2012 © Curr. Issues Pharm. Med. Sci. Vol. 25, No. 4, Pages 452-455

Current Issues in Pharmacy and Medical Sciences

Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA
on-line: www.umlub.pl/pharmacy

Analysis of expression profile of genes encoding cell adhesion proteins in endometrial cancer

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ABSTRACT

Endometrial cancer is one of the most common gynecological malignancies. Unfortunately, molecular pathogenesis of this neoplasm remains poorly understood. Using MacroArray technique, we analyzed expression profile of genes encoding adhesion proteins in neoplastic tissue derived from 40 patients diagnosed with the endometrial cancer between 2003 and 2004. Analysis of statistic dependences between gene expression profiles and tumor grade pointed out thirteen genes encoding adhesion proteins (cadherin-1, -5, -8, -13, -15, integrin alpha-1, -6, integrin beta-1, -8, zyxin related protein-1, paxillin, catenin alpha-1), which are significantly over- or under-expressed (11 and 2 genes respectively) depending of tumor grade. Taking under consideration clinical stage of the cancer we pointed out eleven genes (integrin alpha-8, integrin beta-1, -3, cadherin-8, -15, catenin beta-1, paxillin, trombospondin-2, ninjurin-1, desmocollin-3) encoding proteins crucial for cell-cell interaction those expressions were altered showing statistically significance for different clinical stage according to FIGO classification.

Keywords: endometrial cancer, expression profile of genes, adhesion proteins

INTRODUCTION

Endometrial cancer is one of the most common gynecological malignancies in Polish population with incidence estimated at 15 per 100,000 women per year. The most cases (80-90%) of newly diagnosed sporadic endometrial carcinomas are estrogen-related tumors (type I) and they are associated with endometrial hyperplasia showing expression of estrogen (ER) and progesterone (PR) receptors. These tumors arise in relatively younger pre- or post-menopausal women and are associated with either endogenous or exogenous, unopposed estrogen exposure [12]. 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 [19]. In the last decade, the great effort has been done to understand the molecular pathways leading to neoplastic transformation of endometrial cell. According to accumulated data cancer development is characterized by self-sufficiency in growth signals, insensitivity to growth inhibition, evasion of apoptosis, angiogenesis, invasion of surrounding tissue and metastasis formation. For this last process, impairment of tissue integrity is most crucial. In mammalian epithelial cells, cell-cell adhesion is mediated by protein complexes organized into distinct functional structures termed tight junction, adherens junction and desmosomes. The tight junction forms a diffusion barrier between cells and the extracellular environment [1], desmosomes resist mechanical stress across the epithelium and maintain tissue integrity [16]. Moreover, alternation of expression of certain genes can also modu-

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late the responsiveness of neoplastic cells to standard regimens of chemotherapy [15, 21] and severity of cytotoxic therapy side effects [7-9].

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

MATERIALS 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. Patient's characteristic is presented in Table 1. 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 [?-^{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).

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

Grade	Number of patients	%	Mean age (years)	Last period age (years)
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	%	Mean age (years)	Last period age (years)
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

RESULTS

Analysis of statistic dependences between gene expression profiles and tumor grade pointed out thirteen genes encoding adhesion proteins, which are significantly over- or under-expressed (11 and 2 genes respectively) depending of tumor grade (data presented in Table 2). Most cases of G3 endometrial cancers were characterized by statistically important over-expression of eight genes. These are cadherin 1 (type 1, E-cadherin), cadherin 15 (M-cadherin), cadherin 8 (type 2), integrin beta 1, cadherin 13 (H-cadherin), integrin beta 8, zyxin related protein-1 (ZRP-1) and proteasome activator subunit 1 (PA28 alpha). In comparison to poorly differentiated G3 tumors only two genes were over-expressed in G2 and one in G1 endometrial cancer tissue. These are cadherin 5 (vascular epithelium-cadherin), paxillin and integrin alpha 6 respectively. In contrast, only one gene encoding catenin alpha 1 (cadherin-associated protein) was under-expressed in examined tissue and it was observed in G1 endometrial cancer exclusively.

Table 2. Statistical dependences between expression of selected genes and tumor grade

Gene	Gene name	Grade G	Median	Range		Statistical analysis	
				Min	Max	H	p
A14a	cadherin 1, type 1, E-cadherin (epithelial)	G1	0.00	0.00	0.00	7.00	0.0302
A14a		G2	0.00	0.00	0.00		
A14a		G3	0.00	0.00	0.11		
D02d	cadherin 5, type 2, VE-cadherin (vascularepithelium)	G1	0.59	0.53	0.77	7.15	0.028
D02d		G2	0.37	0.09	1.44		
D02d		G3	0.31	0.21	1.17		
D04d	cadherin 15, M-cadherin (myotubule)	G1	0.00	0.00	0.00	7.00	0.0302
D04d		G2	0.00	0.00	0.00		
D04d		G3	0.00	0.00	0.10		
D05d	cadherin 8, type 2	G1	0.00	0.00	0.00	7.00	0.0302
D05d		G2	0.00	0.00	0.00		
D05d		G3	0.00	0.00	0.18		
D05e	integrin, alpha 6	G1	0.03	0.00	0.20	11.23	0.0036
D05e		G2	0.00	0.00	0.05		
D05e		G3	0.00	0.00	0.12		
D06f	integrin, beta 1 (fibro- nectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)	G1	0.00	0.00	0.00	7.00	0.0302
D06f		G2	0.00	0.00	0.00		
D06f		G3	0.00	0.00	0.36		
D06g	cadherin 13, H-cadherin (heart)	G1	0.00	0.00	0.00	7.00	0.0302
		G2	0.00	0.00	0.00		
D07d	integrin, beta 8	G1	0.88	0.72	1.00	6.89	0.032
D07d		G2	0.51	0.05	1.74		
D07d		G3	0.09	0.00	2.49		
D08f	paxillin	G1	0.30	0.21	0.39	9.38	0.0092
D08f		G2	0.08	0.00	0.67		
D08f		G3	0.00	0.00	0.45		
D09d	catenin (cadherin-associated protein), alpha 1 (102kD)	G1	0.45	0.37	0.73	7.05	0.029
D09d		G2	0.24	0.00	1.62		
D09d		G3	0.60	0.07	2.09		
D11f	Zyxin-related protein ZRP-1	G1	0.00	0.00	0.00	7.00	0.0302
D11f		G2	0.00	0.00	0.00		
D11f		G3	0.00	0.00	010		
F05b	proteasome (prosome, macropain) activatorsubunit 1 (PA28alpha)	G1	0.13	0.00	0.27	11.75	0.0028
F05b							

Analysis of statistic dependences between gene expression profiles and clinical stage of tumor pointed out

eleven genes encoding proteins crucial for cell-cell interaction those expressions were altered showing statistically significance for different clinical stage (data presented at table 3). In the case of nine genes, significant over-expression was noted and what is most important, seven of them were over-expressed in stage III according to FIGO [18]. Statistically important over-expression of genes encoding adhesion proteins for each clinical stage of endometrial cancer is presented below. Integrin alpha 8, cadherin 8 type 2, integrin beta 1, catenin (cadherin-associated protein) alpha 2, thrombospondin 2, ninjurin 1, desmocollin 3 were overexpressed in analyzed tissue derived from FIGO stage III endometrial cancer, whereas over-expression of genes encoding cadherin 15 (M-cadherin) and integrin beta 3 binding protein (beta 3-endonexin) were noted only in FIGO stage II tumors. Only two genes, paxillin and catenin beta 1 (cadherin-associated protein), were under-expressed in examined tissue and this phenomenon was observed only in samples derived from patients in clinical stage III according to FIGO [18].

Table 3. Statistical dependences between expression of selected genes and tumor stage

Gene	Gene name	FIGO stage	Median	Range		Statistical analysis	
				Min	Max	H	p
D04d	cadherin 15, M-cadherin (myotubule)	I	0.00	0.00	0.00	7.00	0.0302
D04d		II	0.00	0.00	0.10		
D04d		III	0.00	0.00	0.00		
D04f	integrin, alpha 8	I	0.00	0.00	0.00	14.35	0.0008
D04f		II	0.00	0.00	0.00		
D04f		III	0.00	0.00	2.08		
D05d	cadherin 8, type 2	I	0.00	0.00	0.00	7.00	0.0302
D05d		II	0.00	0.00	0.00		
D05d		III	0.00	0.00	0.18		
D06f	integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includesMDF2, MSK12)	I	0.00	0.00	0.00	7.00	0.0302
D06f		II	0.00	0.00	0.00		
D06f		III	0.00	0.00	0.36		
D07g	catenin (cadherin-associated protein), alpha 2	I	0.00	0.00	0.00	14.35	0.0008
D07g		II	0.00	0.00	0.00		
D07g		III	0.00	0.00	2.50		
D08d	thrombospondin 2	I	0.00	0.00	0.00	14.35	0.0008
D08d		II	0.00	0.00	0.00		
D08d		III	0.00	0.00	1.54		
D08f	paxillin	I	0.11	0.00	0.67	8.96	0.011
D08f		II	0.26	0.21	0.45		
D08f		III	0.00	0.00	0.08		
D09e	catenin (cadherin-associated protein), beta 1 (88kD)	I	0.00	0.00	0.31	7.13	0.028
D09e		II	0.13	0.00	0.25		
D09e		III	0.00	0.00	0.13		
D09f	integrin beta 3 binding protein (beta3-endonexin)	I	0.00	0.00	0.00	7.00	0.0302
D09f		II	0.00	0.00	0.12		
D09f		III	0.00	0.00	0.00		
D11d	neural cell adhesion molecule 1	I	0.00	0.00	0.00	7.00	0.0302
D11d		II	0.00	0.00	0.00		
D11d		III	0.00	0.00	0.08		
D11f	ninjurin 1	I	0.00	0.00	0.00	7.00	0.0302
D11f		II	0.00	0.00	0.00		
D11f		III	0.00	0.00	0.10		
D14g	desmocollin 3	I	0.00	0.00	0.00	7.00	0.0302
D14g							
D14g							

DISCUSSION

Despite of great effort, molecular pathogenesis of endometrial cancer remains poorly understood. Using Macro-Array technique we have taken attempt to evaluate the expression profile of genes encoding cellular proteins involved in tissue integrity preserving. 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 whose representative for different clinical stage revealed that overexpression of genes encoding cadherin 8, integrin beta 1 or ZRP1 (zyxin related protein 1) usually observed in G3 endometrial cancer is correlated with clinical stage III according to FIGO classification [18]. Moreover, over-expression of cadherin 15 observed in tissue derived also from G3 tumors was correlated with clinical stage II according to FIGO classification. Cadherin family includes cell adhesion molecules responsible for cell-to-cell recognition and adhesion in solid tissue [11, 24]. They can be divided into several groups. The first one named classic cadherins comprises calcium-dependent hemophilic adhesion molecules frequently associated with specific junctional structures. They are expressed in several types of tissue with some specificity. For example, E-cadherin is mostly observed in epithelial cells, N-cadherin in the nervous system, smooth muscle cells, fibroblast and endothelial cells whereas VE-cadherin is specific for the endothelium [6, 11]. The cadherin family includes also desmosomal cadherins (desmocollins, desmogleins), atypical cadherins (T-cadherins) and protocadherins which are characterized by the presence of a variable number of EC domains linked to a cytoplasmic tail which presents no homology with classic cadherins. The loss of cadherin-mediated cell adhesion is typical for transition from a normal epithelium to poorly differentiated carcinoma [14, 23]. Cells undergoing this process lose their polarity, become invasive, and resist apoptosis. Accumulated data suggests that different cadherins are involved in metastatic process. For example, aberrant expression of N-cadherins in selected cell lines promotes invasion and metastasis. N-cadherin has also been shown to increase metastasis from mouse mammary tumors [13]. Another particle, OB-cadherin is expressed by metastatic prostate cancer cells and bone osteoblasts [3]. These observations have led to the hypothesis that this cell adhesion molecule is a modulator of prostate cancer cell metastasis to bone [22]. Furthermore, cadherins can associate to growth factor receptors and modulate their intracellular signaling. It is postulated that, depending on the types of cells and on the receptors expressed, cadherins may play different and cell-specific roles [4]. Another protein playing a crucial role in cellular adhesion stabilization is paxillin.

According to our results, under-expression of paxillin gene was observed in G2 tumor sections and correlated with FIGO clinical stage III. Focal adhesion plaques (FAP) are structures that form at the ends of actin fibers and serve as sites for transmission. Proteins at focal adhesion plaques form multiple interaction and signal transduction networks, which regulate cell adhesion, spreading, motility and convey signals into the nucleus to regulate gene transcription, cell proliferation, differentiation and apoptosis. The proper function of FAP structure depends on LIM domain proteins composed of zyxin, paxillin and several related proteins. They do not have enzymatic domains and serve as adaptor proteins for the assembly of multiple protein complexes in different subcellular compartments. The zyxin subfamily includes zyxin Trip6 (thyroid hormone interacting protein 6), LPP (lipoma preffered partner) and Ajuba [2]. These proteins share high sequence similarity, especially within the LIM domain region. Mammalian paxillin family members include paxillin, Hic-5 and leupaxin [20]. In comparison to zyxin, paxillin family proteins have additional protein interaction domains within the N-terminal domain. Biological function of zyxin/paxillin proteins remains unclear. According to literature, these proteins can affect steroid hormone receptor activity in a hormone-dependent manner [10, 17, 25]. As it was shown by Degenhardt at al [5], zyxin via its LIM domain region interacts with the E6 oncoprotein of human papillomavirus type 6. Because of interaction, zyxin is moved from focal adhesion plaques to nucleus and enhances transcriptional activation by GAL4-zyxin protein. Implementation 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 us to distinguish several genes seemed to be most crucial for cell adhesion impairment during transformation of endometrial cells. We still have to remember that most important is to understand the reason underlying observed genes expression alternation in order to establish new prognostic factors and tailored therapy for endometrial cancer.

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