Application Note: Proteomic Analysis of Laser Microdissected FFPE Tissue Using Mass Spectrometry

Discovery of Novel Protein Biomarkers in Endometrial Cancer Using Formalin-Fixed Paraffin-Embedded Tissue and Mass Spectrometry

Endometrial cancer is the most common cancer of the female reproductive system found in the United States. If the cancer is detected early, a large percentage of patients survive at least five years. A study was undertaken to identify candidate protein biomarkers for early detection of endometrial cancer using formalin fixed paraffin embedded tissue samples. A collection of well documented formalin-fixed endometrial tissues was assembled, and microdissected utilizing the Director® laser microdissection technology to collect both early stage cancerous epithelium and normal epithelium.

Methods

Liquid Tissue® lysates were prepared from microdissected epithelial cells obtained from both early stage endometrial cancer and normal endometrium. Global mass spectrometry profiling of all lysates followed by spectral count quantitation indicate differential expression of proteins that include known cancer biomarkers; low-abundance proteins such as transcription factors and signal pathway proteins; and housekeeping proteins. These differentially expressed proteins also include candidate protein biomarkers of early stage endometrial cancer which can be utilized as improved diagnostic, prognostic, and therapeutic targets (Figure 1). The proteomics platform utilized is diagrammed in Figure 3.

Protein Name	Cancer Samples																																					
Glutathione S-transferase P	5	5	6	6	6	5	7	4	7	2	5	5	2	1	1	3	3	3	7	11	1	8	9	1	0	12	3	6	3	4	8	5						
Transgelin-2	7	5	6	5	5	6	5	9	7	6	4	5	2	1	0	2	3	3	6	6	1	6	7	4	2	5	0	6	5	1	1	6						
6-phosphogluconate dehydrogenase	6	5	4	6	5	7	2	4	3	3	6	4	1	3	1	4	2	2	6	11	1	4	1	0	0	9	0	5	1	11	3	4						
Vinculin	11	17	5	11	11	21	22	12	11	7	14	14	3	9	4	10	8	9	6	9	0	4	16	1	0	4	1	3	1	2	0	4						
ELAV-like protein 1	2	2	1	1	4	2	2	2	1	0	1	1	1	1	1	2	1	2	3	3	1	2	0	0	0	4	1	2	0	1	2	1						
Transmembrane protein Tmp21	3	3	1	2	1	0	2	1	1	1	1	0	0	0	1	0	0	1	2	0	1	1	1	1	1	1	1	1	2	1	3	1						
Galectin-3-binding protein	3	4	6	3	1	0	2	1	1	5	2	1	0	1	0	1	1	2	2	5	0	5	2	0	1	5	0	2	3	4	5	0						
MARCKS-related protein	1	2	2	0	1	2	0	1	1	1	1	1	2	2	1	2	2	2	1	1	1	3	0	0	0	1	0	0	1	0	2	1						
Tenascin	0	9	1	3	6	2	21	6	6	0	1	4	2	3	2	5	1	1	1	2	0	2	5	0	0	4	0	0	6	1	0	1						
14-3-3 protein eta	2	1	2	1	2	1	2	2	1	3	1	1	1	1	1	2	1	1	1	2	1	0	0	0	0	1	0	1	0	1	1	1						
Proteasome activator complex subunit	3	1	4	1	2	4	1	1	3	2	1	1	0	0	0	4	1	2	1	3	0	5	2	1	1	0	1	2	3	0	2	2						
Glucosidase 2 subunit beta	3	4	5	2	3	6	5	4	5	3	3	4	0	2	1	3	2	1	2	3	0	5	3	0	0	5	0	2	1	0	5	1						

Protein Name	Normal Samples																																					
Glutathione S-transferase P	1	1	0	1	2	3	2	1	0	2	0	0																										
Transgelin-2	1	0	2	0	0	3	3	0	0	1	0	0																										
6-phosphogluconate dehydrogenase	0	0	1	0	1	1	1	2	0	0	0	0																										
Vinculin	4	1	0	0	2	0	2	3	0	1	0	0																										
ELAV-like protein 1	1	1	0	1	0	1	0	0	0	0	0	0																										
Transmembrane protein Tmp21	1	0	0	0	0	0	0	0	0	0	0	0																										
Galectin-3-binding protein	1	1	0	1	0	0	0	0	0	0	0	0																										
MARCKS-related protein	0	0	0	0	0	0	0	0	0	0	0	0																										
Tenascin	0	0	0	0	0	0	0	0	0	0	0	0																										
14-3-3 protein eta	0	0	0	0	1	1	0	0	0	0	0	0																										
Proteasome activator complex subunit	0	0	0	0	0	1	1	2	0	0	0	0																										
Glucosidase 2 subunit beta	0	1	0	1	1	0	0	1	0	0	1	0																										

= High Abundance (≥6 peptides)
= Medium Abundance (3-5 peptides)
= Low Abundance (1-2 peptides)
= Not Detected

Figure 1: Heatmap based on the Spectral count analysis of the 33 endometrial cancer samples and 12 normal endometrial samples. The number of peptides identified for each of the 12 proteins in each sample in this study is shown. The color code reflects the spectral count abundance of these proteins in each sample.

Results

Histologically-defined cases of early stage endometrial cancer and normal endometrial epithelium were profiled for protein expression.

- 33 early stage endometrial cancer samples and 12 normal endometrial tissues were microdissected utilizing Director® microdissection technology (Figure 2).

- >13,000 unique proteins and >31,000 unique peptides were identified and analyzed across the entire study by this global proteomic profiling strategy.

- Generation of a heatmap based on the actual spectral count for each protein in each sample indicates increased expression of these 12 proteins in histologically-defined early stage endometrial cancer over histologically-defined normal endometrium (Figure 3).

- Increased expression in cancer vs normal was determined by the total number of unique peptides identified in each cancer sample as compared to each normal sample (Table 1).

- Additional criteria for determination of increased protein expression in cancer vs normal was developed by comparing the ratio of the average number of peptides from a given protein in each cancer sample to the average number of peptides for that same protein in each normal sample (Table 1).

- A total of 140 proteins were found to have significant differential expression in cancer vs normal, of which only the 12 most significant are shown (Table 1).

Figure 2: Histology of normal endometrial epithelium and early stage endometrial cancer.

Normal Endometrium *Cancerous Endometrium*

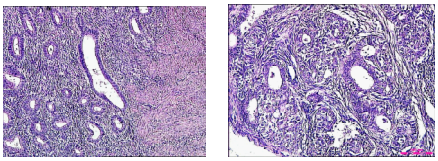


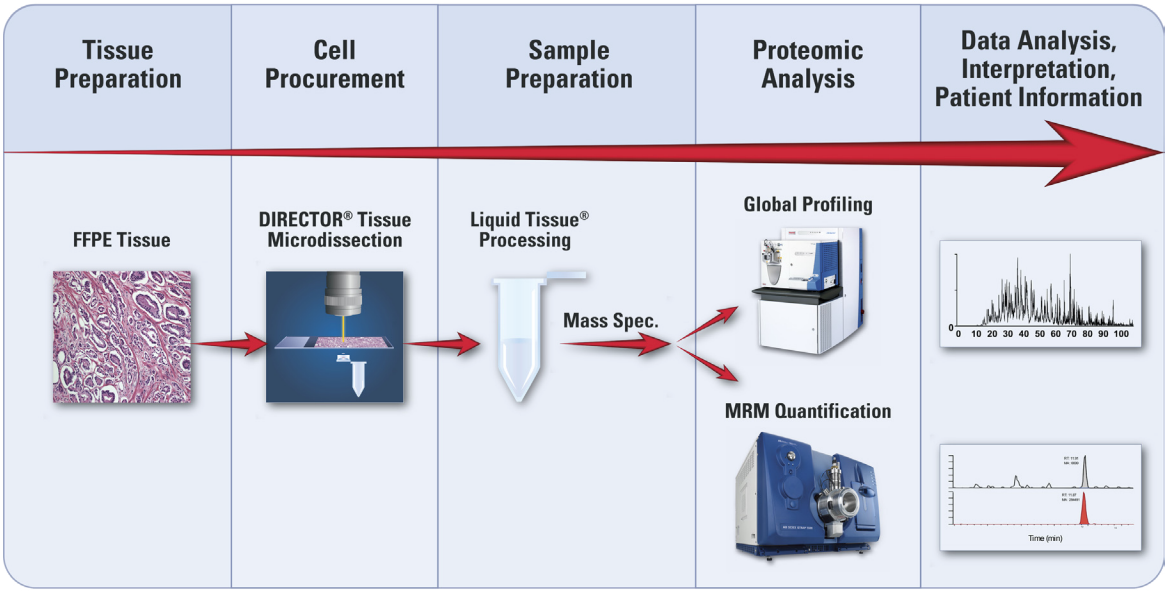
Table 1: Spectral count analysis of the 12 most differentially expressed proteins; defined by total number of unique peptides identified per protein across all cancer/normal samples, average number of peptides per protein identified per cancer/normal sample, ratio between avg. peptides per cancer sample vs avg. peptides per normal sample, and total percentage of cancer/normal samples where each protein expressed.

Protein Name	Accession	# Peptides in Cancers	# Peptides in Normals	Avg. Peptides per Cancer Sample	Avg. Peptides per Normal Sample	Ratio	% Cancers Expressing Protein	% Normals Expressing Protein
Glutathione S-transferase P	P09211	156	13	4.7	1.1	4.4	95%	67%
Transgelin-2	P37802	137	10	4.2	0.8	5.0	86%	42%
6-phosphogluconate dehydrogenase	P52209	124	6	3.8	0.5	7.5	81%	42%
Vinculin	P18206	250	13	7.6	1.1	7.0	81%	50%
ELAV-like protein 1 (Hu-antigen R)	Q15717	47	4	1.4	0.3	4.3	76%	33%
Transmembrane protein Tmp21	P49755	36	1	1.1	0.1	13.1	71%	8%
Galectin-3-binding protein	Q08380	72	3	2.2	0.3	8.7	71%	25%
MARCKS-like protein 1	P49006	35	0	1.1	0.0	--	67%	0%
Tenascin	P24821	95	0	2.9	0.0	--	67%	0%
14-3-3 protein eta (Protein AS1)	Q04917	35	2	1.1	0.2	6.4	67%	17%
PA28-alpha	Q06323	54	4	1.6	0.3	4.9	67%	25%
Glucosidase 2 subunit beta	P14314	83	5	2.5	0.4	6.0	67%	42%

Conclusion

- Liquid Tissue® reagents and protocol enable in-depth proteomics analysis of formalin fixed tissue by high resolution LC-ESI-MS/MS.
- Expression of thousands of proteins can be quantified by the spectral count method for discovery of biomarkers of histologically-defined disease.
- Candidate biomarkers of early stage endometrial cancer were identified by spectral count analysis of global profile data.
- Hundreds of differentially expressed proteins were discovered providing a large number of candidate biomarkers of early stage endometrial cancer.
- Heat map analysis indicates 12 high value proteins that are over-expressed in endometrial cancer cells vs normal endometrial cells.
- Such proteins can provide for biomarkers to improve diagnosis, prognosis, and therapy of endometrial cancer.

Figure 3: Tissue Microproteomics Technology Platform.



Reference

Discovery of Novel Protein Biomarkers in Formalin Fixed Paraffin Embedded Endometrial Cancer Tissue by Mass Spectrometry, David B. Krizman[1]; Marlene M. Darfler[1]; Jill Ray[2]; Mark Stoler[3]; Attila Lorincz[4]

[1]Expression Pathology Inc., Gaithersburg, Maryland; [2]Qiagen, Gaithersburg, MD., USA; [3]University of Virginia, Charlottesville, VA, USA; [4]Cancer Research, UK Centre of Epidemiology, London, UK

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Expression Pathology Inc.
9620 Medical Center Drive
Rockville, MD 20850
United States

Phone: +1.301.977.3654
Fax: +1.301.926.9283
http://www.expressionpathology.com
e-mail: custsvc@expressionpathology.com