Abstracts of the 2009th Alumni Association Medical Research Fund Prize Memorial Lecture (2)

Proteomic Approach to Identify Specific Molecules Related to Ovarian Steroid Hormone-dependent Diseases

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Introduction

Progression of both endometrial cancer arising from glandular epithelial cells of the uterine lining and endometriosis characterized by ectopic endometrium-like tissue depends on ovarian steroid production[1–3]. The normal endometrium consists of a single layer of glandular epithelium and surrounding endometrial stromal cells which demonstrate a wide spectrum of appearances throughout the menstrual cycle. The glandular epithelium proliferates under the influence of estrogen and subsequently demonstrates secretory changes after ovulation under the additional influence of progesterone, which is necessary for blastocyst implantation. Stromal cells also proliferate under the influence of estrogen and demonstrate morphologic and functional changes known as decidualization under the influence of progesterone, which is important for the establishment and maintenance of pregnancy. If the lesions of ovarian steroid-dependent diseases maintain the characteristics of normal endometrium, estrogen and progesterone have stimulatory and suppressive effects, respectively, on the disease progression. The actions of ovarian steroids are mediated through their receptors and transcriptionally regulate the expression of many ovarian steroid-responsive genes. Thus, many molecules are believed to be regulated by the effects of ovarian steroids in the lesion and to be involved in the progression or suppression of ovarian steroid-dependent disease. These molecules are candidate biomarkers and molecular targets for therapy.

In general, molecules to be used as biomarkers and therapeutic targets are expected to be specific to the pathophysiology of the disease. In this respect, a proteomic approach, which analyzes finally translated products rather than transcripts, has an advantage over a genomic approach for screening and identifying these molecules. In the present study, we used a focused differential proteomic approach to identify specific molecules related to ovarian steroid-dependent diseases related to the endometrium.

Methods

Ishikawa, a human endometrial adenocarcinoma cell line of epithelial origin that expresses both estrogen receptor α and progesterone receptor, was cultured in phenol-red free minimum essential medium supplemented with 10% fetal bovine serum, 100 U/mL of penicillin, and 100 mg/mL of streptomycin with ovarian steroid hormone (E: 10 nM beta-estradiol; or E+P: 10 nM beta-estradiol + 10 μM progesterone) or without (Control). After 72 hours, cellular protein was extracted and dissolved in urea. Dissolved protein was labeled with fluorescent dye and subjected to 2-dimensional gel electrophoresis. Protein spots were visualized and analyzed with an image analysis system (Progenesis, Durham, NC). Protein spots demonstrating a change in

Table 1 List of molecules identified by LC-MS/MS analyses (C<E>E+P)

	control	Estrogen	Estrogen + Progesteone	Category
A	1.401	1.605	1.180	Lectin
B	1.352	1.771	1.311	Miscellaneous function
C	1.078	1.456	0.996	Nucleic acid binding
D	1.088	1.683	1.302	Miscellaneous function

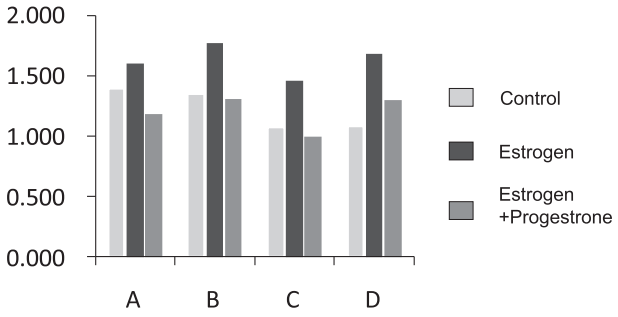

Fig. 1
Quantitative analyses of protein expression patterns (C<E>E+P)

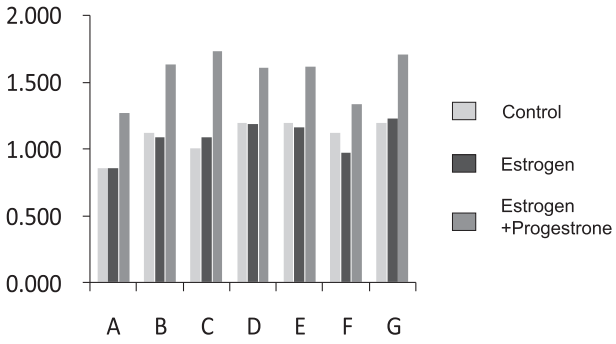

Fig. 2
Quantitative analyses of protein expression patterns (C=E<E+P)

Table 2 List of molecules identified by LC-MS/MS analyses (C=E<E+P)

	control	Estrogen	Estrogen + Progesteone	Category
A	0.855	0.858	1.267	Oxidoreductase
B	1.121	1.086	1.635	Nucleic acid binding
C	1.009	1.085	1.736	Hydrolase
D	1.197	1.189	1.613	Transcription factor
E	1.196	1.160	1.616	Protease
F	1.119	0.976	1.333	Miscellaneous function
G	1.193	1.232	1.706	Lyase

protein expression of more than 140% were analyzed and identified with liquid chromatography-mass spectrometry/mass spectrometry.

Results and Discussion

On differential 2-dimensional gel electrophoresis analysis, 20 protein spots showed a significant difference in expression. Liquid chromatographic-mass spectrometric/mass spectrometric analysis demonstrated that these molecules included heat shock protein, which is overexpressed in endometrial cancer, and galectin-1, whose expression is regulated in relation to the stage of the menstrual cycle. On the basis of quantitative analyses, these expressions can be classified into the following patterns: 1) Control<E<E + P; 2) Control<E>E + P; 3) Control=E<E + P; 4) Control<E=E + P; 5) Control>E<E + P; and 6) Control>E>E + P. The expression of molecules classified in pattern 2 was stimulated under the influence of estrogen and suppressed by the addition of progesterone, which matches the proliferation kinetics of normal endometrial tissue (**Table 1**, **Fig. 1**). Therefore, it is indicated that these molecules are directly involved in normal physiological processes during the ovarian cycle and in the pathophysiology of ovarian steroid-dependent diseases. Molecules classified in

expression pattern 3 are stimulated under the influence of progesterone and are considered to be involved downstream of progesterone signaling (**Table 2**, **Fig. 2**). In this regard, these molecules are candidate therapeutic targets for progestin-resistant cases of low-grade endometrial cancer or endometriosis. Further investigation targeting each molecule to clarify expressive localization and stimulatory or suppressive roles in the lesion will provide insight into the pathophysiology of ovarian steroid-dependent diseases and the foundation for developing novel biomarkers and molecularly targeted therapies.

References

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