SCIENTIFIC REPORT

Urinary bladder cancer is the fifth most common cancer in the western countries. It's incidence is correlated both with age, peaking at the age ranging from 50 to 70 years, and sex, it is three times more common in men than in women. Recently thousands of lncRNAs have been identified and associated to different disease-profiles. In particular it's demonstrated that many lncRNAs are involved in bladder cancer pathogenesis acting as oncogenes or tumor suppressors.

Few years ago Gill Bejerano identified a new class of lncRNAs, the ultra conserved regions (T-UCRs). They rapresent a group of 481 higly conserved sequences. Little is known about their function but their exact conservation in human, rat and mouse genome, suggests an important regulatory role in gene expression. In our previous study has been demonstrated that T-UCRs are consistently deregulated in several human tumors.

To identify the different expression of T-UCRs between Bladder cancer patients (BLCa) and normal tissues (NBE), we evaluated the expression levels of 962 sense and antisense transcripts using a custom microarray. Comparing BLCa patients and normal tissues, we found a differential expression profile of T-UCRs: in particular T-UCR 8+ was the most upregulated and T-UCR 388+ was the most downregulated. However, comparing BLCa patients and Pericancerous bladder cancer patients (PBLCa), we identified other expression profiles: T-UCR 195+ was the most upregulated, T-UCR 8+ the most downregulated. We finally merged these datas and we obtained 57 T-UCRs with a similar trend and other T-UCRs, named "outliers" which expression profile in BLC patients was variable. One of the most deregulated T-UCR, was T-UCR 8+. These datas suggests the presence of different T-UCRs transcription patterns and underline the possible role that the T-UCR 8+ may have in bladder cancer.

By using high stringent conditions for prediction we identified two binding sites (for miR-596 and miR-381-3p, in green) on the T-UCR 8+ sequence. We investigated the correlation between T-UCR 8+ and miR-596 expression in a subset of 20 BLCa tissue samples by qRT–PCR. Our results show consistent lower miR-596 levels in bladder cancer that inversely and significantly correlate with T-UCR 8+ expression. Furthermore, knockdown of T-UCR 8+ resulted in a concomitant increase of mir-596 expression in J82 cell line, supporting an effective biological correlation between the two molecules. We validated the T-UCR 8+::miR-596 interaction by using a fishing approach (through a biotinylated Peptide Nucleic Acid oligomer: PNA). The PNA sequence is complementary to the predicted single strand region of T-UCR 8+. Furthermore we examined the effect of T-UCR 8+ depletion by using RNA-i mediated gene silencing. We observed about 60% reduction compared to control on cell migration and cell proliferation in the human J82 bladder cancer cell lines. These results imply a potential role for T-UCR 8+ in cell motility and other biological activities, such as proliferation.

My aims are to identify possible protein complexes or DNA interaction with T-UCR 8+, using fishing methodic; reveal the specific localization and function of T-UCR 8+ through in situ hybridization and finally make a knockout mouse model silencing T-UCR 8+.

I've done my training at the Institute of Genetics and Biophysics-IGB (National Research Council, Naples, Italy), since my tutor, Luciana Marinelli, has a collaboration with the laboratory of Dr. Cimmino. The host structure is really comfortable and well equipped. Many scientific tools and facilities are available. Everyone in the staff is helpful and knowledgeable. Dr. Luciana Marinelli, but also Dr. Cimmino, are really good person and excellent researchers. Both help me to obtain these preliminary results and encourage me to continue in this way.