

## IDENTIFICATION OF MOLECULAR MARKERS FOR DIAGNOSTICS AND PROGNOSTICS IN CANCER USING DNA MICROARRAYS

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During the development of the thematic project, *Identification of molecular markers for diagnostics and prognostics in cancer using DNA microarrays*, our group identified several long non-coding intronic transcripts, whose levels of expression were correlated to the degree of differentiation of prostate tumors. Results obtained more recently, from analysis of a set of 60 samples of prostate tumor from patients with or without relapse (metastasis), point to a signature of genic expression containing intronic RNAs correlated with the development of metastasis in that cancer. This result is extremely promising and opens the perspective for the development of new molecular tests for the prognosis of cancer of the prostate. Our group has also analyzed profiles of expression of intronic messages in other human neoplasms (kidney, breast, pancreas, leukemias). The results obtained suggest that intronic RNAs have a significant potential as diagnostic/prognostic markers in cancer.

A recent bioinformatic analysis, by our group, revealed that around 75 per cent of human genes have evidence of intronic transcription. Based on this information, we designed a customized oligoarray, containing around 30 thousand probes, for intronic RNAs, which was used to analyze profiles of intronic expression in normal liver and kidney and tumors of the prostate. These experiments revealed the existence of signatures of tissue-specific intronic expression. Surprisingly, we observed that the most abundant of these intronic non-coding transcripts are generated in genic loci involved with the regulation of the transcription, in all three tissues. With the exonic sequences, we did not observe this phenomenon, which suggests a special role for intronic transcripts of the regulatory transcription genes, in the regulation of genic expression.

To explain the possible functional roles exercised

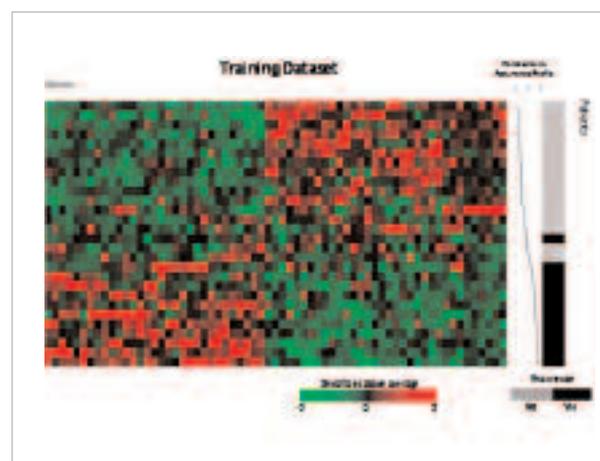


Figure 1 – The figure shows the expression profile of 65 genes (21 exonic protein-coding genes and 44 intronic noncoding RNAs) identified as most correlated to cancer recurrence ( $p$ -value  $< 0.01$  based on Signal-to-Noise Ratio analysis with permutations) in a set of 28 samples from patients with clinical follow-up longer than 5 years. The known clinical status of these patients (recurrence/non-recurrence) is shown on the right grey/black bar. The 65 genes are represented in the columns and each patient sample in one line. Patients are ordered according to the correlation value ( $r$ ) of its profile with respect to the mean expression profile of patients with tumor recurrence. The expression level of each gene is represented as number of standard deviations above (red) or below (green) the mean expression of that gene across all samples

by this new non-coding class of RNAs, we are testing *in vitro* the effect of modulators of genic expression on the intronic transcription. In this direction, we demonstrated recently, that the expression of a set of intronic transcripts may be modulated by the androgen hormone in a prostate lineage, and that these changes of expression affect the stability and/or pattern of splicing of the corresponding coding genes for protein.

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Our project *Identification of molecular markers for diagnosis and prognosis in cancer using DNA microarrays* has identified a large number of long intronic noncoding RNA transcripts whose expression levels were correlated to the degree of differentiation of prostate tumors. Recent results have been obtained in the analysis of a set of 60 prostate tumor samples from patients with long follow-up (longer than 5 years) with or without recurrence (metastasis). The results point to a gene expression signature including noncoding intronic RNAs, that is correlated with recurrence of prostate cancer. This result is extremely promising and opens the way to developing new molecular tests for the diagnosis and prognosis of prostate cancer. Our group has been also analyzing the expression profiles of intronic RNA messages in other human neoplasias (kidney, breast, pancreas, leukemia). Our results suggest that intronic noncoding RNAs have a significant potential as markers of diagnosis and prognosis in all types of cancer.

A recent bioinformatics analysis of our group revealed that over 75% of all human genes have evidence of transcription of noncoding RNAs from their intronic regions. With this information, we have designed a custom oligoarray containing approximately 30 thousand probes for intronic noncoding RNAs that has been used to measure the intronic expression profile in normal liver and kidney human tissues as well as in prostate tumors. These experiments revealed a tissue-specific expression signature of intronic RNAs. Surprisingly, we observed that the most highly expressed intronic noncoding transcripts in each of the three tissues are mainly transcribed from gene *loci* involved with Regulation of Transcription (Nakaya et al., 2007). For exonic RNA this pattern was not observed, which suggests a special role of intronic noncoding RNAs in regulating gene expression.

In order to explore possible functional roles, exerted by this novel class of noncoding RNAs, we are testing *in vitro* cell cultures the effect of modulators of gene expression on the intronic transcription. We have recently demonstrated that the expression of a set of intronic noncoding RNAs can be modulated by androgen hormone in a prostate cell line, and that these changes of expression affects the stability and/or the splicing pattern of the corresponding protein coding genes.

## MAIN PUBLICATIONS

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