Conclusions: miRNA deregulation could have an impact on the diagnosis of prostate cancer. It is possible to define signal transduction pathways that may be influenced by miRNA deregulation.

miRNA expression patterns have the potential of being valuable biomarkers in the diagnosis of prostate cancer. It is possible to define signal transduction pathways that may be influenced by miRNA deregulation.

Introduction & Objectives: miRNAs are non-coding RNA molecules of about 21-25 nucleotides that are able to regulate gene expression on a post-transcriptional level via inhibition of protein synthesis. Hence, miRNAs can act as tumor suppressors or oncogenes. miRNA deregulation is commonly found in all types of malignancies and is supposed to play a major role in carcinogenesis. We examined the differential expression of miRNAs in tissue samples of prostate carcinomas.

Materials & Methods: We applied four independent methods to assess miRNA expression in two patient cohorts (deep sequencing, miRNA microarrays, real-time PCR and miRNA in situ hybridization). The ability of miRNA expression patterns to discriminate between cancerous and nonmalignant tissue samples was analyzed by binary logistic regression and filtered according to the posterior probability of equivalent expression and fold difference in expression. Results were validated by RT-qPCR in individual samples. To determine whether differences between CAF and NF extended to the protein level, secreted chemokines were evaluated with a multiplexed ELISA assay. CAF/NF effects on THP1 monocyte heterotypic cell adhesion, proliferation, metalloprotease activation and chemotaxis were evaluated.

Results: CAF and NF were 100% positive for vimentin and negative for pan-cytokeratins. Frequency of αSMA and smoothelin expression was lower (21.8±6% and 63.3±5%, respectively) in CAF vs. NF. CAF showed increased proliferative, migratory and invasive properties compared to NF. Thirty-five genes were up-regulated and fifty-seven genes were down-regulated in CAF vs. NF (fold-change>2; adjusted p-value<0.05). Known functions of all these genes relate to extracellular matrix organization and immune response (p<0.005). Array data and RT-qPCR yielded concordant results for up-regulated (CCL2, CCL7, CCL11) and down-regulated (CXCL1, CXCL6, CXCL8) chemokines. Analysis of chemokine profile in culture media we confirmed 2.49- and 7.87-fold up-regulation of CCL2 and CCL7, respectively (p<0.05), and 2.58-fold down-regulation of CXCL1 (p<0.05). In CAF vs. NF. Adhesion assays showed enhancement of monocyte adhesion to CAF compared to NF (1.7-fold; p=0.03). CAF conditioned media elicited a stronger chemotactic response on THP1 monocytes (1.4-fold; p=0.02) and a significative activation of THP1 metalloprotease-9 (p=0.001).

Conclusions: Identified altered gene expression in these cells likely contributes to carcinoma progression by modifying the extracellular matrix and the inflammatory chemokine profile, and modulating the biological response of inflammatory cells. Supported by Instituto de Salud Carlos III (FIS PI07-0536), Ministerio de Ciencia e Innovacion, Spain.

ANDROSTENEDIONE IS THE MOST EFFICIENT PRECURSOR OF INTRAPROSTATIC TESTOSTERONE PRODUCTION IN PRIMARY HUMAN PROSTATE CANCER TISSUE

Introduction & Objectives: Androgen-deprivation therapy for prostate cancer (PC) eventually leads to the castration-resistant phase of the disease (CRPC). Despite castrate levels of serum androgens, intraprostatic levels remain high and high androgen receptor gene expression is maintained. De novo steroidogenesis of androgens in situ has been proposed as a potential mechanism for the development of CRPC. We sought to determine the steroidogenic and metabolic potential of freshly cultured CRPC tissue and non-cancerous benign prostatic hyperplasia (BPH) tissue from patients.

Materials & Methods: Freshly excised prostatic tissue specimens were obtained from 15 patients undergoing transurethral resection of prostate (TURP); 7 patients had BPH only whilst 8 patients had pathologically confirmed PC with clinical CRPC. Tissue specimens were separately cultured in an excess of cholesterol, progesterone, androstenedione, Dehydroepiandrosterone (DHEA) or testosterone for 4 days. Samples were treated for steroid metabolite identification by GC/MS using a Shimadzu GCMS-QP2010 (n=15) with DHEA, testosterone, androgens of interest and two internal standards. Array data were analyzed using the HumanHT-12 v4 BeadChip (Illumina) and read using a BeadChip reader. Statistical analysis was performed on BeadStudio (Illumina).

Results: Analysis of some 15 different steroid metabolites indicated that non-cancerous prostatic tissue from BPH patients did possess the capacity to initiate de novo steroidogenesis and synthesize testosterone from cholesterol, albeit to very low levels. This property was lost in the CRPC tissue however, with no detectable testosterone or Dihydrotestosterone (DHT) production from cholesterol or progesterone in the prostate cancer tissue. Similarly, very low levels of testosterone were detected with DHEA as the precursor though this was upregulated in the PC samples. In marked contrast, Androstenedione was efficiently converted to testosterone in BPH tissue (levels 200 fold higher than with any other precursor) and this was upregulated in the PC tissue (500 fold higher). Testosterone was efficiently converted to DHT in BPH tissue and this conversion was upregulated 2.5 fold in the PC tissue. Steroidogenic enzyme gene expression levels correlated with the observed metabolic profiles of the tissue samples.

Conclusions: Direct analysis of the steroidogenic and metabolic potential of fresh