Translatomics in Invasive Breast Cancer: a Better Comprehension from a Translation Control Perspective.

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BACKGROUND: Breast cancer is the most prevalent type among the female sex and has a unique complexity, with considerable heterogeneity. In the last decades, important advances in the knowledge of breast tumors identified molecular subgroups (Luminal A, Luminal B, HER2 positive and Basal Like), which allowed the development of specific therapeutic guidelines and improved survival. However, even within these subgroups there is a wide range of prognostic perspectives and clinical behavior that until now can not be fully understood, even by high through put methods using “omics” approaches, such as massive DNA and total RNA sequencing. A complicating factor is that the information regarding post-transcriptional regulation of gene expression is lost in projects using massive DNA and RNA sequencing. Thus, information regarding abundance of RNA does not necessarily reflect the protein expression levels. Therefore, to identify mRNAs subjected to translational control can not only lead to the design of gene expression profiles that better reflect the population of proteins, but also suggests more accurate molecular subgroups. HYPOTHESIS: The identification of a population of differentially translated mRNAs can give new insights into molecular mechanisms associated with tumorigenesis in the breast and suggest new biomarkers associated with improved survival or treatment response. METHODS: 249 Invasive Ductal Carcinoma (IDC) and Invasive Lobular Carcinoma (ILC) frozen tissue samples from the A.C.Camargo Cancer Center BioBank were used. Polysomes were isolated by ultracentrifugation in a non-linear sucrose gradient. RNA extraction from whole cell lysates and isolated polysomes was made using Trizol. Whole exome sequencing was made applying Smart-seq2 methodology, which allows the generation of full-length cDNA and sequencing libraries with very low concentrations of RNA. RESULTS: Clinical information from all the patients was retrieved. Mitotic index was associated with worse prognosis, increasing among the molecular subgroups Luminal A<Luminal B< Basal like, and also being related with higher incidence of necrosis. In addition, the presence of necrosis is increscent considering the molecular classification Luminal A<Luminal B<Basal like and tumor size T1<T2<T3. Moreover, progesterone receptor expression under 60% of the cells was related to higher mitotic index and, as consequence, with worse prognosis. Mutations on p53 were much more frequent in IDC comparing to ILC ($p>0.05$). Polysomes were successfully isolated from all samples. Polysome profiles indicated that triple negative breast tumor samples had increased translation rates when compared to Luminal A and B tumors, revealing a possible relationship between higher translation rates and worse prognosis. During RNA extraction, approximately 35% of all samples were lost due to RNA degradation, and only samples with RIN (RNA Integrity Number) above 5 were selected for cDNA synthesis. Currently, libraries from 161 patients are being sequenced for future data analysis using ANOTA package, which was developed to use linear regression in order to compare total and polysomal RNA for translatomics studies and, equally important, gene expression patterns identified will be compared to the expression of main components of translational machinery.

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