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 <i>et al</i> . Human Repr. <b>2009</b> , 24, 4, 954-965
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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

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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 bio-

markers 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

appearance of cancer. Although the specificity for these responses is high, typically only 5-20% of patients demonstrate a response to any given antigen, which has limited the usefulness of single antigen responses as biomarkers. The recent development of protein microarrays may offer an ideal tool for screening for immune response to tumor antigens. These arrays offer the advantage that hundreds to thousands of different proteins can be printed and screened simultaneously and only require a few microliters of serum per assay.

Prof. LaBaer's group (Harvard Institute of Proteomics) has developed a novel method for producing protein microarrays called nucleic acid programmable protein arrays (NAPPA) that avoids the need to express and purify the proteins by substituting the printing of cDNAs on the arrays, which are then

transcribed and translated in situ as needed at the time of the assay. NAPPA has been used successfully to map the pairwise protein interactions of the human DNA replication complex. Here, we propose adapting the NAPPA protein microarray technology for use in the rapid and efficient screening of sera from cancer patients for antibodies to 1000 known and potential tumor antigens in a multiplex format in order to better characterize the immune response to known tumor antigens, identify new informative tumor antigens and evaluate the value of using patterns of tumor antigen immune responses as biomarkers in Colorectal Cancer. For the validation of the possible biomarkers found in 20 different patients (pre- & post-chemotherapy), currently we are using iFISH and SNPs approaches with the main goal to correlate genomics and functional proteomics.

# **Detection of Prostate Cancer by Urine Proteomics**

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#### Introduction

Prostate-specific-antigen (PSA) serum measurement in combination with a digital rectal examination (DRE) and transrectal ultrasound-guided biopsy (TURS) is currently the gold standard for prostate cancer (PCa) screening in Europe [1]. Nevertheless, PSA and DRE lack significant specificity and biopsy lacks ideal sensitivity (12-30% false negatives) [2, 3]. Therefore additional biomarkers are needed to supplement or potentially replace the currently used diagnostic techniques. As the secreted products from both, normal prostate epithelial cells as well as PCa cells, can be detected in the urine of men, their use as a proximal body fluid to detect PCa is very attractive since they could be the best compromise between a minimal invasive technique accepted by a wider range of the male population and the possibility to obtain enough cells for a correct diagnosis [4, 5].

# **Objective**

We sought to determine a proteomic profile in urine able to distinguish between the presence and absence of PCa.

# Material&Methods

We used a combination of proteomic technologies in aged matched post-Digital Rectal Exam (DRE) urine supernatants specimens to identify differentially expressed proteins in patients with PCa. Firstly, we depleted 9 histological confirmed PCa urine samples and 9 control samples (age-matched patients with the typical background of benign prostate hiperplasy (BPH), atrophy and chronic inflammation) using ProteoMiner (BioRad), a novel depletion technique that reduces the level of the most abundant species, while strongly concentrating the more dilute and rare ones. Then, Two-dimensional