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[1] Department of Chemotherapy, St John's Cancer Center, Lublin, Poland;
[2] Department of Clinical Pathomorphology, Medical University in Lublin, Lublin, Poland;
[3] St John's Cancer Center, Lublin, Poland;
[4] Occupational Hygiene and Ergonomic Dept., Lublin University of Technology, Lublin, Poland
[5] Radio-Diagnostic Department, St John's Cancer Center, Lublin, Poland;
[6] Department of Medical Genetics Medical University in Lublin, Lublin, Poland;

TOMASZ KUBIATOWSKI[1], ELŻBIETA STAROSŁAWSKA[1],
JUSTYNA SZUMIŁO[2], KRZYSZTOF J. CZARNOCKI[3,4],
FRANCISZEK BURDAN[5], JACEK WOJCIEROWSKI[6]

Analysis of expression profile of genes encoding cellular receptor proteins in endometrial cancer

Analiza profilu ekspresji genów kodujących białka receptorowe w komórkach raka endometrium

INTRODUCTION

Endometrial cancer is one of the most common gynecological malignancies. For the Polish population its incidence is estimated at 15 per 100,000 women per year. Most cases (90%) of newly diagnosed endometrial cancer are sporadic while remaining 10% arise from genetic background and are diagnosed in younger women with positive family history of this cancer. The majority (80-90%) of sporadic endometrial carcinomas, designated as Type I carcinomas, are estrogen-related tumors and they are associated with endometrial hyperplasia showing expression of estrogen and progesterone receptors. These tumors arise in relatively younger pre or post-menopausal women and are associated with either endogenous or exogenous, unopposed estrogen exposure [3]. Histologically, they are low-grade, endometrioid endometrial carcinomas but the rare mucinous adenocarcinomas are also considered Type I carcinomas, since they usually express estrogen and/or progesterone receptors. Clinically, Type I carcinomas are usually characterized by a favorable prognosis. About 10-20% of sporadic endometrial cancers, designated as Type II, are not associated with estrogen stimulation and showing negative or weakly positive ER and PR expression. These tumors usually arise at atrophic endometrium and occur at an older age. Histologically, they are high-grade carcinomas of nonendometrioid differentiation, most frequently serous, less frequently clear cell. Type II carcinomas are characterized by an aggressive clinical course and poor prognosis [11]. This dualistic model for endometrial cancer was established over 20 years ago and, in general, it describes morphological differences between Type I and II very well. In the last decade the great effort

was done to understand the molecular pathways leading to neoplastic transformation of endometrial cell. According to accumulated data it is well known that each type of endometrial cancer is characterized by specific molecular alternations. Microsatellite instability [4], PTEN inactivation [6] or β-catenin mutations [12] are typical for Type I, while p53 mutations [14] and HER2/neu alternation are often seen in Type II of endometrial cancer [2,10]. Correlation of histological and molecular features of endometrial cancer revealed inconsistency because some tumors show mixed or overlapping morphological and molecular features of both Type I and Type II endometrial carcinomas. For example it has been proposed that occasional serous carcinomas could develop through the dedifferentiation of pre-existing endometrioid carcinomas [5]. Therefore, in daily practice many endometrial carcinomas are in the grey zone with overlapping clinical, morphologic, immunohistochemical and molecular features.

The aim of our study was to profile expression of cellular receptor genes to candidate those typical for tumor grade or clinical stage by the mean of macroArray technique.

MATERIAL AND METHODS

Histopatologically confirmed neoplastic tissue derived from 40 patients diagnosed with endometrial cancer was used for RNA isolation performed according to TRI reagent procedure. Patients' characteristic is presented in Table 1. The control group comprised 10 healthy patients who underwent hysterectomy by the reason other than tumor disease. After quantity and quality estimation isolated RNA was used in MacroArray experiments. Into our work we incorporated BD Atlas ™ Human Cancer 1.2 Array (BD Bioscience Clontec, Palo Alto, USA) and all experimental procedure was done according to recommended manual. Briefly, during the first step DNA contamination was removed and pure RNA was used for reverse transcription in order to prepare probe labeled with radioisotope [α-^{32}P]. Radiolabeled probe was then hybridized to expression array "carrying" 1180 of cDNAs representing genes important for neoplastic transformation. Plasmid and bacteriophage DNAs spotted on the array served as negative controls to confirm hybridization specificity. Several housekeeping cDNAs also spotted on the array served as positive controls for normalizing mRNA abundance. When the hybridization step was completed intensive membrane washing was performed and the result of experiment was visualized using radioactivity scanner (Cyclone, Perkin Elmer, USA). In order to obtain hard copy of each result the autoradiography was done. After signal normalization final results were analyzed using AtlasImage™ software (BD Biosciences Clontech, Palo Alto, USA). In order to analyze statistic dependences between gene expression profiles and tumor grade or clinical stage we performed Kruskal-Willis test, using Statistica software (Statsoft v 8.0).

RESULTS

The analysis of statistic dependences between gene expression profiles and tumor grade pointed out nine genes encoding cellular receptor proteins, which are significantly over- or under-expressed (4 and 5 genes respectively) depending of tumor grade (data presented in Table 2). What is interesting these alternations are observed only in material derived from G2 and G3 tumors and they are not detected in G1. Most cases

of G2 endometrial cancers were characterized by statistically important over-expression of three genes encoding proteins possessing tyrosine kinase activities. These are met proto-oncogene (hepatocyte growth factor receptor), TYRO protein tyrosine kinase binding protein and Fc fragment of IgG, receptor, transporter, alpha. In G2 group of tumors statistically significant under-expression of genes encoding cellular receptors were not observed and it was, according to our data, typical only for G3 tumors. Statistically important signal distribution in the group of G3 tumors was distinctive from that noted for G2 tumors. Aryl hydrocarbon receptor gene, SEX gene, TRK-fused gene, bone morphogenetic protein receptor gene and oncostatin M receptor gene were significantly under-expressed in the analyzed G3 endometrial cancers. In comparison to G2 tumors only one gene encoding golgi apparatus protein 1 was over-expressed in poorly differentiated G3 endometrial cancers.

The analysis of statistic dependences between gene expression profiles and clinical stage of tumor pointed out 15 genes encoding cellular receptor proteins. Those expressions were altered showing statistically significance for different clinical stage (data presented in Table 3). In the case of 14 genes significant over-expression was noted and, what is most important, 11 of them were over-expressed in stage III according to FIGO. Statistically important over-expression of genes encoding cellular receptor proteins for each clinical stage of endometrial cancer is shown below. TYRO protein tyrosine kinase binding protein gene, met proto-oncogene (hepatocyte growth factor receptor) were over-expressed in analyzed tissue derived from FIGO stage I endometrial cancer, whereas low density lipoprotein-related protein 2 gene was noted only in FIGO stage II tumors. Genes encoding Notch homolog 1, Notch homolog 4, retinoic acid receptor beta, retinoid X receptor beta, tumor necrosis factor receptor superfamily member 1B, tumor necrosis factor receptor superfamily member 10b, retinoic acid receptor gamma, fms-related tyrosine kinase 4, PTK7 protein tyrosine kinase 7, G protein-coupled receptor 4, TEK tyrosine kinase, were over-expressed in RNA isolated from tissue derived from FIGO stage III endometrial cancer. As shown in Table 3, only one gene, TRK-fused gene, presented statistically significant under-expression in FIGO stage III endometrial cancer.

Table 1. Characteristics of patients diagnosed with endometrial cancer included into this study

Grade	Number of patients	%	Median Age	Last menses
G1	6	12.2	63.6	51.3
G2	29	59.2	62.6	51.6
G3	5	10.2	71.8	51.0
Control	9	18.4	50.1	47.6
FIGO stage	Number of patients	%	Median age	Last menses
I	30	61.2	61.6	51.2
II	5	10.2	67.7	51.5
III	5	10.2	52.2	50.7
Control	9	18.4	50.1	47.6

Table 2. Statistic dependences between expression of selected gene and tumor grade

Gene name	G grade	Median	Range		p-value	
			Min	Max	H	p
SEX gene	G1	0.24	0.00	0.55	7.04	0.029
	G2	0.06	0.00	0.60		
	G3	0.00	0.00	0.00		
Oncostatin M receptor	G1	0.08	0.00	0.20	10.34	0.005
	G2	0.00	0.00	0.24		
	G3	0.00	0.00	0.00		
golgi apparatus protein 1	G1	0.19	0.12	0.29	6.25	0.043
	G2	0.07	0.00	0.50		
	G3	0.05	0.00	1.20		
Fc fragment of IgG, receptor, transporter, alpha	G1	0.69	0.00	0.88	6.44	0.039
	G2	0.18	0.00	1.44		
	G3	0.00	0.00	0.41		
Met proto-oncogene	G1	0.10	0.00	0.31	6.75	0.034
	G2	0.04	0.00	0.69		
	G3	0.00	0.00	0.00		
TRK-fused gene	G1	0.36	0.21	0.69	9.67	0.007
	G2	0.13	0.00	0.48		
	G3	0.00	0.00	0.23		
Bone morphogenetic protein receptor, type II	G1	0.35	0.16	0.58	13.38	0.001
	G2	0.10	0.00	0.62		
	G3	0.00	0.00	0.15		
Aryl hydrocarbon receptor	G1	0.27	0.17	0.58	10.48	0.005
	G2	0.09	0.00	0.95		
	G3	0.00	0.00	0.00		
TYRO protein tyrosine kinase binding protein	G1	0.46	0.00	0.85	6.41	0.040
	G2	0.11	0.00	9.13		
	G3	0.00	0.00	0.09		

Table 3. Statistic dependences between expression of selected gene and tumor stage

Gene name	FIGO	Median	Range		p-value	
			Min	Max	H	p
G protein-coupled receptor 4	I	0.00	0.00	0.00	15.35	0.0008
	II	0.00	0.00	0.00		
	III	0.00	0.00	2.22		
Notch homolog 1,	I	0.00	0.00	0.17	10.15	0.006
	II	0.00	0.00	0.07		
	III	0.02	0.00	1.23		
Notch homolog 4	I	0.00	0.00	0.00	7.00	0.030
	II	0.00	0.00	0.00		
	III	0.00	0.00	0.76		
Low density lipoprotein-related protein 2	I	0.00	0.00	0.00	14.35	0.0008
	II	0.00	0.00	0.36		
	III	0.00	0.00	0.00		
TYRO protein tyrosine kinase binding protein	I	0.12	0.00	9.13	7.00	0.030
	II	0.49	0.09	0.85		
	III	0.00	0.00	0.10		
Met proto-oncogene	I	0.04	0.00	0.69	6.94	0.031
	II	0.13	0.00	0.31		
	III	0.00	0.00	0.00		
TRK-fused gene	I	0.15	0.00	0.69	6.14	0.046
	II	0.25	0.20	0.52		
	III	0.00	0.00	0.22		
PTK7 protein tyrosine kinase 7	I	0.00	0.00	0.00	14.35	0.0008
	II	0.00	0.00	0.00		
	III	0.00	0.00	2.16		
TEK tyrosine kinase, endothelial	I	0.00	0.00	0.00	14.35	0.0008
	II	0.00	0.00	0.00		
	III	0.00	0.00	1.06		
Tumor necrosis factor receptor superfamily, member 1B	I	0.00	0.00	0.26	7.39	0.024
	II	0.00	0.00	0.07		
	III	0.00	0.00	1.24		
Tumor necrosis factor receptor superfamily, member 10b	I	0.00	0.00	0.12	9.05	0.010
	II	0.00	0.00	0.00		
	III	0.00	0.00	1.19		
Retinoic acid receptor, beta	I	0.00	0.00	0.18	6.98	0.030
	II	0.00	0.00	0.15		
	III	0.00	0.00	1.00		
Retinoid X receptor, beta	I	0.00	0.00	0.00	7.00	0.030
	II	0.00	0.00	0.00		
	III	0.00	0.00	0.95		
Retinoic acid receptor, gamma	I	0.00	0.00	0.06	9.05	0.010
	II	0.00	0.00	0.00		
	III	0.00	0.00	0.77		
Fms-related tyrosine kinase 4	I	0.00	0.00	0.00	7.00	0.030
	II	0.00	0.00	0.00		
	III	0.00	0.00	0.27		

DISCUSSION

Molecular pathogenesis of endometrial cancer remains poorly understood. It is well known that all genes encoding proteins involved in cell cycle regulation, cell differentiation or surrounding tissue invasion contribute to tumor and metastases development. It has been stressed that different factors such as VEGF, which play a key role in neoplastic angiogenesis, are also very important for tumor growth. Complexity of molecular mechanisms leading to endometrial cancer development implies that only experiments utilizing array technique can collect enough data to understand all relationships between different molecular pathways during tumor development. Using MacroArray technique we have taken attempt to evaluate the expression profile of genes encoding cellular receptor proteins. Depending on analyzed parameter we selected several genes, which are able to characterize different grade or clinical stage of endometrial cancers. Comparison of genes describing grade of tumor with the representative of which for different clinical stage revealed that over-expression of c-met proto-oncogene and TYRO protein encoding gene usually observed in G2 endometrial cancer is correlated with clinical stage I according to FIGO classification. Hepatocyte growth factor (HGF) is a masenchymal-derived cytokine, which interact with its tyrosine kinase receptor encoded by c-met proto-oncogene [15]. The over-expression of HGF/c-met complexes has been observed in many malignancies such as breast [1], gastrointestinal [8], renal [7] or endometrial cancer [16]. It is postulated that in case of endometrial cancer the interaction between HGF and c-met is related to tumor invasion [9], angiogenesis [16] or stimulates anti-apoptotic pathways [13]. Moreover, previous studies have reported, that HGF activates mainly two kinase cascades, the extra-cellular signal-related kinase (ERK) and PI3K/AKT signaling pathways, which interact with PTEN protein. TRK-fused gene (TFG) is another gene, which can be involved in etiopathogenesis of endometrial cancer. It is a novel gene first discovered in the rearranged form. TFG is involved in the generation of a class of ALK oncogenes in anaplastic large cell lymphoma and has been detected as a novel fusion partner for NORI in extraskeletal myxoid chondrosarcoma. As shown in previous studies TFG gene is ubiquitously expressed in human adult tissues. The major characteristic of TFG is the presence of a coiled-coil domain, which exerts a crucial role in TRK-T3 oncogenic activation by mediating the formation of protein complexes. In addition to the coiled-coil domain, the TFG protein also contains several consensus sites, which suggest possible interactions with other proteins. These include a PB1 domain, putative phosphorylation sites for PKC and CK2, glycosylation sites, as well as SH2- and SH3-binding sites. In the case of our study the expression of TRK-fused gene was down-regulated in tissue material derived form patient diagnosed with G3 endometrial cancer showing clinical stage III according to FIGO classification.

Introducing of gene expression profiling based on array analysis put a great effort to understand the role of different molecular pathways in neoplastic transformation. It allowed distinguishing several genes that seemed to be most crucial for tumor formation. It has to be stressed that gene expression profiling is only the first step on the way towards of apprehension of cancer. Understanding of mechanisms underlying alternation of genes expression is the real key to establish new prognostic factors and tailored therapy for endometrial cancer.

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SUMMARY

Endometrial cancer (EC) is one of the most common gynaecological malignancy. Unfortunatelly, molecular pathogenesis of this neoplasm remains poorly understood. More than 90% od EC cases are sporadic and they can be divided into two main subgroups. The first group consists of oestrogen-related tumours that occur in pre and post-menopausal women and are frequently proceded by endometrial hyperplasia. These tumours are usually associated with abnormalities of DNA-mismatch repair genes, k-ras, PTEN and beta-catenin. The second group consists of tumours that occurs mainly in post-menopausal women and which are usually not related to oestrogen. These tumours are characterized by abnormalities of p53 and HER2/neu. The aim of our study was to profile expression of cellular receptor genes to candidate those typical for tumor grade or clinical stage by the mean of macroArray technique.

Based on Kruskal-Wilis test we pointed out nine genes encoding cellular receptor proteins showing statistically significant over or under-expression (4 and 5 genes respectively) depending tumor grade. The analysis of statistic dependences between gene expression profiles and clinical stage of tumor pointed out 15 genes encoding cellular receptor proteins the expressions of which were altered showing statistical significance for different clinical stages. It is worthy to note that understanding of mechanisms underlying alternation of genes expression is a real key to establish new prognostic factors and tailored therapy for endometrial cancer.

Keywords: endometrial cancer, MacroArray Technique, gene expression profile

STRESZCZENIE

Rak endometrium jest jednym z najczęstszych nowotworów narządu rodnego. Mechanizmy molekularne leżące u jego podłoża są jednak wciąż bardzo mało poznane. Ponad 90% raków endometrium pozbawionych jest podłoża rodzinnego i zaliczanych jest do jednej z dwóch grup. Grupę I stanowią nowotwory estogenozależne diagnozowane u kobiet przed lub po menopauzie, powstajace na podłożu zmian hyperplastycznych endometrium. Cechują się one zaburzeniem funkcji genów systemu naprawy DNA, KRAS, PTEN oraz beta-kateniny. Grupa druga obejmuje nowotwory estrogenowo niezależne, diagnozowane w wieku pomenopauzalnym i cechujące się zaburzeniem funkcji p53 oraz HER2/neu.

Celem niniejszej pracy było opisanie, przy użyciu techniki makromacierzy, profilu ekspresji genów kodujących białka spełniające funkcje receptorowe w komórce reprezentatywnego dla stopnia zróżnicowania histopatologicznego lub zaawansowania klinicznego nowotworu. Zastosowanie testu Kruskala-Wilisa pozwoliło na wyodrębnienie 9 genów wykazujących istotne statystycznie zwiększenie lub obniżenie ekspresji w zależności od stopnia złośliwości histologicznej nowotworu. Analiza statystyczna zależności ekspresji genów oraz klinicznego stopnia zaawansowania pozwoliła na wyodrębnienie 15 genów wykazujących istotne zmiany ekspresji. Dla pełnego poznania czynników prognostycznych oraz opracowania terapii celowanych w raku endometrium konieczne jest jednak poznanie mechanizmów leżących u podłoża opisywanych zmian.

Słowa kluczowe: rak endometrium, technika makromacierzy, profilowanie ekspresji genów