptor; ER: estrogen receptor; PR: progesterone receptor; EGFR-MB: membranous expression of epidermal growth factor receptor; KLK7-C: cytoplasmic expression of kallikrein 7.

Table 3. Comparative immunohistochemical analysis of the expression of 40 markers in the first set of BRCA1, BRCA2, and BRCAX carcinomas (60 carcinomas).

MARKER		BRCA1 n (%)	p†	BRCA2 n (%)	p ‡	BRCAX n (%)	p§
ER	Positive	14/22 (63%)	NS*	9/13 (69%)	NS*	16/25 (64%)	NS*
PR	Positive	17/22 (77%)	0.033*	5/13 (39%)	NS*	9/25 (36%)	0.009*
AR	Positive	15/22 (68%)	NS*	9/13 (69%)	NS*	14/25 (56%)	NS*
P53 (≥50%)	Positive	18/22 (81%)	NS (0.123*)	7/13 (53%)	NS*	10/25 (40%)	0.007*
TOPOISOMERASE II ALPHA (Median IRS)	Positive	2/22 (9%)	NS*	4/13 (30%)	NS*	5/25 (20%)	NS*
KI-67 (MEDIAN %)	Positive	14/22 (63%)	NS*	10/13 (77%)	NS (0.086*)	11/25 (44%)	NS*
CYCLIN D1 (10%)	Positive	19/22 (86%)	0.05*	7/13 (53%)	NS*	18/25 (72%)	NS*
CYCLIN E (10%)	Positive	20/22 (90%)	NS*	12/13 (92%)	NS*	24/25 (96%)	NS*
P21 (10%)	Positive	15/22 (68%)	NS*	6/13 (47%)	NS*	16/25 (64%)	NS*
P27 (50%)	Positive	8/22 (36%)	NS*	2/13 (15%)	NS*	6/25 (24%)	NS*
RB (10%)	Positive	17/22 (77%)	NS*	9/13 (69%)	NS*	22/25 (88%)	NS*
P16	Positive (IRS SCORE 7-12)	10/22 (45%)	NS*	7/13 (53%)	NS*	12/25 (48%)	NS*
BCL2 IRS SCORE	Low (0-1)	6 (27%)	NS	6/13 (47%)	NS	7/25 (28%)	NS
	Medium (2-6)	6 (27%)	NS	3/13 (23%)	NS	7/25 (28%)	NS
	High (8-12)	10 (46%)	NS	4/13 (30%)	NS	11/25 (44%)	NS
BCL-XL IRS SCORE	Low (0-6)	9/22 (40%)	NS (0.078*)	10/13 (77%)	0.043*	10/25 (40%)	NS*
	High (7-12)	13/22 (59%)	NS (0.078*)	3/13 (23%)	0.043*	15/25 (60%)	NS*
SURVIVIN NUCLEAR (≥10%)		16/22 (72%)	NS*	9/13 (69%)	NS*	17/25 (68%)	NS*
SURVIVIN CYTOPLASMIC (≥10%)		14/22 (63%)	NS*	9/13 (69%)	NS*	16/25 (64%)	NS*
E-CADHERIN CYTOPLASMIC	Positive	5/22 (22%)	NS*	4/13 (30%)	NS*	13/25 (52%)	NS (0.07*)
E-CADHERIN NUCLEAR	Positive	0/22	NS*	0/13	NS*	0/25	NS*
E-CADHERIN MEMBRANE	Preserved	16/22 (72%)	NS*	8/13 (61%)	NS*	18/25 (72%)	NS*
ß-CATENIN CYTOPLASMIC	Positive	7/22 (31%)	NS*	5/13 (39%)	NS*	11/25 (44%)	NS*
ß-CATENIN NUCLEAR	Positive	1/22 (4%)	NS*	1/13 (7%)	NS*	3/25 (12%)	NS*
ß-CATENIN MEMBRANE	Preserved	19/22 (86%)	NS*	11/13 (84%)	NS*	21/25 (84%)	NS*
G-CATENIN CYTOPLASMIC	Positive	11/22 (50%)	NS*	5/13 (39%)	NS*	15/25 (60%)	NS*
G-CATENIN NUCLEAR	Positive	3/22 (13%)	NS*	1/13 (7%)	NS*	3/25 (12%)	NS*
G-CATENIN MEMBRANE	Preserved	18/22 (81%)	NS*	7/13 (53%)	NS*	20/25 (80%)	NS*
KALLIKREIN 7 NUCLEAR	Positive	7/22 (31%)	NS*	5/13 (39%)	NS*	14/25 (56%)	NS*
KALLIKREIN 7 SCORE MEAN ± SD		5.59±2.66	NS**	6.46±2.06	NS**	5.4±2.29	NS**
KALLIKREIN 6 SCORE MEAN ± SD		5.55±2.55	NS**	4.38±2.9	NS**	4.71±2.01	NS**
EMA	0	0/22	NS*	0/13	NS*	1/25 (4%)	NS*
	1	0/22	NS*	1/13 (7%)	NS*	0/25	NS*
	2	4/22 (18%)	NS*	5/13 (39%)	NS*	9/25 (36%)	NS*
	3	18/22 (81%)	NS*	7/13 (53%)	NS*	15/25 (60%)	NS*
High level expression (2-3)		22/22 (100%)	NS*	12/13 (92%)	NS*	26/25 (96%)	NS*
MMP7 NUCLEAR	Positive	7/22 (31%)	NS*	6/13 (46%)	NS*	9/25 (36%)	NS*
MMP7 CYTOPLASMIC (MEAN ± SD)		81.93±66.36	NS (0.096**)	135.38±119.25	0.024**	65.5±64.77	NS**
CD105	Positive	3/22 (13%)	NS*	1/13 (7%)	NS*	4/25 (16%)	NS*
VEGF	Low	19/22 (86%)	NS*	10/13 (77%)	NS*	20/25 (80%)	NS*
	High	3/22 (13%)	NS*	3/13 (23%)	NS*	5/25 (20%)	NS*
HER-2	Positive	0/22	NS*	0/13	NS*	1/25 (4%)	NS*
C-KIT	Positive	0/22	NS*	0/13	NS*	1/25 (4%)	NS*
EGFR MEMBRANE	Positive	1/22 (4%)	NS*	1/13 (7%)	NS*	5/25 (20%)	NS*
EGFR NUCLEAR	Positive	14/22 (63%)	0.035*	3/13 (23%)	0.038*	15/25 (60%)	NS*
EGFR CYTOPLASMIC	Positive	6/22 (27%)	NS*	5/13 (39%)	NS*	11/25 (44%)	NS*
COX-2	Positive	4/22 (18%)	NS*	4/13 (30%)	NS*	6/25 (24%)	NS*
TUBB3 (10% + INT 2-3)	Positive	6/22 (27%)	NS*	7/13 (53%)	NS*	13/25 (52%)	NS*
ERCC1	Positive	10/22 (45%)	NS*	3/13 (23%)	NS*	11/25 (44%)	NS*
XPG	Positive	4/22 (18%)	NS*	3/13 (23%)	NS*	3/25 (12%)	NS*
XPF	Positive	18/22 (81%)	NS*	11/13 (84%)	NS*	19/25 (76%)	NS*
RAD50 (% x Intensity)	Positive	18/22 (81%)	NS*	9/13 (69%)	NS*	17/25 (68%)	NS*
RAD51 CYTOPLASMIC	Positive	6/22 (27%)	NS*	2/13 (15%)	NS*	8/25 (32%)	NS*
RAD51 NUCLEAR	Positive	9/22 (40%)	NS*	4/13 (30%)	NS*	10/25 (40%)	NS*
CHEK2	Positive	17/22 (77%)	NS*	8/13 (61%)	NS*	20/25 (80%)	NS*
IQGAP1	Positive	20/22 (90%)	NS*	9/13 (69%)	NS*	14/25 (56%)	0.003*
PARK2	Positive (High level expression: 2-3)	11/22 (50%)	NS*	7/13 (53%)	NS*	17/25 (68%)	NS*
CUL4A	Positive	7/22 (31%)	NS*	3/13 (23%)	NS*	13/25 (52%)	NS*
PIK3CA NUCLEAR	Positive	14/22 (63%)	NS*	10/13 (77%)	NS*	18/25 (72%)	NS*
PIK3CA CYTOPLASMIC	Positive	11/22 (50%)	NS*	8/13 (61%)	NS*	10/25 (40%)	NS*
NM23 NUCLEAR	Positive	12/22 (55%)	NS*	7/13 (53%)	NS*	11/25 (44%)	NS*
NM23 CYTOPLASMIC	Positive (Strong intensity)	8/22 (36%)	NS*	3/13 (23%)	NS*	7/25 (28%)	NS*

ER: estrogen receptor; PR: progesterone receptor; AR: androgen receptor; RB: retinoblastoma 1; EMA: epithelial membrane antigen; MMP7: matrix metalloproteinase 7; VEGF: vascular endothelial growth factor; HER-2: human epidermal growth factor receptor-2; EGFR: epidermal growth factor receptor; COX-2: cyclooxygenase 2; TUBB3: ß-tubullin III; ERCC1: excision repair cross-complementing rodent repair deficiency, complementation group 1; XPG: xeroderma pigmentosum complementation group G; XPF: xeroderma pigmentosum complementation group F; PARK2: Parkinson protein 2. *: Fisher´s exact test. **: Student's t-test. NS: not statistically significant. †: P value for comparison between BRCA1 and BRCA2 carcinomas. ‡: P value for comparison between BRCA2 and BRCAX carcinomas. §: P value for comparison between BRCA1 and BRCAX carcinomas.

carcinomas (p=0.025 and p=0.004, respectively) (Table 4).

Survival analysis

We collected clinical information related to CR, PFS and OS from 39 of the patients (14 BRCA1 mutation carriers, 8 BRCA2 mutation carriers, and 17 BRCAX cases). Using Fisher's exact test we compared CR among the three groups of familial carcinomas and no statistically significant differences were observed, although most of the *BRCA1* mutation carriers achieved CR (around 40%). On the other hand, based on a log-rank test, BRCA1 mutation carriers had better PFS than *BRCA2* and *BRCAX* mutation carriers (mean 38.7 months vs. 16.6 and 17.3 months, respectively, p=0.048). There was also a trend for *BRCAX* patients and those with BRCA1 mutations to have better OS than patients with *BRCA2* mutations (mean 99.3 and 102.1 vs. 69.9 months, respectively) (Table 2).

We have combined the results from these analyses in order to establish the clinicopathological, IHC and clinical outcome characteristics typical of the three case groups (Table 5).

Hierarchical clustering

Since the amount of information generated in this study using TMAs was large, we carried out an unsupervised hierarchical clustering analysis. By applying this algorithm, the 60 carcinomas in the first TMA were clustered based on similarities in patterns of expression of the 40 IHC markers plus histological grade (Fig. 2) and statistical values with a FDR <0.05. Two main branches were observed which were differentiated principally by ERCC1 expression. While no tumours in branch A (left) showed positive expression of ERCC1, the vast majority of those in B (right) did (p<0.0001) (Fig. 2).The distribution of familial case groups and histological subtypes was very heterogeneous in both branches, with similar percentages of subtypes as well as high and low grade tumors. Carcinomas grouped in branch B more frequently over-expressed PIK3CA, PARK2, nuclear survivin, topoisomerase IIα, and XPF (p<0.001), plus other markers such as ki-67, cytoplasmic nm23, Bcl-2, CD105, nuclear p27, cul4a, ER, e-cadherin and Rb (p≤0.043). In contrast, branch A carcinomas more often over-expressed IQGAP1 (p=0.021) and showed loss of expression of e-cadherin (p=0.043).

Table 4. Validation of IHC results for PR, p53, ki-67, cyclin D1, BCL-XL, nuclear EGFR and cytoplasmic MMP7 in the whole set of carcinomas.

MARKER	BRCA1 n (%)	p†	BRCA2 n (%)	p‡	BRCAX n (%)	p§	
PR	Positive	20/28 (71%)	NS (0.054*)	6/16 (38%)	NS*	11/29 (38%)	0.015*
P53 (≥50%)	Positive	22/28 (79%)	NS (0.091*)	8/16 (50%)	NS*	12/29 (41%)	0.013*
Ki-67 (MEDIAN %)	Positive	19/28 (68%)	NS*	13/16 (81%)	0.025*	12/29 (41%)	NS*
Cyclin D1 (10%)	Positive	22/28 (79%)	NS*	9/16 (56%)	NS*	22/29 (76%)	NS*
BCL-XL IRS SCORE	Low (0-6)	10/28 (36%)	NS (0.059*)	11/16 (69%)	NS*	12/29 (41%)	NS*
	High (7-12)	18/28 (64%)	NS (0.059*)	5/16 (31%)	NS*	17/29 (59%)	NS*
Nuclear EGFR	Positive	16/28 (57%)	0.025*	3/16 (19%)	0.004*	19/29 (66%)	NS*
Cytoplasmic MMP7 (Mean ± SD)	90.15±69.45	NS**	110.48±91.25	NS**	81.5±60.52	NS**	

NOTE: There were 28 BRCA1, 16 BRCA2 and 29 BRCAX carcinomas studied. PR: progesterone receptor; EGFR: epidermal growth factor receptor; MMP7: matrix metalloproteinase 7. *Fisher´s exact test. **: Student's t-test. †: P value for comparison between BRCA1 and BRCA2 carcinomas. ‡: P value for comparison between BRCA2 and BRCAX carcinomas. §: P value for comparison between BRCA1 and BRCAX carcinomas. NS: not statistically significant.

Table 5. Summary of the clinicopathological and immunohistochemical characteristics of BRCA1, BRCA2 and BRCAX carcinomas.

Clinicopathological, immunohistochemical and clinical outcome features	BRCA1	BRCA2	BRCAX
Mean age of diagnosis	50.27	Older (58.23)	49.78
High grade	60%	64%	31%
FIGO stage	III-IV (71%)	III-IV (76%)	I-II(55%)
PR(% positive expression)	71%	38%	41%
P53(% positive expression)	78%	50%	41%
Ki-67(% positive expression)	68%	81%	41%
Nuclear EGFR(% positive expression)	57%	19%	65%
Complete remission	39%	25%	34.5%
PFS	38.73±11.42	16.67±4.25	17.33±5.48
OS	102.05±13.5	69.92±6.36	99.26±15.92

PR: progesterone receptor; EGFR: epidermal growth factor receptor. PFS: progression free survival; OS: overall survival.

We investigated whether there were differences regarding OS and PFS between these two branches. A trend of better clinical evolution was found for women with tumors in branch B, although these differences were not statistically significant (mean PFS: 31.8 versus 19.7 months; mean OS: 101.4 versus 84.4 months, respectively). A consistent trend was observed when comparing the main marker that differentiates the two branches; cases with tumors positive for ERCC1 had longer mean PFS (43.1 vs. 21.2 months) and OS (110.4 vs. 85.3 months) than cases with tumors negative for it (data not shown).

Discussion

In the present study we carried out a morphological and IHC analysis of familial primary ovarian epithelial carcinomas using TMAs and considering 40 markers involved in different genetic pathways. We found that the three familial case groups considered (BRCA1, BRCA2 and BRCAX) have different IHC profiles and identified a marker that might point towards new alternative treatment, as well as a prognostic marker of better clinical outcome (Table 5).

We found that BRCA1 and BRCAX cases have an earlier age of onset (mean 50.3 and 49.8 years, respectively) than BRCA2 cases (58.2 years). This result for BRCA1 versus BRCA2 mutation carriers is consistent with those from studies carried out by other groups (Boyd et al., 2000, Cass et al., 2003). Our finding that mutation-negative familial ovarian cancer (BRCAX) cases also tend to be diagnosed at a younger age than BRCA2 mutation carriers is novel. Regarding histopathological characteristics, we observed that SC is the predominant histological subtype in all 3 familial groups. In addition, all 5 borderline tumors in our series were diagnosed in women negative for mutations in BRCA1 and BRCA2. This subtype represents 7% of all tumors of our series and 17% of BRCAX tumors. We also observed that there were two endometrioid carcinomas and one clear-cell carcinoma in the BRCA1 group, and one clear-cell carcinoma in the BRCA2 group. These data show that, in contrast to what is currently presupposed, women with other histological subtypes of ovarian cancer may also carry mutations in BRCA1 or BRCA2.

In our series the major differences in the histopathological profiles of the three familial case groups were due to histological grade and FIGO staging. Thus, we found that carcinomas in BRCA1 and BRCA2 mutation carriers are mainly high-grade neoplasms diagnosed at a more advanced stage (FIGO III-IV) than tumors in non-carriers. Bilaterality and ascites were present in a similar percentage in all three groups (Table 2).

Based on an analysis of 40 markers by IHC, we found that BRCA1 carcinomas more often over-express p53 and PR, and show positive nuclear EGFR expression in more cases (Table 5). PR over-expression seems to be associated with BRCA1 mutation carrier status and, as far as we know, there is no data in the literature regarding the expression of hormone receptors (ER, PR, and AR) in hereditary epithelial ovarian cancer. Our results therefore suggest for the first time that BRCA1 mutation carriers in particular could benefit from hormonal therapy as an alternative treatment for ovarian carcinomas with high expression of PR. Positive nuclear EGFR expression was more frequent in BRCA1 carcinomas (57%) and BRCAX carcinomas (65%) than in BRCA2 carcinomas (19%). There is only one study on the nuclear expression of EGFR in sporadic ovarian carcinomas, which reported that 28% of 221 carcinomas were positive for nuclear EGFR expression and these cases were statistically significant associated with poor OS (Xia et al., 2009). In that study nuclear expression of EGFR was also correlated with increased levels of cyclin D1 and ki-67, both indicators of cell proliferation. In our series of BRCA1 and BRCAX carcinomas, we didn't observe this positive correlation, and in fact the highest expression of ki-67 corresponded to BRCA2 carcinomas, which tended to have negative nuclear expression of EGFR and worse OS. Further studies are therefore necessary to clarify this issue in FBOC.

BRCA2 carcinomas are proliferative tumors that tend to over-express ki-67. The fact that the ki-67 protein is present during all active phases of the cell cycle (G1, S, G2 and mitosis), but absent from resting cells (G0), makes it an excellent marker of the growth fraction within tissues. Of the several known proliferative markers, ki-67 is the most frequently used in OC, and several studies have demonstrated that ki-67 over-expression is associated with poorer outcome for women with these tumors (Anttila et al., 1998; Kaern et al., 2005). These findings are concordant with our observation of lower PFS and OS for cases with BRCA2 mutations, the group with highest expression of ki-67.

BRCAX carcinomas are the least proliferative group and contained the only mucinous tumor with over-expression of HER2 in our case series. Given that trastuzumab therapy is effective in other tumors with HER2 over-expression, it would be interesting to evaluate the effectiveness of this drug in this type of patients. However, to our knowledge no such clinical trials have been carried out to date.

Our results in relation to clinical outcome have shown that cases with BRCA1 mutations present a high percentage of CR (around 40%), have longer mean PFS than patients with BRCA2 and BRCAX mutations (38.7 vs. 16.6 and 17.3 months, respectively), and have better OS than patients with BRCA2 mutations (mean 102 vs. 69.9 months) (Table 2). These results are in opposition to those from two studies reporting a significantly increased death rate in BRCA1 mutation carriers compared with BRCA2 mutation carriers, and poorer long-term survival for early-stage disease (5-year and 10-year survival stage I and II), but no difference in survival for late-stage disease (Miller et al., 1981; Milne et al., 2008). Larger studies are thus needed to clarify the

effect of germline BRCA1 and BRCA2 mutations on clinical outcome in ovarian cancer.

We used unsupervised hierarchical clustering to classify the full set of familial carcinomas and found that they clustered into two branches that were differentiated principally by ERCC1 expression; one (branch A) with negative expression and the other primarily with positive expression (branch B) (Fig. 2). Cases with branch B tumors tend to have better PFS and OS than those with branch A tumors (mean PFS: 31.8 versus 19.7 months; mean OS: 101.4 versus 84.4 months) although these results were not statistically significant. ERCC1 is a DNA excision repair protein belonging to the nucleotide excision repair (NER) pathway and its positive expression has been associated with poor PFS and OS in sporadic ovarian carcinomas (Steffensen et al., 2009). In our series of familial ovarian cancer cases, positive expression of ERCC1 was non-statistically-significantly associated with longer PFS (43.1 vs. 23.6 months) and OS (110.4 vs. 85.3 months). These apparently contradictory results in sporadic and familial ovarian cancer could be related to alterations, in the familial cases, in the BRCA1 and BRCA2 genes and their level of expression. BRCA1 and BRCA2 are involved in double strand DNA repair and work in cooperation with other DNA repair pathways such as NER. A recent study of advanced sarcomas (Schoffski et al., 2011) identified that low BRCA1 and high ERCC1 and/or XPG mRNA expression levels were a marker of sensitivity to trabectedin, a drug approved for use in Europe and in other countries in patients with sarcomas, and subsequently in patients with ovarian cancer (Monk et al., 2010). It will therefore be important to validate our result in an independent series of patients because of the potential value of ERCC1 as a prognostic and treatment marker.

In conclusion, we have defined the clinico-morphological, IHC and clinical outcome features of familial ovarian carcinomas, as summarized in Table 5. New series of patients are required to validate some of these results, particularly those referring to PR over-expression in BRCA1 carcinomas and positive expression of ERCC1 in familial carcinomas in general, because of the possible implications that they could have in the development of alternative treatments and prognosis.

Acknowledgements. This article has been partially fund by the Spanish Association Against Cancer (AECC), the Spanish Cancer Research Network (RTICC RD06/0020/1060 and RTICC RD06/0020/0015), the Fondo de Investigación Sanitaria (FIS PI06-0950 and FIS PS09-01094), the Fondo Europeo de Desarrollo Regional "Una manera de hacer Europa", Department of Health (Spain), the Fundación Mutua Madrileña-07 and La Caixa. IM is sponsored by the Biomedical Rare Diseases Network (CIBERER). We would like to thank the CNIO Tumor Bank and the Immunohistochemical Unit for their help in the management of the tumors and the construction of TMAs. We also thank Victoria Fernandez and Maika Gonzalez for their technical support and Ron Harton for his help with the writing of the manuscript.

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Accepted July 25, 2012