1. INTRODUCTION

Colorectal cancer (CRC) is the second cause of cancer death in western countries. The success of the therapy depends on an early diagnosis; the knowledge of the biological behavior in each tumor, and its susceptibility to different therapies. The 5-year survival rate after surgery depends on the ability to correctly classify it into different stages. This work was performed using an intermediate stage (C1) as reference. We determined the number of matched spots from the individual matchsets obtained from each patient. From these, we determined the number of matched spots from the individual matchsets obtained from each patient. From these, we determined the number of matched spots from the individual matchsets obtained from each patient.

2. MATERIALS AND METHODS

Tissues and patients: A total of 120 tissue samples (120 from patients with CRC in different stages, and 12 samples from patients without CRC) were obtained from Cruces Biologic (Santurtzi, Gipuzkoa, Spain). The samples were collected in 200 μl of RNA Later Stabilization Solution (Qiagen) and stored at -80°C.

RNA extraction: Total RNA was extracted from all the samples using the RNeasy Mini Kit (Qiagen). RNA quantity and quality was determined with the Agilent 2100 Bioanalyzer (Calyxo, Barcelona, Spain). We used the RNA Integrity Number, a quality standard to select the samples. We synthesized and labeled the cRNA using the Agilent Low RNA input Fluorometric Linear Amplification Kit (Agilent Technologies). cRNA hybridization: The cRNA hybridized on the Agilent Human 1A 4×40-tissue microarrays (Agilent Technologies) and the microarrays were scanned using the Axon GenePix 4000B scanner (Axon Instruments). Images were analyzed using GenePix 6.0 (Axon Instruments) and data were filtered and normalized with RMA 4.1 (Axon Instruments).

Experimental design: Tumoral and non-tumoral samples were hybridized against a common RNA control. As a control, six non-tumoral samples were used. A control RNA sample was collected from normal tissue of the tumor. The tumor samples were labeled using Cy5-dye (red) and the "pool" was labeled with Cy3-dye (green).

2.1 GENOMICS

Tissue samples were preserved in RNA later Stabilization Solution (Qiagen) and stored at -80°C.

RNA expression: Total RNA was extracted from all the samples using the RNeasy Mini Kit (Qiagen). RNA quantity and quality was determined with the Agilent 2100 Bioanalyzer (Calyxo, Barcelona, Spain). We used the RNA Integrity Number, a quality standard to select the samples. We synthesized and labeled the cRNA using the Agilent Low RNA input Fluorometric Linear Amplification Kit (Agilent Technologies). cRNA hybridization: The cRNA hybridized on the Agilent Human 1A 4×40-tissue microarrays (Agilent Technologies) and the microarrays were scanned using the Axon GenePix 4000B scanner (Axon Instruments). Images were analyzed using GenePix 6.0 (Axon Instruments) and data were filtered and normalized with RMA 4.1 (Axon Instruments).

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2.2 PROTEOMICS

Protein Extraction and Quantification: Sequential extraction was made using ReadyPrep sequential extraction kit (Bio-Rad) based on the differential solubility of the proteins. We obtained a total of 35kVh. 2D-DS2D assays. IPG strips were loaded on Criterion gels 2D Precast (Bio-Rad) and were run for 80 minutes on a Dodeca Criterion Cell at 200V. The gels were stained with SYPRO Ruby dye (Bio-Rad). The gel images were captured with VersaDoc (Bio-Rad) and analyzed using the PDQuest software. Protein identification was made by MALDI-TOF analysis.

3. RESULTS

3.1 GENOMICS

After the quality analysis of all the RNA samples and based on the different expression values, we were able to group the samples into six different classes with cardinalities: 33 non-tumoral, 12 tumoral, 13 non-tumoral, 13 tumoral, 13 tumoral, and 20 tumoral.

Discretisation and classification: The number of matched spots from the individual matchsets obtained from each patient. From these, we determined the number of matched spots from the individual matchsets obtained from each patient. From these, we determined the number of matched spots from the individual matchsets obtained from each patient. From these, we determined the number of matched spots from the individual matchsets obtained from each patient.

3.2 PROTEOMICS

Protein quantification was estimated before and after clean up step. The low quality sample buffer was used to interface with both Bradford and DC-DC (Bradford) methods. We used a novel method based on fluorescence (E2O Protein Quantitation Kit, Molecular Probes) and we found that this method does not interfere with any sample buffer used in our experiments (Fig. 7).

Protein fraction of Fraction 1 is being performed by 2-dimensional gels. In Fig. 8 a proteome analysis example of a colorectal cancer patient (Stage C1) is shown. The assays were made by batchwise, and a masspectrometry containing all the spots from each gel was generated and analyzed using P/Quest software. In this case, a total of 283 spots were found, with variability among gels of 19.08% (229 matched spots, and 54 unmatched spots). 6 spots were selected, picked and identified by MALDI-TOF.

A proteomic analysis of 3 patients of different colorectal cancer stages (B3, C1, and D) is shown in Fig. 9. A high level masspeak was created from the individual matchsets obtained from each patient gel as described above. The spot color gene is a total of 239 spots in the HLG level of patients. Difference among cancer stages was 33.89%, indicating differences in proteins associated to the progression of the disease.

Using an intermediate stage (C1) as reference, we determined the unmatched spots falls in other cancer stages (B3, and B2). Stage D showed a 34% of difference of unmatched spots, while stage B2 showed a 21.3% of unmatched spots.

CONCLUSIONS

We have already a tentative model (first ten genes are shown in table 2) for the classification of cancers and non-cancers samples based on their gene expression profile. We are now in the validation process of this model, and it is of the outmost importance for us to check its potentiality for diagnosis/prognosis. From the machine learning point of view we envision the building of different classification models. Furthermore, the search statistical reliability dependencies could bring us some light regarding the complex nature of human CRC. An interesting approach would be as well to try to look to the inherent relationships between the genomics and the proteomics analysis.

Our preliminary results from proteomics studies suggest that differences in protein expression could be related to different stages of disease. From the identified proteins, Nagnopsis was found to be present in both patient M7 (stage B2) and M12 (stage C1), while Peg3D was present in all samples of the 3 stages.