Analysis of the translatome by ribosome profiling in colorectal cancer

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Colorectal cancer (CRC) has a high incidence and mortality rates worldwide [1]. CRC carcinogenesis is a continuous accumulation of genetic alterations with concomitant variations in the gene expression profiles [2]. To study the variations of gene expression profiles involved in cancer progression, the genome-wide analyses of gene expression have so far focused on the abundance of mRNA species as measured either by microarray or RNA sequencing [3,4]. However, neither approach provides information on protein synthesis, which is the true end-point of gene expression [3-5]. Ribosome profiling emerges to monitor *in vivo* translation, providing global and quantitative measurements of translation by deep sequencing of ribosome-protected mRNA fragments (RPFs) [5,6].

The main goal of this project is to determine the changes between the translatome of CRC and normal colorectal cells and their role in CRC tumorigenesis.

We will analyze ribosome profiling data already available for the CRC HCT116 cell line, as well as for other cancer and non-neoplasic cell lines. Gene ontology and network interaction analysis of the differentially translated mRNAs will elucidate the main molecular pathways through which the corresponding proteins are involved in CRC progression. Furthermore, we aim to analyze the potential of translatable short open reading frames (ORFs) and/or the corresponding peptides to regulate CRC progression.

Our computational analysis of ribosome profiling data from HCT116 and non-neoplasic mammary gland (MCF-10A) cell lines identified 1666 5' untranslated regions (5'UTRs) differentially expressing RPFs. Among these, 702 5'UTRs showed an increased accumulation of RPFs in HCT116/MCF-10A and were enriched in cell cycle regulatory genes. The remaining had a decreased RPFs accumulation and was enriched in genes involved in cell adhesion, migration, and angiogenesis. Based on these analysis and others previously published [7], ABCE1, ABCF1, ABCF2 and ABCF3 mRNAs were chosen for further studies. Semi-quantitative RT-PCR has shown a down-regulation of these transcripts in HCT116 cells in comparison to the non-neoplasic colorectal cell line (NCM460) and two CRC cell lines (CaCo-2 and SW480). In addition, we are testing the potential function of several upstream ORFs (uORFs) present in the ABCE1 and ABCF3 5'UTRs. For this purpose, we are first mapping the exact 5'-end of these 5'UTRs by cRACE.

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