number of these proteins were further validated by western-blotting confirming previous results.

In summary, we have investigated the regulation and biological activity of 1α,25(OH)2D3 in EMT of colon cancer cells. Through the use of proteomics tools we have identified numerous proteins whose expression levels were altered with 1α,25(OH)2D3 treatment, and some of them have been further validated. Our results show evidence of the direct role of 1α,25(OH)2D3 treatment in EMT in colon cancer through the transcriptional regulation of a number of genes mainly related to cell morphology, cell assembly, cell organization as well as cellular repair.

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Comprehensive proteomic analysis of human endometrial fluid aspirate

Juan Casado-Vela[1†], Eva Rodriguez-Suarez[1†], Ibon Iloro[1†], Amagoia Ametzazurra[2], Nere Alkorta[1], Juan Antonio García-Velasco[3], Roberto Matorras [4,5], Begoña Prieto[4,5], Sandra González[5], Daniel Nagore[2], Laureano Simón[2], Felix Elortza[1]*

[1]Proteomics Unit, CIC bioGUNE, CIBERehd, ProteoRed, Technology Park of Bizkaia, Derio, Spain, [2]Proteomika S.L.; Technology Park of Bizkaia. Derio, Spain. [3]Instituto Valenciano de Infertilidad, Madrid, Spain. [4]Hospital de Cruces, Barakaldo, Spain. [5]Instituto Valenciano de Infertilidad, Bilbao, Spain

Abstract

The endometrial fluid is a non-invasive sample which contains numerous secreted proteins representative of endometrial function. We show here, for the first time, an in depth analysis of the protein content of the EFA using proteomic techniques [1]. To achieve this objective, three different but complementary strategies were used. First, in solution digestion followed by reverse phase high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS); second, protein separation by denaturing one dimensional electrophoresis (SDS-PAGE) followed by HPLC-MS/MS analysis. Finally, two dimensional polyacrylamide gel electrophoresis (2D-PAGE) followed by MALDI-TOF/TOF analysis. The combination of the three strategies led to the successful identification of 803 different proteins. An extensive description of the endometrial fluid proteome will help provide the basis for a better understanding of a number of diseases and processes, including endometriosis, endometrial cancer and embryo implantation. We believe that the thorough catalogue of proteins presented here can serve as a valuable reference for the study of embryo implantation and for future biomar-

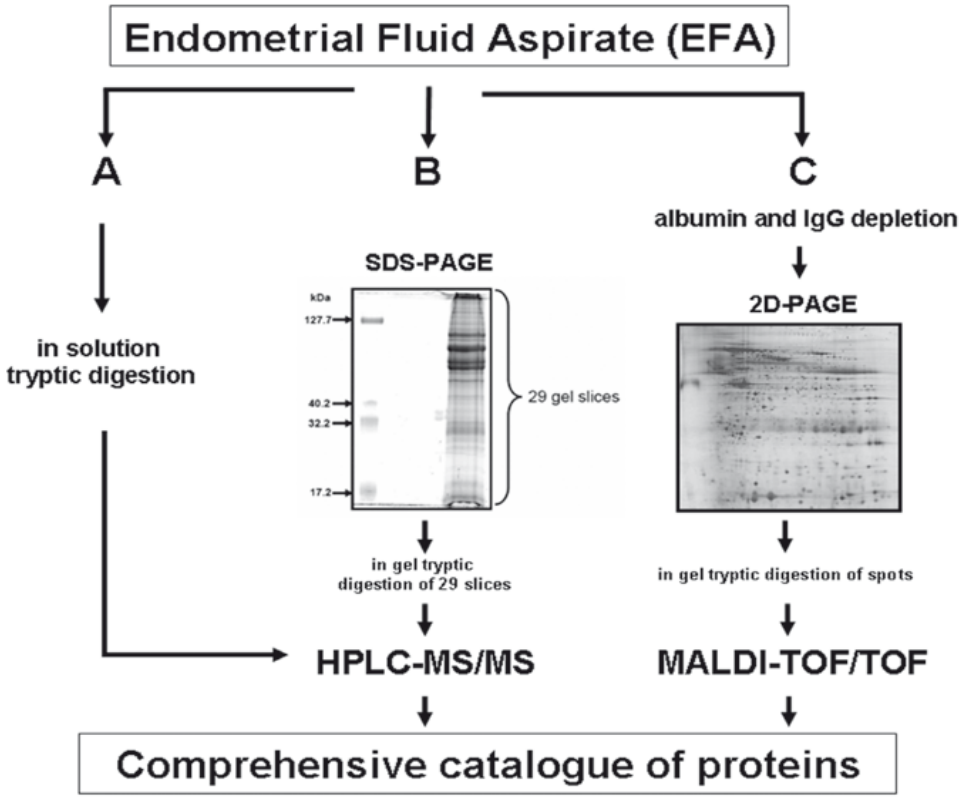

Figure 1. *Schematic view of the three strategies (A, B and C) followed for the analysis of endometrial fluid aspirate.*

ker discovery involved in pathologic alterations of endometrial function.

Communication

The endometrial fluid is a complex biological fluid which is in direct contact with the endometrial cavity and contains a multitude of proteins and proteolytic enzymes secreted from the endometrium. Currently the diagnosis of endometrial diseases such as endometriosis is achieved by invasive methods that often include laparoscopic surgery under general anaesthesia. The endometrial fluid can be collected by aspiration in a painless manner using non-invasive methods. Interest in the protein content of endometrial secretions has gained much momentum in recent years and has been suggested to play a key role in the embryo implantation process. Therefore, differential proteomics of the endometrial fluid aspirate proteome could give a new insight to understand the mechanisms involved in the onset of endometrial pathologies and the process of embryo implantation [2].

With the aim of achieving a thorough description of the proteome of the endometrial fluid aspirate we used three different methodological strategies (A, B and C) that combine chromatographic and gel separation methods, as depicted in Figure 1. The integrated proteomic approaches used here led, for the first time, to a thorough identification of the catalogue of proteins present in EFA.

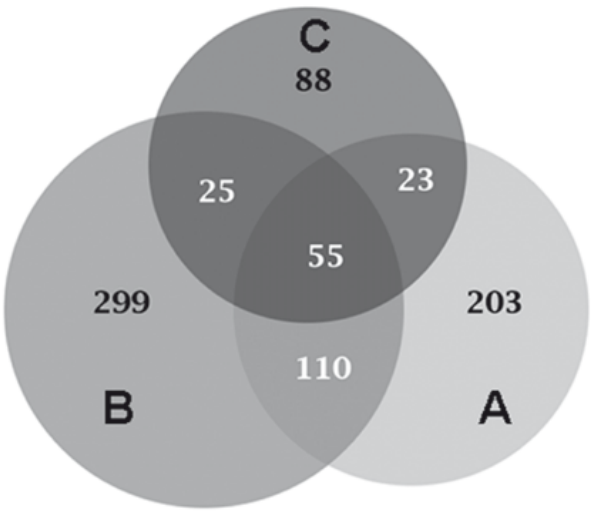

Figure 2. *Venn diagram comparing the number of proteins identified after the analysis of endometrial fluid aspirate with the three different strategies (A, B and C).*

Table 1. *Proteins identified in endometrial fluid aspirate (EFA) involved in endometrial alterations or in embryo implantation.*

Protein description	Endometriosis / e ctopic endometrium	endometrial cancer / vulvar cancer	embryo implantation	references
Matrix metalloproteinase 9	**X**			Kyama *et al*. Fertil. Steril. **2008**, 89, 301-310.
Tissue inhibitor of matrix metal-loproteinase	**X**			Kyama *et al*. Fertil. Steril. **2008**, 89, 301-310.
Annexin A1	**X**			Chun-yan *et al.* Chin. Med. J. **2008**. 121, 927-931. Ametzazurra *et al*. Human Repr. **2009**, 24, (4), 954-965
Mucin 16 / CA125	**X**	**X**		Lenhard *et al*. Clin. Chem. Lab. Med. **2009**, 537-542. Mol *et al.* Fertil Steril. **1998**, 70, 101–1108 Moore *et al*. Ultrasound Obstet Gynecol. **2002**, 20, 630-634. Gupta *et al.* **2006**, 13, 126-134.
Cyclophilin A/ peptidyl-prolyl cis-trans isomerase A		**X**		Li *et al.* Mol. Cell. Proteomics. **2008,** 7, 1810-1823.
Interleukin-18	**X**		**X**	Luo *et al.* Reprod. Immunol. **2006**, 72, 108-117.
Pyruvate kinase-M1/M2		**X**		De Souza *et al.* Mol. Cell. Proteomics. **2007**, 6, 1170-1182. De Souza *et al.* Proteomics. **2005**, 5, 270-281. De Souza *et al.* J. Proteome Res. **2008**, 7, 3525-3534
Alpha 1-antitrypsin/ Alpha-1 protease inhibitor/ Serpin 1		**X**		DeSouza *et al.* Mol. Cell. Proteomics. **2007**, 6, 1170-1182. DeSouza *et al.* Proteomics. **2005**, 5, 270-281. Hefler *et al*. Int. J. Cancer. **1999**, 83, 167-170.
Fatty acid-binding protein		**X**		**Li** *et al*. Int. J. Cancer. **2008**, 123, 2377-2383.
Vimentin Heat shock protein 70	**X**			ten Have *et al*. Proteomics Clin. Appl.. **2007**, 1, 1243-1251.
Vitamin D binding protein	**X**			Ferrero *et al.* J. Soc. Gynecol. Investig. **2005**. 12, 272-277
Carbonic anhydrase 1	**X**			Zhang *et al*. Fertil. Steril. **2006**, 86, 274-282
Heat shock protein 90 Annexin A2 Peroxiredoxin 2 Apolipoprotein A1	**X**			Fowler *et al*. Proteomics. **2006**, 7, 130-142.
Mucin 1			**X**	Achache *et al.* Hum. Reprod. Update. **2006,** 12, 731-746.
Protein S100-A8 Apolipoprotein A-1 Alpha 1-antitrypsin	**X**			Ferrero *et al*. JPR. **2007**, 6, 3402-3411

Moesin **Beta-actin** **Tubulin beta chain** **F-actin capping protein subunit beta** **WD repeat protein 1** **Heat-shock protein beta-1** **Rho GDP-dissociation inhibitor 1 and 2** **14-3-3 sigma and gamma** **Glycodelin** **Beta-2-glycoprotein 1** **Sialic acid synthase** **Alcohol dehydrogenase [NADP+]** **Glutathione transferase omega-1** **Ribose-phosphate pyrophosphokinase II** **Poly(rC)-binding protein 1** **Ferritin heavy chain**	**X**			Ametzazurra *et al*. Human Repr. **2009**, 24, 4, 954-965

The three different strategies followed for EFA analysis (A, B and C) led to the identification of 391, 489 and 191 proteins, respectively (Figure 2). Combining the three strategies, we successfully identified 803 proteins. To conclude, the most relevant proteins identified in EFA and involved in endometrial alterations or in embryo implantation according to the literature are shown in Table 1.

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Identification of Biomarkers in Colorectal Cancer (pre- & post-chemotherapy) by Nucleic Acids Programmable Protein Microarrays (NAPPA), iFISH and SNPs approaches

María González[1], Raquel Bartolomé[1], Jose María Sayagues[1], María González[1], Ana Laura Moro[1], Elena Andrada[1], Sahar Sibani[2], Josh LaBaer[2], Jacinto García[3], Alberto Orfao[1], Manuel Fuentes[1,2]

[1]Centro de Investigación del Cáncer. Universidad de Salamanca-CSIC. Salamanca. Spain. [2]Harvard Institute of Proteomics. Harvard Medical School. Boston. USA. [3]Departamento Cirugía. Hospital Clínico Universitario. Salamanca. Spain

Biomarkers, particularly those with strong positive and negative predictive value, have many potential uses in the diagnosis and treatment of cancer, including monitoring treatment success, indicating disease progression and detecting early disease. One potentially powerful approach to finding biomarkers is to exploit patients' own immune systems, which produce humoral responses to cancer antigens released by their tumors due to alterations in protein expression, mutation, degradation, or localization. Antibodies to tumor antigens have been detected as early as several years before the clinical