Regulation of epithelial-mesenchymal transition in colon cancer by 1α,25-dihydroxyvitamin D3: a proteomics approach

Iván Cristobo[1], María Jesús Larriba[2], Vivian De los Ríos[3], Ingrid Babel[1], Rodrigo Barderas[1], Alberto Muñoz[2], José Ignacio Casal[1]

[1] Functional Proteomics Laboratory, Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas. Madrid. [2] Department of Cancer Biology, Instituto de Investigaciones Biomédicas "Alberto Sols", Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid, Madrid. [3] Proteomics and Genomics Facility, Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, Madrid

Epithelial-mesenchymal transition (EMT) is a typical feature of cells undergoing proliferation and it is a fundamental process in the early stages of carcinoma invasion and metastasis [1-3]. It is a process characterized by loss of cell adhesion, repression of E-cadherin expression, and increased cell mobility (Figure 1).

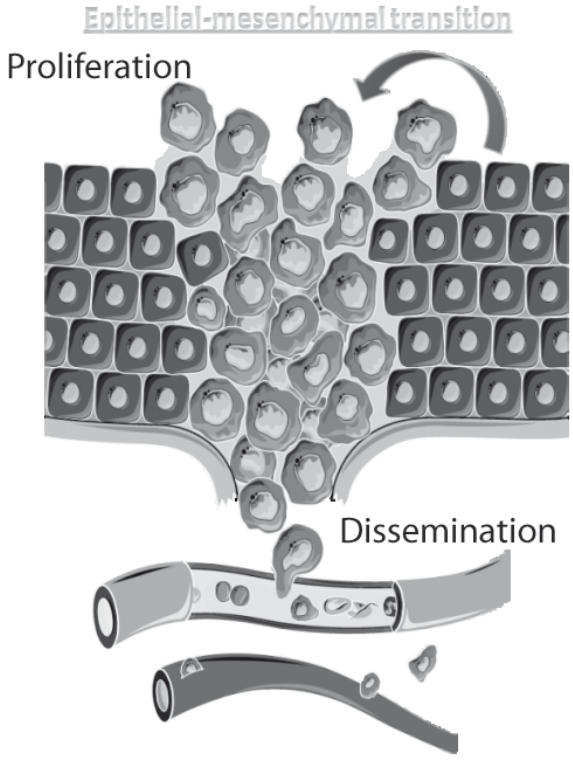

Figure 1. *Epithelial-mesenchymal transition. During EMT epithelial cells lose polarity and cell-cell contacts, become migratory, and divide at a faster rate. The cells can intravasate into lymph or blood vessels, allowing their passive transport to distant organs.*

There is an increasing interest in the active vitamin D metabolite 1α,25-dihydroxyvitamin D3 [1α,25(OH)2D3] and its analogs as preventive and therapeutic anticancer agents [4, 5]. 1α,25(OH)2D3 drastically alters the gene expression profile of many cell types, inhibiting the proliferation and promoting the differentiation to a normal adhesive epithelial phenotype of human colon cancer cells [6].

In this study we have investigated the regulation and biological activity of 1α,25(OH)2D3 in EMT of colon cancer cells. We present evidence of the direct regulation of a number of proteins by 1α,25(OH)2D3 in colon cancer cells.

SW480-ADH colon cancer cells were treated with 1α,25(OH)2D3 for 8 and 48 hours. Nuclear extracts of treated and non-treated cells were used by triplicate for a proteomic analysis. Proteins were labeled with CyDye Fluor for their subsequent separation by 2D-DIGE. Comparative analysis of protein expression patterns was performed with Progenesis SameSpots and Ludesi Redfin software. Differentially expressed proteins were identified by mass spectrometry (MS) MALDI-TOF/TOF and subsequently validated by western-blotting.

An average of 1600 spots was detected on 2D-DIGE gels. A total of 31 and 19 spots were found to be differentially expressed at 8 hours with Ludesi and Progenesis software ($p < 0.05$; fold-change >1.2), respectively; and a total of 84 and 71 spots at 48 hours ($p < 0.05$; fold-change >1.3). A 36% of overlapping in spot detection was found between the 2 software. By MS and MS/MS were unequivocally identified 17 and 72 proteins from treatment at 8 and 48 hours, respectively. These proteins were mainly involved in DNA and RNA regulation, affecting cell morphology, cell assembly, cell organization as well as cellular repair. A

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.

References

[1] Peinado H, Olmeda D, Cano A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? Nat Rev Cancer 2007; 7: 415-428.

[2] Perl AK, Wilgenbus P, Dahl U, Semb H, Christofori G. A causal role for E-cadherin in the transition from adenoma to carcinoma. Nature 1998; 392: 190-193.

[3] Vleminckx K, Vakaet L, Jr., Mareel M, Fiers W, van Roy F. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. Cell 1991; 66: 107-119.

[4] Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. Nat Rev Cancer 2007; 7: 684-700.

[5] Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. Am J Clin Nutr 2007; 85: 1586-1591.

[6] Palmer HG, Gonzalez-Sancho JM, Espada J, Berciano MT, Puig I, Baulida J, et al. Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling. J Cell Biol 2001; 154: 369-387.

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-