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Östrogen-Rezeptor alpha (*ESR1*) Gen-Amplifikation ist selten bei malignen Ovarialtumoren

Dissertation

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To all whom I love

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1. Introduction

1.1. Ovarian cancers
1.1.1 Incidence/ Mortality

Worldwide, ovarian cancer is the fifth most frequent malignant tumor in women and the most common cause of death amongst cancers of the reproductive system.

In Germany, about 9.660 new cases are recorded every year with an average age of onset of 65 years (GEKID, Krebs in Deutschland" 5. Ausgabe, Saarbrücken, 2006). In the US, approximately 1.4% of women will develop ovarian cancers in there lifetime (Kurman 2002). Prognosis is generally poor as these cancers are often detected at late stage. The median overall survival in these patients is 24 to 38 months after diagnosis (Smyth et al 2007).

1.1.2. Risk factors

Many factors have been suggested to prevent or cause ovarian cancer. For example, it was established that increasing parity, oral contraceptive use, hysterectomy and tubal ligation play a role as protective factors against ovarian cancers, but there is much still debate about these factors (Kurman 2002). On the other hand, reproductive factors like early menarche, late menopause, infertility and fertility drugs were demonstrated as risk factors for developing ovarian cancers. Hormone replacement therapy, age, weight, breastfeeding, viral infections during childhood, talc, smoking, diet, and ionizing radiation are other risk factors of ovarian cancers. In addition, the genetic factors play an important role in ovarian cancers and are responsible for about 10% of cases (Kurman 2002).

For first degree relatives, the possibility of having ovarian cancer varies from 1.94 to 25.5 (WHO, Tumors of the breast and female genital organs).

1.1.3. Pathology / Histology

Ovarian cancers were divided into three main groups according to the possible histogenesis and direction of differentiation.

Table 1: WHO 2003, histological classification of tumours of the ovary (Tavassoli 2003)

Ovarian tumours	
1 Surface epithelial-stromal tumours	
1.1 Serous tumours 1.2 Mucinous tumours, endozervicale und intestinal type 1.3 Endometrioid tumors including variant with squamous differentiation 1.4 Clear cell tumours	Benign Borderline tumours Malignant tumours
1.5 Transitional cell tumours	1.5.1 Benign 1.5.2 Borderline 1.5.3 Malignant Brenner-Tumor 1.5.4 transitional cell carcinoma (non-Brenner type)
1.6 Squamous cell tumours	
1.7 Mixed epithelial tumours (specify components)	1.7.1 Benign 1.7.2 Borderline-Malignität 1.7.3 Malignant
1.8 Undifferentiated tumours	1.8.1 Undifferentiated carcinoma 1.8.2 Adenocarcinoma not otherwise specified
2 Sex cord-stromal tumours	
2.1 Granulosa-stromal cell tumours	2.1.1 Granulosa cell tumour group 2.1.2 Thecoma-Fibroma group
2.2 Sertoli-stromal cell tumors	2.2.1 Sertoli-Leydig cell-Tumour group 2.2.2 Sertoli cell tumour 2.2.3 Stromal-Leydig

Ovarian tumours	
	cell tumour
2.3 Sex cord-stromal tumoursof mixed or unclassified cell type	2.3.1 Sex cord tumour with annular tubules 2.3.2 Gynandroblastoma 2.3.3 Unclassified
2.6 Steroid cell tumors	2.6.1 Stromal luteoma 2.6.2 Leydig cell tumour group 2.6.3 Steroid cell tumor, not otherwise specified
3 Germ cell Tumours	
3.1 Primitive germ cell tumours	3.1.1.Dysgerminoma 3.1.2 Yolk sac tumour 3.1.3 Embryonal carcinoma 3.1.4 Polyembryoma 3.1.5 Non gestational Chorioncarcinoma 3.1.6 Mixed germ cell tumour
3.2 Biphasic or triphasic Teratoma	3.2.1 immature 3.2.2 mature
3.3 Monodermal teratoma and somatic-type tumours associated with dermal cysts	
4 Germ cell sex cord-stromal Tumours	
4.1 Gonadoblastoma	
4.2 Mixed germ cell-sex cord-stromal tumour	
5 Tumours of rete ovarii	
5.1 Adenocarcinoma 5.2 Adenoma 5.3 Cystadenoma 5.4 Cystadenofibroma	
6 Miscellaneous tumours	
6.1 Small cell carcinoma, hypercalcaemic type 6.2 Small cell carcinoma, pulmonary type 6.3 Large neuroendocrine carcinoma 6.4 Hepatoid carcinoma 6.5 Primary ovarian mesothelioma 6.6 Wilms tumour 6.7 Gestational choriocarcinoma 6.8.Hytatidiform mole 6.9 Adenoid cystic carcinoma 6.10 Basal cell tumour 6.11 Ovarian wolffian tumour	

Ovarian tumours	
6.12 Paraganglioma 6.13 Myxoma 6.14 Soft tissue tumors not specific to ovary 6.15 Other	
7 Tumour-like conditions	
8 Lymphoid and haematopoetic tumours	
9 Secondary tumours	

The main group of the ovarian tumors is the epithelial tumors, which comprises about 50- 60% of all ovarian tumors (Boecker and Denk 2004), and accounting for about 90% of malignant tumors (WHO).

Surface epithelial tumors were classified according to the following characters:

- Cell types: serous, endometrioid and mucinous tumors.
- Growth pattern: cystic, solid, papillary.
- Accompanying fibrous tissue.
- Atypia and invasivness.

It is important to say that borderline tumors rank between benign and malignant tumors, as they don't fulfill all criteria of malignant one and have better prognosis. This group accounts for about 5-10% of ovarian tumors.

10-20% of all ovarian tumors are germ cell tumors which is the second most frequent tumors. Most of them happen in children and young adults (Rosai and Ackermann 2004). 5-10% of ovarian tumors are sex cord-stromal tumors (Boecker and Denk, 2004).

1.1.4. Treatment

Treatment options include surgical removal of the tumor mass with a maximal reduction of the peritoneal cancer mass in case of local tumor extension. In addition, topical and systemic cytotoxic therapy is applied. Ovarian cancer belongs to the group of cancers with frequent expression of steroid hormone receptors. The frequency of estrogen receptor (ER) expression varies greatly between different studies, and has been reported in 25 % to 86% of ovarian cancers with highest percentages reported in endometroid and serous subtypes (Vang et al 2001, Teufel et al 1983, De Sousa Damião et al 2007, Kommoss et al 1992, Rosen et al 2004, Vang et al 2006, Van Doom et al 2000, Lindgren et al 2004, Lindgren et al 2001, Van Mieghem et al 2005, Cardillo et al 1998, Farinola et al 2007, Ho 2003, Høgdall et al 2007). Accordingly, endocrine therapy is a recognized option in the treatment of chemo-resistant ovarian cancer after failure of first and second line therapies. However, not all ER positive ovarian cancers respond to anti-estrogen therapy, and it was suggested that might be due to the facts that most of the studies have been retrospective, small in size, without adequate selection of the patients and generally used hormonal therapy as a last-line therapy for the refractory or resistant ovarian cancers; moreover, concerning tamoxifen, it has not been definitely clarified whether it only acts as a pure estrogen antagonist in ovarian tissue, or it has also an agonist effects (Perez-Gracia et al 2002, Langdon et al 1994, Makar 2000, Clinton Hua,1997, Cunat et al 2004).

1.1.5. Prognostic factors

There are many factors known to influence the prognosis of ovarian cancers but the most important are the following: extension of the tumors beyond the organ limits, the volume, ascites, DNA ploidy, age of the patient, serum CA-125, P53, histological grade and type (Kurman 2002).

The most reliable and well spread, world wide applied prognostically relevant classification is the FIGO Stage (International Federation for Gynecology and Obstetric) (Table 2).

Table 2: FIGO – Staging of ovarian cancers

01 - FIGO Stage I

01	**FIGO Stage I**	**Tumour limited to ovaries.**
01A	IA	Tumour limited to one ovary; capsule intact, no tumour on ovarian surface; no malignant cells in ascites or peritoneal washings.
01B	IB	Tumour limited to both ovaries; capsule intact, no tumour on ovarian surface; no malignant cells in ascites or peritoneal washings.
01C	IC	Tumour limited to one or both ovaries with any of the following: capsule ruptured, tumour on ovarian surface, malignant cells in ascites or peritoneal washings.

02 - FIGO Stage II

02	FIGO Stage II	Tumour one or both ovaries with pelvic extension.
02A	IIA	Extension and/or implants on uterus and/or tube(s); no malignant cells in ascites or peritoneal washings.
02B	IIB	Extension to other pelvic tissues; no malignant cells in ascites or peritoneal washings.
02C	IIC	Pelvic extension (2a or 2b) with malignant cells in ascites or peritoneal washings.

03 - FIGO Stage III

03	FIGO Stage III	Tumour involves one or both ovaries with microscopically confirmed peritoneal metastasis outside the pelvis and/or regional lymph node metastasis.
03A	IIIA	Microscopic peritoneal metastasis beyond pelvis.
03B	IIIB	Macroscopic peritoneal metastasis beyond pelvis 2 cm or less in greatest dimension.
03C	IIIC	Peritoneal metastasis beyond pelvis more than 2 cm in greatest dimension and/or regional lymph node metastasis.

04 - FIGO Stage IV

04	FIGO Stage IV	Distant metastasis (excludes peritoneal metastasis)

1.2. Pathogenesis of ovarian cancers

The pathogeneses of the ovarian cancers is still unknown and in comparison to the carcinomas of the colon there is no reliable tumor progression model. Many efforts were done to propose a model to clarify the pathway of these cancers' development and were based on the clinicopathological and molecular studies. In this proposed model the surface epithelial tumors were divided into low and high grade groups. The low grade group arises in a stepwise manner and contains low grade serous carcinomas, mucinous carcinomas, endomedrioid carcinomas, malignant Brenner tumors and clear cell carcinomas. The most molecular changes that associated with Type I are *BRAF* and *KRAS* mutations in serous tumors, *KRAS* mutation in mucinous tumors and B-catenin and PTEN mutations and microsatellite instability in endometrioid carcinomas. Type II includes high grade serous carcinomas, malignant mixed mesodermal tumor, and undifferentiated carcinomas. The well known molecular change in this group of tumors is p53 mutations (Figure 1) (Shih and Kurman 2004).

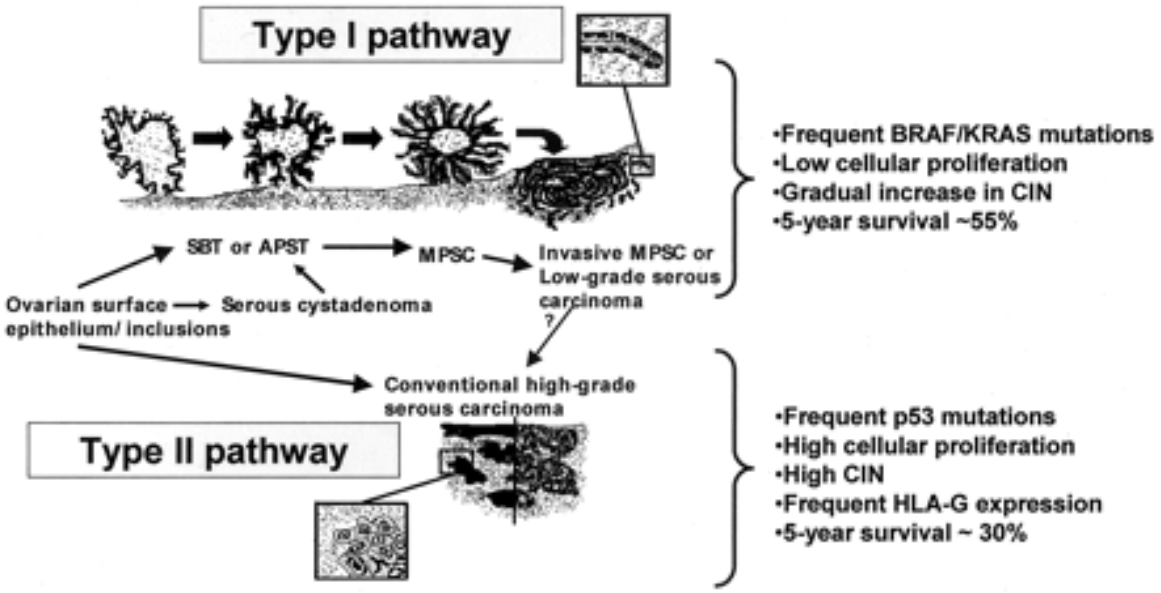

Figure1. Schematic representation of the dualistic model depicting the development of ovarian serous carcinomas, the most common type of ovarian cancer (Shih and Kurman 2004).
Low-grade serous carcinoma (MPSC) represents the prototypic type I tumor and develops in a stepwise manner from an atypical proliferative tumor through a noninvasive stage of MPSC (both of these tumors qualified as borderline) before becoming invasive. These tumors are associated with frequent *KRAS* or *BRAF* mutations. High-grade serous carcinoma represents the prototypic type II tumor and develops from the ovarian surface epithelium or inclusion cysts without morphologically recognizable intermediate stages. *KRAS* and *BRAF* mutations have been rarely found in these neoplasms. CIN, chromosomal instability.

Recently, the epidemiological and experimental studies have pointed out to the possible carcinogenetic role of estrogen in promoting the development of ovarian cancers in postmenopausal women (Cunat et al, 2004).

1.3. Estrogen receptors

Estrogen is a steroidal hormone can pass through the phospholipid cytoplasmic membrane without any need to membrane bound receptors (figure 2).

ER is a nuclear receptor and is activated by 17β-estradiol hormone. There are two types of ER referred to as α and β, each encoded by a separate gene *ESR1* and *ESR2* respectively.

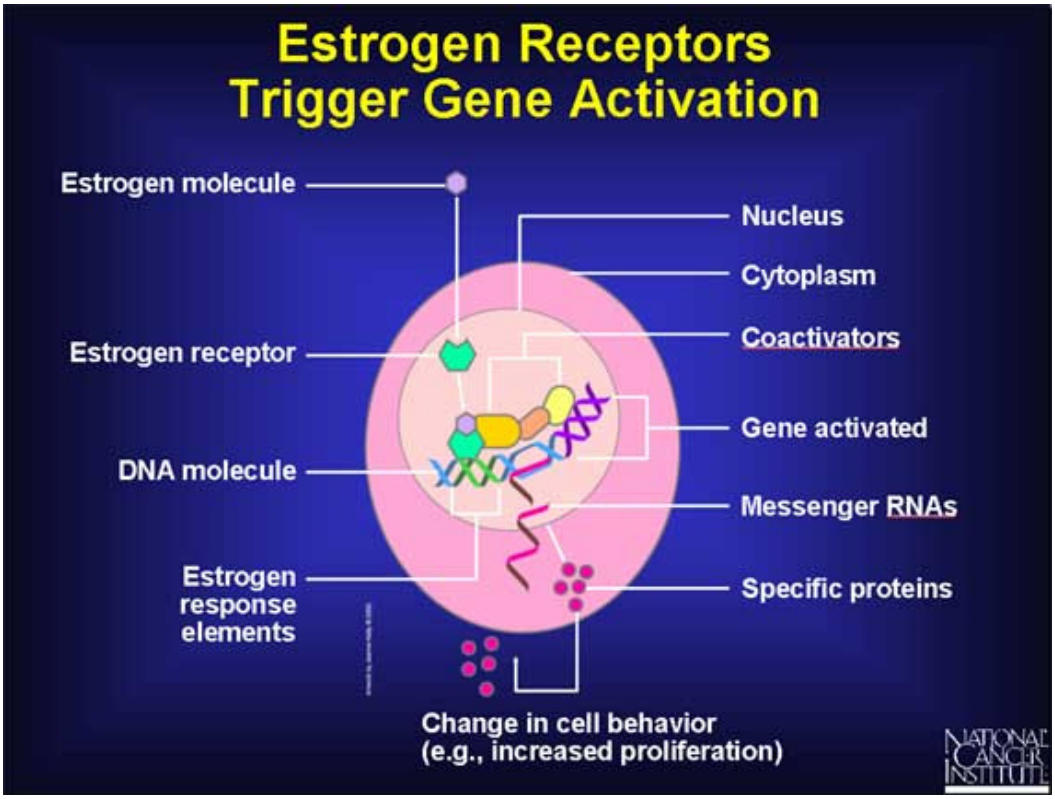

Figure2. Binding of hormone molecule with ER and migration through the nuclear membrane, then hormone-receptor complex binding to DNA double strand (hormone response element) (http://www.cancer.gov/cancertopics/understandingcancer/estrogenreceptors/).

After binding to the hormone, dimerization of the receptor happens, then binding of the receptor dimer to specific sequences of DNA known as hormone response elements (Klinge CM 2001, Ito I et al 2010).

The DNA/receptor complex recruits other proteins, which are responsible for the transcription of downstream DNA into mRNA and finally protein. Different ligands may vary in their affinity for α and ß isoforms of the estrogen receptor. Thus, 17-ß-estradiol binds equally well to both receptors, estrone binds preferentially to the

α receptor, and estriol prefers the ß receptor (Zhu et al 2006). Additionally, the same ligand may be an agonist in some tissues, and an antagonist in other tissues. For example Tamoxifen is an antagonist in breast and is therefore used as a breast cancer treatment, but acts as an ER agonist in endometrium and skeletal tissue, preserving bone density (Dahlman-Wright et al 2006).

1.4. Purpose of the work

In breast cancer, estrogen receptor alpha (*ESR1*) gene amplification has been recently described as a frequent mechanism for ER overexpression. More than 20% of breast cancers showed *ESR1* gene amplification and more than 15% additional cases low level ESR1 gene copy number gains (Holst et al 2007). Preliminary data also suggested that *ESR1* amplified breast cancers may exhibit a high responsiveness to tamoxifen (Holst et al 2007).

To determine, whether *ESR1* amplifications also occur in ovarian cancer, we analyzed a set of more than 420 primary ovarian cancers for *ESR1* gene amplification. The results of this study suggest that *ESR1* amplification is a mechanism for ER overexpression only in a very small subset of ovarian cancers.

2. Material and Methods.

2.1. Material

Primary tumors of 428 ovarian cancer patients were used for this study. Tumors have been collected from Institute of Pathology at Basel University and Institute of Pathology at UKE, Hamburg. The median patient age was 58.1 (range 24–84) years. The mean follow up time was 41.85 months (range 1–210).

Formalin fixed (neutral buffered aqueous four percent solution), paraffin embedded tumor material was utilized. The pathologic stage was obtained from the primary pathology reports. All slides from all tumors were reviewed by two pathologists (HM, RI) to define the histological grade and the histological tumor type. The composition of the TMA is described in detail in table 3.

Table3. The composition of the TMA in details.

Histologie	Nr. Cases (Basel)	Nr. Cases (Hamburg)
Papillary, Serous Carcinoma	112	63
Mucinous Carcinoma	38	46
Endometrioid Carcinoma	68	22
Clear Cell Carcinoma	24	0
Malignant Brenner Tumor	5	0
Squamous Cell Carcinoma	1	0
Mullerian Mixed Cancer	15	0
Sex Cord- Stromal Tumors	10	0
Yolk Sack Tumor	4	0
Undifferentiated	15	0
other rare types	5	0
Sum	297	131

2.2. Tissues Microarray

A single 0.6 mm tissue core was punched from each of the 428 donor blocks and placed in the recipient paraffin block. The patients were treated at Hamburg and Basel University Hospitals between 1980 and 2001.

Follow up data was available from 169 patients. The mean follow up time was 41.85 months. The composition of the TMA was shown in details in table2.

A picture of a hematoxilin and eosin stained arrays section is shown in figure 3.

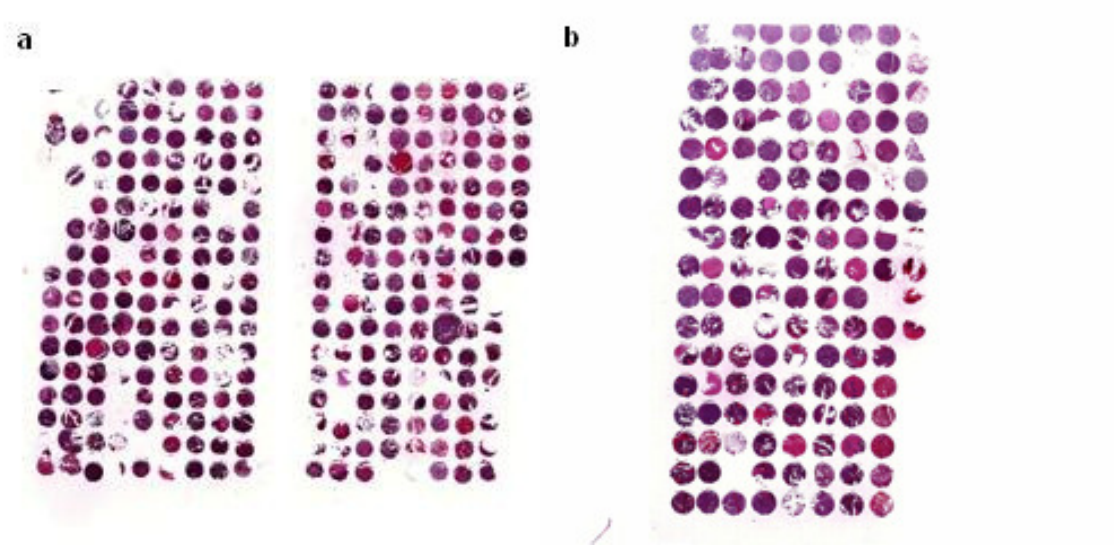

Figure 3: Hematoxilin & eosin stained sections of our ovarian cancers TMA.

a) TMA block, Basel cases, 297 tumors' spots. The diameter of each is 0.6 mm.

b) TMA block, Hamburg cases, 131 tumors' spots with 16 spots of different body tissues.

2.3. Immunohistochemistry

Immunohistochemical detection of ER alpha protein was performed using a mouse monoclonal antibody (DAKO #M7047, clone 1D5). A 4µm TMA section was deparaffinized in xylol and rehydrated in a descending series of ethanol (96%, 90%, 80%, and 70%). For heat induced antigen retrieval, slides were

incubated with DAKO's antigen retrieval solution pH9 (DAKO #S2368). The primary antibody was diluted 1:50 and incubated for 30 min at room temperature. The primary antibody was omitted for negative control. All spots were analyzed by one pathologist (R.I.). IHC scoring was performed according to the Allred score (Harvey et al 1999). In brief, ER staining intensity was recorded in a 4-step scale (0-3) and the fraction of ER positive tumor cells in a 5-step 1-5 scale (0: none- 1: < 1/100- 2: 1/100 to 1/10- 3:1/10 to 1/3- 4: 1/3 to 2/3- and 5: > 2/3) (Allred score was illustrated in figure 4)(Choudhury et al 2009). Combination of both parameters results in an 8-step score, where all samples with score >2 are regarded as ER positive.

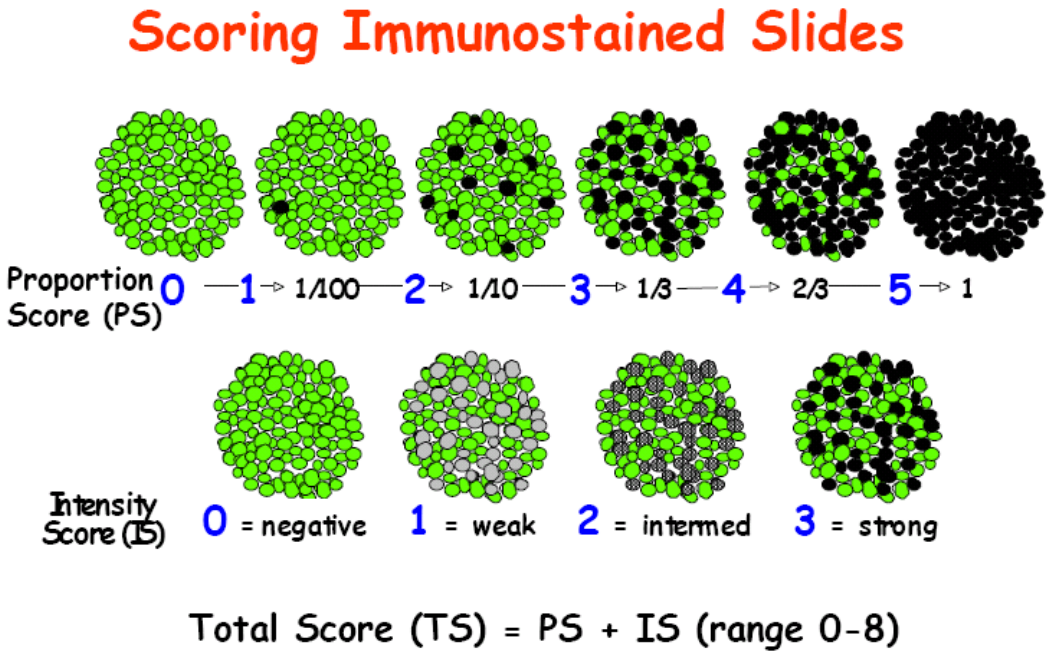

Figure4. Scoring of immunohistochemstry.

Two series of cartoons depicting the methodology for calculation of the Allred score. The green color identifies unstained cells, whereas the gray, dark gray, and black colors identify cells stained to different intensities. (A) Series in which the stain intensity is constant (at maximum), and the proportion of stained cells increases from left to right. (B) Series in which the proportion of stained cells is constant (at 1/3), and the stain intensity increases from left to right (from none to maximum). Allred (2008). http://www. asbd.org/images/D3S9%20-%20Craig%20Allred.pdf

2.4. Fluorescence in situ hybridization (FISH)

TMA sections were treated according to the Paraffin Pretreatment Reagent Kit protocol (Vysis, Downers Grove, IL) before hybridization. FISH was performed with a digoxigenated BAC probe (BAC RP11-450E24, RZPD, Germany) containing a part of the ESR1 gene and a Spectrum-Orange labeled chromosome 6 centromeric probe (CEP6) as a reference (purchased from Vysis). Hybridization and post hybridization washes were according to the 'LSI procedure' (Vysis). Probe visualization using fluorescent isothiocyanate (FITC)-conjugated sheep anti-digoxigenin (Roche Diagnostics, Rotkreuz, Switzerland) was as described (Wagneret al 1997). Slides were counterstained with 125 ng/ml 4', 6-diamino-2-phenylindole in an antifade solution. Hybridization and post hybridization washes were according to the 'LSI procedure' (Vysis). Slides were then counterstained with 125 ng/ml 4',6-diamino-2-phenylindole in an antifade solution. The number of fluorescence signals was estimated by an experienced person (FH) in each tissue spot for the centromere 6 and the ESR1 gene probes. ESR1 alterations were defined based on the ratio of gene copy numbers of ESR1 and centromere 6. Tissues with more at least two-fold more ESR1 than cen. 6 copies (ratio ≥2.0) were considered "ESR1 amplified". Tissues with more ESR1 than centromere 6 copies not reaching the criteria for amplification were considered "ESR1 gained" (ratio >1.0 but <2.0). All other analyzable tissues (Ratio 1.0) were considered "ESR1 normal".

2.5. Statistical analysis

Contingency table analysis and chi-square tests were used to study the relationship between clinicopathological parameters of the analysed tissues and ER expression levels. Kaplan-Meier plots and log-rank tests were employed to analyze the relationship between ER expression status and patient survival.

3. Results

3.1. ER Expression

Immunohistochemical ER analysis was successful in 384/428 (89, 7%) arrayed samples. Analysis failure was due to lack of tumor cells in tissue spots (n=19, 4.4%) or missing tissue spots (n=24, 5.6%). More than one third (148/384, 37.2%) of tumors showed at least weak ER expression. Strongest staining (score 7-8 according to Allred) was found in 36/384 (9.4%) of samples, and was linked to high grade cancers (p=0.038). ER expression was unrelated to patient prognosis (p=0.2491, figure 5). Examples of IHC positive and negative tumors are shown in figure 6. All IHC results are summarized in table 4.

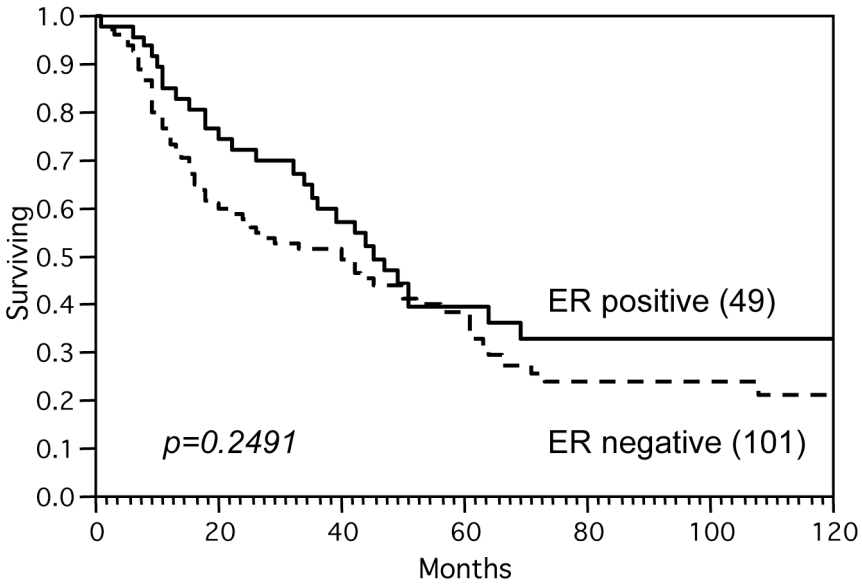

Figure 5: Kaplan-Meier survival analysis of ER positive and ER negative ovarian cancers.

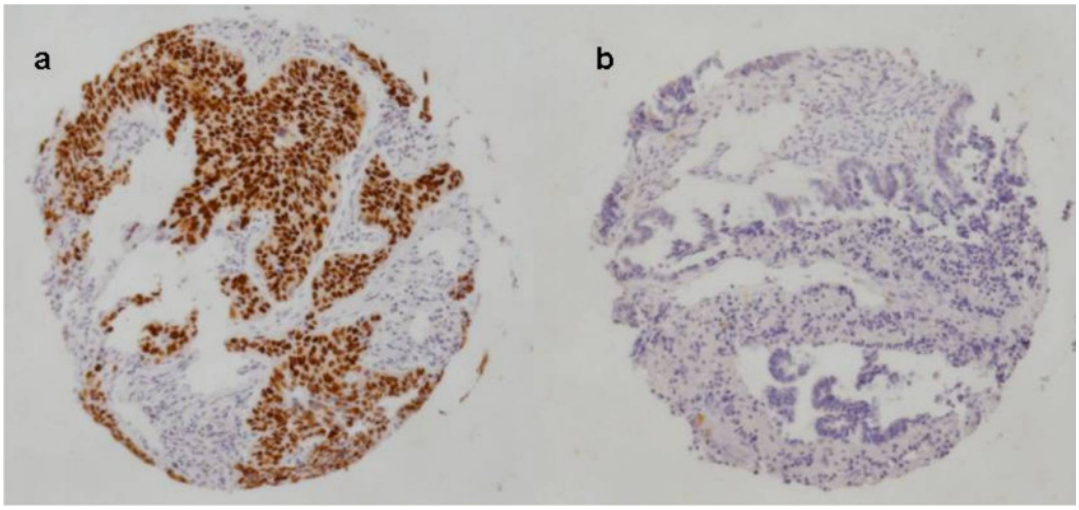

Figure6: Examples of ER positive (a) and ER negative (b) ovarian cancer. Immunohistochemistry, 100x magnifications.

Table4. Results of Immunohistochemistry and scoring by using Allred score.

Histology	ER-ICH Results		Allred score			
	Nr. cases	analyzable	(0-2)	(3-4)	(5-6)	(7-8)
papillary, serous Carcinoma	175	158	77	26	36	19
Mucinous Carcinoma	84	69	58	2	5	4
Endometrioid Carcinoma	90	80	44	11	17	8
Mullerian Mixed Cancer	15	14	14	0	0	0
Clear Cell Carcinoma	24	24	24	0	0	0
Malignant Brenner Tumor	5	4	2	0	2	0
Squamous cell Carcinoma	1	1	1	0	0	0
Sex Cord- Stromal Tumors	10	10	6	1	0	3
Yolk Sack Tumor	4	4	3	1	0	0
Undifferentiated	15	15	7	2	4	2
other rare types	5	5	5	0	0	0
sum	428	384	241	43	64	36

3. 2. *ESR1* Amplification

ESR1 FISH analysis was successful in 243/428 arrayed tissue samples. Missing results were either due to missing tissue samples on the TMA (n=80) or lack of interpretable FISH signals (n=105). *ESR1* amplification (ratio *ESR1*/centromere 6 ≥ 2.0) was found in 5/243 (2.1%) tumors. Amplifications were usually low level

with 4-8 FISH signals. One sample had a high level amplification (>10 signals). Examples of *ESR1* amplified and non-amplified tumors are shown in figure 7.

ESR1 amplification was unrelated to histopathological parameters including histological subtype, tumor stage, and grade. No survival analysis was performed because of the small number of cases with *ESR1* amplification. All 5 tumors with *ESR1* amplification were variably positive for *ER* protein expression with strong positivity in 3 out of 5 cases. All IHC and FISH results are summarized in table 5.

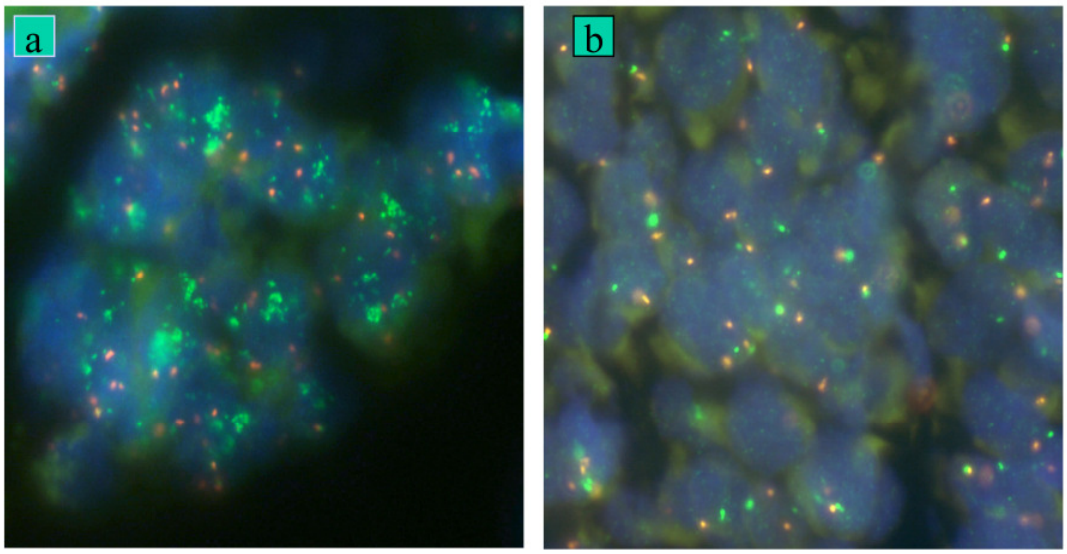

Figure 7: Examples of ovarian cancers with *ESR1* amplification (a) and with normal *ESR1* copy numbers (b). Red signals indicate copy number of centromere 7; green signals indicate *ESR1* copy numbers. FISH analysis, 630 x magnifications.

Table5: Association between histopathological data of ovarian cancers and ER protein expression and ESR1 amplification.

Estrogen receptor immunohistochemistry result (ALLRED Score) (%)									ESR1 FISH Results		
		on TMA	**analyzed (n)**	**0-2 (%)**	**3-4 (%)**	**5-6 (%)**	**7-8 (%)**	**p-value**	**analyzed (n)**	**amp (%)**	**p-Value**
Histology	All Cancers	428	384	62.7	11.2	16.7	9.4		243	2.1	
	Serous Ca.	175	158	48.7	16.5	22.8	12.0		105	1.9	
	Mucinous Ca.	84	69	84.1	2.9	7.2	5.8		40	2.5	0.6098*
	Endometrioid	90	80	55.0	13.8	21.2	10.0		44	4.5	
	Mullerian Mixed Cancer	15	14	100.0	0.0	0.0	0.0		7	0.0	
	Clear Cell Cancer	24	24	100.0	0.0	0.0	0.0		13	0.0	
	Malignant Brenner Tumor	5	4	50.0	0.0	50.0	0.0		3	0.0	
	Squamous Cell Ca.	1	1	100.0	0.0	0.0	0.0		1	0.0	
	Sex cord- Stromal tumors	10	10	60.0	10.0	0.0	30.0		8	0.0	
	Yolk Sack Tumor	4	4	75.0	25.0	0.0	0.0		2	0.0	
	Undifferentiated Ca.	15	15	46.7	13.3	26.7	13.3		10	0.0	
	Other Rare Types	5	5	100.0	0.0	0.0	0.0		10	0.0	
pT Stage	pT1	58	54	75.7	5.6	13.0	5.7	0.1343	25	0.0	
	pT2	36	32	78.0	6.3	6.3	9.4		19	0.0	
	pT3	99	88	58.0	15.9	18.1	8.0		58	1.7	
Silverberg Grade	G1	81	71	71.8	5.6	14.1	8.5	0.038	33	0.0	
	G2	91	82	72.0	11.0	12.1	4.9		52	0.0	
	G3	91	85	51.7	20.0	21.2	7.1		55	1.8	

- *mucinous versus endometroid

4. Discussion

The results of this study show that *ESR1* amplification is rare in ovarian cancers (2.1%).

More than one third of ovarian tumors showed immunohistochemically detectable *ER* protein expression, most abundant in serous and endometroid subtypes. This is in line with previous studies done on the classical paraffin blocks. The good concordance between our data and previous studies demonstrates the representativity of our TMA data obtained on a 0.6 mm tissue spot per tumor (Rosen, 2004).

A small subset of *ESR1* amplified *ER* positive cases was indeed found in ovarian cancers. In comparison, some other genes showed higher rates of amplifications in these cancers. For example, the amplification of *ERBB2* ranges (0-66%) (Wu et al 2003, Leary et al 1992), *EGFR* (3.65-12%) (Lassus et al 2006, Dimova et al 2006), *CCND1* (0-19%) (Masciullo et al 1997, Courjal et al 1996, Diebold 2000), *C-MYC* up to 54.5 (Wu et al 2003, Xin 1993, Bian et al 1995), and *KRAS* (31%) (Bian et al 1995).

The significant frequency of *ER* positivity in ovarian cancers had prompted treatment efforts using hormonal therapy early on (Long RT and Evans AM 1963). In addition their relatively little toxicity was another provoking factor to continue going on to achieve more advance in this therapeutic field. Monotherapy studies using tamoxifen, Aromatase inhibitors and GnRH analogues had yielded variable results with objective response rates ranging between 0 and 56% (Perez-Gracia and Carrasco 2002, Makar 2000, Clinton and Hua 1997, Cunat et al 2004, Li et al 2007, Papadimitriou et al 2004, Balbi et al 2004, Trope et al 2000, Levine et al 2007).

Combinatorial treatment regimens combining tamoxifen and goserelin or tamoxifen and Gefitinib had obtained results with objective response rates of up to 11, 5% (Hasan et al 2005, Wagner et al 2007). Few of these studies had selected patients based on the immunohistochemically determined *ER* status. It is therefore unclear, whether the *ER* expression level has any impact on the likelihood of response, or this just reflects the lack of establishment of well organized treatment strategy in previously heavily treated patients and who in significant part already suffered from advanced disease.

The role of ER expression for response prediction to anti-hormonal drugs has been much better studied in breast cancer, where a strong association between *ER* positivity and response to anti-hormonal drugs is well established. However, also in breast cancer, not all *ER* positive cancers respond to tamoxifen and related drugs (Massarweh and Schiff 2006, Higgins and Stearns 2009). In a recent study we had found that *ESR1* amplification may be strongly predict tamoxifen response among *ER* positive breast cancers. More than 20% of breast cancers had amplified or at least elevated *ESR1* copy number (Holst et al 2007). Possible explanations for the predictive effect of *ESR1* amplification could be a particularly high expression of amplified as compared to non amplified cancers. Alternatively, it could be speculated, that *ESR1* amplified are more dependent on the *ER*-pathway than other tumors that express *ER* together with many other growth receptors. If this latter hypothesis was true, visualization of *ESR1* amplification would pinpoint towards an “Achilles tendon” of a tumor that could be most successfully targeted.

The frequency of *ESR1* amplified ovarian cancers (2.1%) is much lower than that in breast cancer. Interestingly, this fraction somehow parallels the percentage of ovarian cancers reported to show strong responses to hormonal therapies. For example, in retrospective analysis was conducted of patients who received tamoxifen at a dose 20 mg twice daily for the treatment of advanced epithelial ovarian cancer, Karagola et al found that out of twenty-nine eligible patients were included to the study there were 1 (3%) complete response, 2 (7%) partial response, 6 (21%) stable disease, and 20 (69%) progressive disease (Karagol et al 2007) (41). Papadimitriou et al have studied response rate in twenty-seven patients treated with letrozole at a dose of 2.5 mg once a day. Patients with measurable or evaluable disease (n = 21) and those with only increasing CA 125 serum levels (n = 6) were eligible. Among the 21 patients with measurable or evaluable disease, observed one complete response (5%) and two partial responses (10%) for an objective response rate of 15%. Other studies, in which the combined regiment had been implicated, Patients were given oral tamoxifen 20 mg twice daily on a continuous basis and subcutaneous goserelin 3.6 mg once a month until disease progression. In total 26 patients entered this study, of which 17 had platinum-resistant disease, using the definition of endocrine response that included patients with stable disease (SD) of 6 months or greater, the overall response rate (clinical benefit rate) was 50%. This included one complete response (CR) (3.8%), two partial responses (PR) (7.7%) and 10 patients with SD (38.5%).

5. Conclusion

ESR1 amplification is an uncommon mechanism for *ER* overexpression in ovarian cancer occurring in about 2.1% of the total number of ovarian cancers. In general, this frequency parallels the fraction of ovarian cancers reported to show complete response to anti-estrogenic therapies. Given the strong predictive power of *ESR1* amplification for response to tamoxifen in breast cancer, an evaluation of such treatments in *ESR1* amplified ovarian cancers appears justified.

6. Abstract:

Amplification of the gene encoding estrogen receptor alpha occurs in about 20% of breast cancers and is an important mechanism for estrogen receptor overexpression in this tumor type. In ovarian cancer, overexpression of estrogen receptor protein has been described in more than two thirds of cases. To study a potential role of estrogen receptor alpha gene amplification for estrogen receptor overexpression in ovarian cancer, a tumor tissue microarray containing 428 ovarian caners was analyzed by fluorescence in-situ hybridization for estrogen receptor alpha gene amplification and immunohistochemistry for estrogen receptors expression. The estrogen receptor alpha gene status was successfully determined in 243/428 arrayed cancers. Estrogen receptor gene amplification was found in 5/243 (2%) of tumors. Amplification levels were usually low with 4-8 estrogen receptor alpha gene copies. However, one case had a high level amplification with more than 30 estrogen receptor alpha gene copies. All 5 amplified tumors were estrogen receptors positive with 3/5 tumors showing highest (Allred score 7-8) estrogen receptor levels. The data demonstrate that estrogen receptor alpha amplification occurs only rarely in ovarian cancer.

Keywords: Ovarian cancers, Estrogen receptor alpha gene, Estrogen receptors, Fluorescence in-situ hybridization, Immunohistochemistry.

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10. Eidesstattliche Versicherung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.

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