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OVEREXPRESSION OF THE ONCOGENE C-ERBB-1 (HER1/NEU) BY TISSUE MICROARRAY ANALYSIS (TMA) IN OVARIAN CANCER

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ABSTRACT

This study was accomplished by using a technology (Tissue microarrays (TMA) and Fluorescence In Situ Hybridization (FISH)) on different type of ovarian tumors, The method of FISH applied on TMA with HER1 and HER2 specific probes proved itself as the most commonly used and valuable analysis for routine HER1 status detection and copy number changes. The results showed in Out of all 417 ovarian carcinomas examined, the number of successfully hybridized for HER1 oncogene is 376 (90.61%), 224 of them are malignancies tumors (218 epithelial and 6 non epithelial) ,25 low malignant ovarian tumors(just epithelial), 127 – Benign ovarian tumors(103 epithelial and 24 non epithelial). We established HER1 amplification in malignancies tumors only 11 (4.91%) ,10 (4.58%) epithelial and in 1 (1.16%) non epithelial). Not found amplification in tumors with low malignant potential & Benign ovarian tumors. While we established HER1 gains in malignancies tumors, low malignant ovarian tumors and Benign ovarian tumors only 28, malignancies 18 about (8.03%), low malignant 2 (8%) and in Benign ovarian tumors 8 (6.29%). Epithelial malignant tumors among the highest incidence of HER1 amplification was detected in Serous (4.71%), Mucinous (4%), Combined (4.54%), Undifferentiated tumors(4.34%) and Unclassification (14.28%). HER1 amplification is found in 1 of 6 non epithelial malignant tumors, Sex-cord stromal tumour (16.16%). HER1 gains in malignancies tumors was detected in serous (5.71%), Mucinous (8%), endometrioid (10.52%), Clear cell (11.11%), Combined (13.63%), Undifferentiated tumors(8.69%) and Unclassification (7.14%).and in epithelial malignant tumors was detected only in Embryonal carcinoma (16.16%). In low malignant ovarian tumors we was detected HER1 gain only in Mucinous (18.18%). In Benign ovarian tumors was detected HER1 gain in epithelial malignant tumor in serous

(7.14%), Mucinous (5%), Endometriod (5.71%), and in non epithelial malignant was found in sex – cord stromal tumor (10%), Embryonal carcinoma (8.33%). Were preserved at the grouping of tumor samples according to the WHO classification.

KEYWORDS: Overian cancer, EGFR, HER1, Tissue microarray, FISH.

INTRODUCTION

Ovarian cancer accounts for approximately 3% of all cancers in women and is been the fifth leading cause of cancer-related death among women in the United States.[1] after breast, lung, and colorectal cancer.[2] Approximately Estimated new cases 21,550 and deaths 14,600 from ovarian cancer in the United States in 2009.[3] The histological classification of ovarian cancer is complex, with a large number of histological subtypes. According to WHO, types of ovarian cancers in women age 20+ are as follows: 1-Surface epithelial-stromal tumour, also known as ovarian epithelial carcinoma, is the most common type of ovarian cancer. It includes serous tumour, endometrioid tumor and mucinous cystadenocarcinoma. 2- Sex cord-stromal tumor, including estrogen-producing granulosa cell tumor and virilizing Sertoli-Leydig cell tumor or arrhenoblastoma, accounts for 8% of ovarian cancers. 3-Germ cell tumor accounts for approximately 30% of ovarian tumors but only 5% of ovarian cancers. 4-Mixed tumors, containing elements of more than one of the above classes of tumor histology. During the last years, several new agents targeting specific and critical pathways for ovarian cancer.[4,5] HER1/neu (also known as ErbB-1, ERBB1) its once of agents stands for "Human Epidermal growth factor Receptor 1" and is a protein giving higher aggressiveness in ovarian cancer.[6] HER1-neu is member of the erbB gene family and encode for transmembrane receptor-type tyrosine-protein kinases.[4,5] It is normally involved in the signal transduction pathways leading to cell growth and differentiation in ovarian cancer.[6,7] wild type HER-1 has been overexpressed in 20–30% of ovarian cancers, associated with a poor prognosis in several, but not all, studies.[8,9] HER-1 determined by several methods. The most commonly used methods are Fluorescence in situ hybridization (FISH)[10], and detects gene amplification by measuring the number of copies of the HER1 gene in the nuclei of tumor cells[11] HER1 amplification and overexpression are established through several molecular-biological and biochemical techniques – Southern blotting, Western blotting, PCR, ELISA[12] The FISH method assists to know the alterations in HER1 copy number,the highly informative technology of tissue microarray has enabled the simultaneous analysis of a great number of ovarian tumors,thus, the FISH method applied on TMA with specific probe for the

examined HER1 Oncogene, has realized amplification and genetic gain frequency detection, thus enabling evaluation of the input of this oncogene rearrangements for the ovarian tumor occurrence and progression.[13]

MATERIALS AND METHODS

A total of 417 tissue samples of ovarian carcinomas where hybridized for HER1 oncogene is 257 of them malignancies tumors (220 epithelial and 10 non epithelial) 27 low malignant ovarian tumors(just epithelial), 160 – Benign ovarian tumors (125 epithelial and 35 non epithelial). The first step was to create a database of patients' data including the year of disease diagnosis, passport data, histological diagnosis, disease stage, number of each sample and clinical course information. Then, the paraffin blocks, according to their numbers in the database, were collected. The paraffin blocks, arranged by number and year of obtainment, were preserved at the Pathologo anatomic Department of the Clinic of Thoracic Surgery. A grouping of tumor samples according to the WHO classification and their histology followed

Specimens were fixed in cold ethanol (4°C) for 16 h and then embedded in paraffin. A H&E-stained section was made from each block to define representative tumor regions.Tissue cylinders with a diameter of 0.6-mm were then punched from tumor areas of each "donor" tissue block and brought into a recipient paraffin block using a custommade precision instrument as described (30) Five-mm sections of the resulting multitumor tissue microarray block were transferred to glass slides using the paraffin sectioning aid system [adhesivecoated slides (PSA-CS4x), adhesive tape, and UV lamp; Instrumedics, Inc., Hackensack, NJ], supporting the cohesion of 0.6-mm array elements.

FISH.

The tissue microarray sections were treated according to the Paraffin Pretreatment Reagent kit protocol (Vysis, Downers Grove, IL) before hybridization.FISH was performed with Spectrum Orange-labeled (HER1): LSI HER1 probes. Spectrum Green-labeled centromeric probes CEP7 were used as a reference (Vysis). Hybridization and posthybridization washes were according to the "LSI procedure" (Vysis).Slides were then counterstained with 125 ng/ml 49,6-diamino-2-phenylindole in antifade solution. FISH signals were scored with a Zeiss fluorescence microscope equipped with double-band pass filters for simultaneous visualization of Spectrum Green and Spectrum Orange signals (Vysis).

Amplification was defined as The analysis is based on counting the signals for the gene and respective centromere chromosome region in the cells using the appropriate filter. Normal diploid cells have dual signals corresponding to two homologous chromosomes. The presence of one or more than two signals in centromere probes is assessed as numerical aberration. The presence of one gene signal and two centromere is accepted as deletion. The presence of one centromeric signal is assessed as monosomy.

The slide is carefully examined. For each tissue cylinder, a maximum number of cells is analyzed, as the signals for the gene and centromere are compared. The green signal corresponds to the centromeric region of the examined chromosome 7 , and the red signal corresponds to the examined gene (HER1). Normally, the number of signals for the gene and centromere is equal to two (red signal: green signal = 2 : 2).

Gene oncogene amplification is assessed in case of at least 3-fold increase of gene signals compared to centromeric for the same chromosome. Genetic gain (additional genetic material i.e. increased gene copy number) is assessed in case of at least 1.5-fold (but less than 3-fold) gene signals compared to centromeric.

RESULTS AND DISCUSSION

Out of all 417 ovarian carcinomas examined, the number of successfully hybridized for HER1 oncogene is 376 (90.16%), 224 of them are malignancies tumors (218 epithelial and 6 non epithelial), 25 low malignant ovarian tumors(just epithelial), 127 – Benign ovarian tumors(103 epithelial and 24 non epithelial).

The availability of unsuccessfully hybridized tumors is due to various reasons: may be a lack of signal after hybridization or loss of material and may be an insufficient number of cells or irrelevant specimen acquisition from the donor block during tissue microchip preparation.

We established HER1 amplification in malignancies tumors only 11 (4.91%), 10 (4.58%) epithelial and in 1 (1.16%) non epithelial), Figure(1).

Not found amplification in tumors with low malignant potential & Benign ovarian tumors. While we established HER1 gains in malignancies tumors , low malignant ovarian tumors and Benign ovarian tumors only 28, malignancies 18 about (8.03%) ,low malignant 2 (8%) and in Benign ovarian tumors 8 (6.29%). Table (1,2,3).

Table 1. Frequency of HER1 copy number in ovarian carcinomas with different Histological Type in Malignant Tumors.

Histological Type Malignant Tumors		***Normal of HER1 signals***	***HER1 genetic gain***	***HER1 amplificatio***	***TOTAL***
Epithelial	N	**191**	**17**	**10**	**218**
	%	87.61	7.79	4.58	
Serous	N	94	6	5	105
	%	89.52	5.71	4.71	
Mucinous	N	22	2	1	25
	%	88	8	4	
Endometrioid	N	17	2	0	19
	%	89.47	10.52		
Clear cell	N	8	1	0	9
	%	88.88	11.11		
Brenner tumors	N	1	0	0	1
	%	100.0			
Combined	N	18	3	1	22
	%	81.81	13.63	4.54	
Undifferentiated tumors	N	20	2	1	23
	%	86.95	8.69	4.34	
Unclassification	N	11	1	2	14
	%	78.57	7.14	14.28	
Non epithelial	N	**4**	**1**	**1**	**6**
	%	66.66	16.66	16.66	
Sex-cord stromal tumours	N	2	0	1	3
	%	66.66		33.33	
Granulosa cell tumours	N	1	0	0	1
	%	100.0			
Embryonal carcinoma	N	1	1	0	2
	%	50	50		
TOTAL	N	195	18	11	224
	%	87.05	8.03	4.91	

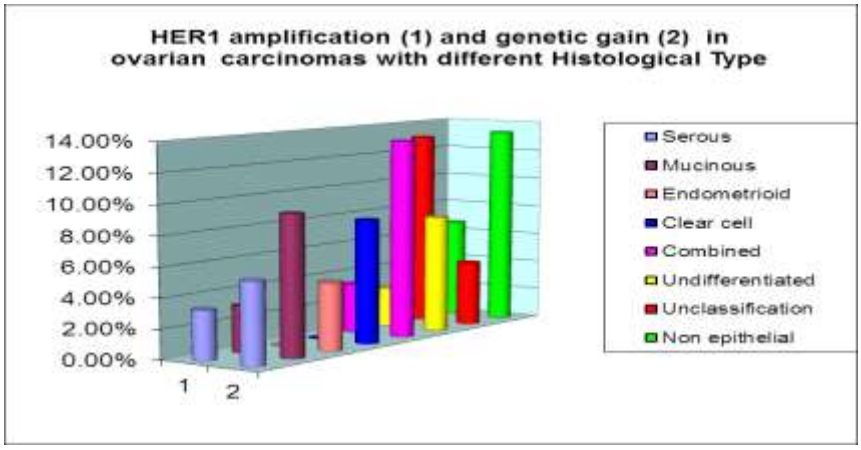

Figure 1. Description HER1 copy number in ovarian carcinomas with different Histological Type in Malignant Tumors

Table 2. Frequency of HER1 copy number in ovarian carcinomas with different Histological Type in Low Malignant Tumors.

Malignant Tumors Low		**Normal of HER1 signals**	**HER1 genetic gain**	**TOTAL**
Epithelial	N	**23**	**2**	**25**
	%	92%	8%	
Serous	N	12	0	12
	%	100.0		
Mucinous	N	9	2	11
	%	81.81	18.18	
Endometrioid	N	0	0	0
	%			
Clear cell	N	0	0	0
	%			
Brenner tumors	N	0	0	0
	%			
Combined	N	2	0	2
	%	100.0		
Undifferentiated tumors	N	0	0	0
	%			
Unclassification	N	0	0	0
	%			
Non epithelial	N	0	0	0
	%			
Sex-cord stromal tumours	N	0	0	0
	%			
Granulosa cell tumours	N	0	0	0
	%			
Embryonal carcinoma	N	0	0	0
	%			
TOTAL	N	23	2	25
	%	92	8	

Table 3. Frequency of HER1 copy number in ovarian carcinomas with different Histological Type in Benign Tumors.

Benign Tumors		Normal of HER1 signals	HER1 genetic gain	TOTAL
Epithelial	N	**97**	**6**	**103**
	%	94.17	5.82	
Serous	N	39	3	42
	%	92.85	7.14	
Mucinous	N	19	1	20
	%	95	5	
Endometrioid	N	33	2	35
	%	94.28	5.71	
Clear cell	N	0	0	0
	%			
Brenner tumors	N	1	0	1
	%	100.0		
Combined	N	3	0	3
	%	100.0		
Undifferentiated tumors	N	0	0	0
	%			
Unclassification	N	2	0	2
	%	100.0		
Non epithelial	N	**22**	**2**	**24**
	%	91.66	8.33	
Sex-cord stromal tumours	N	9	1	10
	%	90	10	
Granulosa cell tumours	N	2	0	2
	%	100.0		
Embryonal carcinoma	N	11	1	12
	%	91.66	8.33	
TOTAL	N	119	8	127
	%	93.70	6.29	

DISCUSSION

the purpose of our study was to analyze the correlations between gene copy number changes and tumor phenotype and determine the frequency of epidermal growth factor receptor gene amplification and gain in a large number of ovarian carcinomas, arranged in a tissue microarray(TMA), in order to assess their role in ovarian cancer development . Literature data for the occurrence of frequency of HER1 gene amplifications and gains in ovarian tumors are insufficient(88). We used fluorescence in situ hybridization (FISH)for evaluation of the HER1 copy number changes.[14,15] In our collection there were ovarian tumors from all histological types and subtypes[13] Examining the relationship between the increased HER1 concentrations and invasive phenotype, Alper et al. have found that most of ovarian

carcinomas analyzed (70-100%) express high HER1 levels.[16] On examination of 40 patients with ovarian carcinomas and 7 with low malignant potential tumors (LMP), Nagai et al. analyzed HER1 expression. They have found HER1 expression in 76.6% (30/39) of malignant tumors and in 42.9% (3/7) of low malignant potential tumors.[17] In another study, the authors have established 58.7% HER1 activity in malignant tumors and 50% in benign ones.[18] On examination of 60 serous cystadenocarcinomas, Ilekis et al. demonstrated that more than 98% of ovarian tumors expressed significant HER1 levels.[19] The reason for the detected high expression percentage probably lies in the fact that the other studies have used different techniques for analysis of the same carcinoma type.[20] The reason for result differences may lie in varying range of detection method sensitivities. Microaaray technology is the optimal for evaluating the relationship between increased copy number of a certain molecular marker and tumor phenotype. In the study presented, we used the FISH method applied on ovarian tumor TMAs with HER1 specific probe to assess HER1 amplifications and gains and to evaluate their input in the development of benign, low malignant potential and malignant ovarian tumors of various stage and grade. Microarray technology enabled us to determine HER1 oncogene role in ovarian carcinoma progression.[13, 20] In the present study, we have found that frequency of aberrant HER1 copy numbers in epithelial carcinomas is 12.37%. The frequency of HER1 gains in epithelial malignant tumors is 7.79% and in non-epithelial, it is comparatively higher – 16.66%. Using comparative genome hybridization (CGH), studies on ovarian carcinomas have found additional genetic material in 7p.[21,22,23] On examination of 24 differentiated ovarian carcinomas, most frequent genetic alterations have been found in chromosomes 1, 2, 7, 8, 9, 13 and 17.[22] The frequencies, by which aberrant oncogene copy numbers are determined in different studies, depend greatly on the detection method used, amplification definition and number of tumors analyzed.[23] Alterations of HER1 are probably an early event in ovarian carcinoma development as HER1 gains have been also found in benign tumors. The genetic gain is mostly due to numerical or structural chromosome aberrations involving the short arm of 7 - 7p chromosome. In these cases, gene activation is influenced by adjacent sequences or other more distantly localized factors.[24] An increased (Gains) HER1 copy number has been found in almost all epithelial carcinomas except for Brenner tumor (only one of this type has been analyzed). The highest frequency of HER1 amplifications has been observed in non-classifiable carcinomas (14.28%) and this of gains – in combined epithelial carcinomas (13.63%). Kiechle et al. analyzed 106 primary ovarian carcinomas through CGH and found amplification mostly in 7p and 8q chromosome areas.[25] Our results suggest that alterations in HER1 copy number are

particularly characteristic for the light cell ovarian tumors. We not found amplification in tumors with low malignant and Benign ovarian tumors . This is in consent with Ross et al., who reported that HER1 amplification is of exceptional occurrence in low malignant ovarian tumors compared to malignant ones.[26] Another study has found significantly decreased expression of HER1 protein in low malignant potential tumors and Benign ovarian tumors compared to carcinomas.[27] The authors have suggested that the number of HER1 positive adenocarcinomas increases with disease progression.[25] while genetic gain HER1 alterations was detected in low malignant just in Mucinous , and in Benign the highest frequency of HER1 has been observed in serous (7.14%), which means that HER1 alterations are not an early phenomenon in ovarian tumorogenesis .[26] This is supported by the study of Werness et al., suggesting that increased HER1 expression occurs later in invasive tumor development and has its role in the late pathogenesis of ovarian carcinoma.[28] In conclusion, the high number of ovarian tumors analyzed in our TMA allowed us to rapidly identify statistically significant associations between HER1 copy number changes and histological type. The data provide apicture of the incidence of HER1 gains and amplifications in Benign tumors, in tumors with Low malignant potency, and in Malignant ovarian tumors, and the gene being of great importance to cancerogenesis, and to examine it in the contest of various tumor phenotypes.

REFERENCE

1. Bartlen ,J.S Methods in Molecular Medicine."Ovarian cancer methods and protocols". Humana Press., 39(1): 3-5, Inc., Totowa, NJ.
2. Jemal, A., Thomas, A., Murray, T., and Thun, M. Cancer statistics, 2007. CA ancer J Clin, 2007; 57: 43-66.
3. Ohsaki, Y. et al. Epidermal growth factor receptor expression correlates with poor prognosis in non-small cell lung cancer patients with p53 overexpression. Oncol. Rep., 2000; 7: 603-607.
4. Robert C. Bast, Jr. and Gordon B. Mills." Alterations in Oncogenes, Tumor Suppressor Genes,and Growth Factors Associatedwith Epithelial Ovarian Cancers". Methods in Molecular Medicine, Humana Press, Inc., Totowa, NJ., 39.
5. Olayioye MA. "Update on HER-2 as a target for cancer therapy: intracellular signaling pathways of ErbB2/HER-2 and family members". Breast Cancer Res., 2001; 3(6): 385–389.

6. Santin AD, Bellone S, Roman JJ, McKenney JK, Pecorelli S. "Trastuzumab treatment in patients with advanced or recurrent endometrial carcinoma overexpressing HER2/neu". Int J Gynaecol Obstet., 2008; 102(2): 128–31.
7. Coussens L. "Tyrosine Kinase Receptor with Extensive Homology to EGF Receptor Shares Chromosomal Location with neu Oncogene". Science., 1985; 230: 1132–1139
8. Slamon, D. J., Godolphin, W., Jones, L. A., et al. Studies of the HER-2/neu protooncogene inhuman breast and ovarian cancer. *Science.*, 1989; 244: 707–712.
9. . Berchuck, A., Kamel, A., Whitaker, R., et al. Overexpression of HER-2/*neu* is associatedwith poor survival in advanced epithelial ovarian cancer. *Cancer Res.*, 1990; 50: 4087–4091.
10. Wolff AC, Hammond EH, Schwartz JN, et al. Arch Pathol Lab Med., 2007; 131: 18-43.
11. Sliwkowski MX. In: Harris JR, Lippman ME, Morrow M, Osborne CK, eds. *Diseases of the Breast*. 3rd ed. Philadelphia, PA: Lippincott Williams & Wilkins., 2004; 415-426.
12. Rubin S.C., Finstad C.L., Wong G.Y. et al. Prognostic significance of HER-2/neu expression in advanced epithelial ovarian cancer: A multivariate analysis. Am J Obstet Gynecol., 1993; 168: 162-169.
13. -Zeyade, Mohammed." Tissue Microarray Analysis of EGFR Copy Number Changes In Lung Cancer". Msc. degree. Sofia University-Faculty of biology., 2008.
14. Wang MB, Alavi S, Engstrom M, et al. Detection of chromosome 11q13 amplification in head and neck cancer using fluorescence in situ hybridization. Anticancer Res., 1999; 19: 925_/31.
15. Hui R, Campbell DH, Lee CS, et al. EMS1 amplification can occur independently of CCND1 or INT-2 amplification at 11q13 and may identify different phenotypes in primary breast cancer. Oncogene., 1997; 15: 1617_/23.
16. Alper-O; Bergmann-Leitner,-E-S; Bennett,-T-A; Hacker,-N-F; Stromberg,-K; Stetler-Stevenson,-W-G. Epidermal growth factor receptor signaling and the invasive phenotype of ovarian carcinoma cells. J-Natl-Cancer-Inst., 2001 Sep 19; 93(18): 1375-1384.
17. Nagai,-N; Oshita,-T; Fujii,-T; Katsube,-Y; Matsubayashi,-S; Ohama,-K. Are DNA ploidy and epidermal growth factor receptor prognostic factors for untreated ovarian cancer? A prospective study. Am-J-Clin-Oncol., 2001 Jun; 24(3): 215-221.
18. Harlozinska,-A; Bar,-J-K; Sobanska,-E; Goluda,-M. Epidermal growth factor receptor and c-erbB-2 oncoproteins in tissue and tumor effusion cells of histopathologically different ovarian neoplasms. Tumour-Biol., 1998; 19(5): 364-373.

19. Ilekis,-J-V; Connor,-J-P; Prins,-G-S; Ferrer,-K; Niederberger,-C; Scoccia,-B. Expression of epidermal growth factor and androgen receptors in ovarian cancer. Gynecol-Oncol., 1997 Aug; 66(2): 250-254.
20. Henzen-Logmans SC, Berns EM, Klijn JG, van der Burg ME, Foekens JA. Epidermal growth factor receptor in ovarian tumours: correlation of immunohistochemistry with ligand binding assay. Br J Cancer., 1992 Dec; 66(6): 1015-1021.
21. Hauptmann S., Denkert C., Koch I., Petersen S., Schluns K., Reles A., Dietel M., Petersen I. Genetic alterations in epithelial ovarian tumors analyzed by comparative genomic hybridization. Hum Pathol., 2002 (Jun); 33(6): 632-641.
22. Kraggerud,-S-M; Szymanska,-J; Abeler,-V-M; Kaern,-J; Eknaes,-M; Heim,-S; Teixeira,-M-R; Trope,-C-G; Peltomaki,-P; Lothe,-R-A. DNA copy number changes in malignant ovarian germ cell tumors. Cancer-Res., 2000 Jun 1; 60(11): 3025-3030.
23. 95-Tapper,-J; Butzow,-R; Wahlstrom,-T; Seppala,-M; Knuutila,-S. Evidence for divergence of DNA copy number changes in serous, mucinous and endometrioid ovarian carcinomas. Br-J-Cancer., 1997; 75(12): 1782-1787.
24. Dimova, I., Zaharieva, B. , Raicheva, S.,et al. (2004) Association of cyclinD1 copy number changes with histological type in ovarian tumors Acta Oncologica., 2004; 43(7): 675_/679.
25. Kiechle M., Jacobsen A., Schwarz Boeger U., Hedderich J., Pfisterer J., Arnold N. Comparative genomic hybridization detects genetic imbalances in primary ovarian carcinomas as correlated with grade of differentiation. Cancer., 2001; Feb 1; 91(3): 534-540.
26. Hunter T. Cooperation between oncogenes. Cell., 1991; 64: 249.
27. Egan S.E., Wright J.A., Jarolim L. at al. Transformation by oncogenes encoding protein kinases induces the metastatic phenotype. Science., 1987; 238: 202-205.
28. Hart I.R. Mechanisms of tumor cell invasion. Cancer Biol Rev., 1981; 2: 29-58.
29. Johnson-Early et al., 1980; Kawahara et al., 1998; Knudsen, Schulman, Fowler, & van den Hoek, 1984; Nordquist, Simon, Cantor, Alberts, & Gepler, 2004; Richardson et al., 1993; Zhou et al., 2006.
30. Kononen, J., Bubendorf, L., Kallioniemi, A., Barlund, M., Schraml,P., Leighton, S., Torhorst, J., Mihatsch, M. J., Sauter, G., and Kallioniemi, O. P. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat. Med., 1998; 4: 844–847.