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Phospholipase D in Citrus EST database - Structure and function studies

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Short Abstract: Phospholipases D in plants are involved in several cellular responses. The in silico analyze of CitrusESTs database revealed 457 sequences related to phospholipases as 13 contigs and 12 singlets. A theoretical model, validated by Procheck, revealed common domains and therefore functions. It is the first evidence of PLD in Citrus.

Long Abstract:
Phospholipases D in plants are involved in cellular responses, as growth, development, stress and defense. The in silico analyze of Citrus ESTs database revealed 457 sequences related to phospholipases as 13 contigs and 12 singlets. The homology modeling of a PLD from Citrus revealed common structures and therefore functions. The model was validated by Procheck server (http://biotech.ebi.ac.uk:8400/cgi-bin/sendquery). Our results suggest that the phospholipase D shares structure-function relationship and perhaps same reaction mechanism. Structural analysis suggests that the occurs via phosphohistidine intermediate and provide the identification of a catalytic water molecule. It is the first evidence of PLD in Citrus.

Poster L-36

Characterization of gene expression during erytrhoid differentiation by Serial Analysis of Gene Expression (SAGE)

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Short Abstract: SAGE enables the analysis of thousands of genes and a total quantification of each transcript. We used SAGE to quantify expression profiles during the differentiation of human erythroid cells. Results may contribute to the comprehension of erythroid differentiation and identification of new targets genes involved in some erythroid diseases.

Long Abstract:

Erythroid differentiation is a dynamic and complex process in which a pluripotent stem cell undergoes a series of developmental changes that commit it to a specific lineage. These alterations involve changes in gene expression profiles. Extensive studies have led to a considerable understanding of the cellular and molecular control of hemoglobin production during red blood cell differentiation, however, a complete understanding of human erythropoiesis will require a robust description of the entire transcriptome of these cells during differentiation. From a global point of view of cell metabolic regulation, where genomic information could be complemented with gene expression, the use of methods that enable quantification of the entire transcriptome of red blood cell during the differentiation is of great importance. Serial analysis of gene expression (SAGE), a technique developed by Velculescu et al. (1995) [1] enables the analysis of thousands of expressed genes and a total quantification of each transcript. In this study, we used SAGE to quantify the gene expression profiles during differentiation of Human erythroid cells in a two phase liquid culture. To do this, we performed SAGE experiments in cells collected at the beginning, immediately before the addition of erythropoeitin (0 hour), during the culture and at the end of the second phase of culture, i.e., 192 hours and 336 hours after the addition of this hormone, respectively. We generated, after automatic sequencing, a total of 19328 tags at 0 hour, 19783 tags at 192 hours and 19562 tags at 336 hours, representing 8497, 8482 and 8175 unique tags, respectively. In the 0 hour library, a high expression of ferritin genes and CD74 antigen gene was observed. The beta globin, gamma globin and ribosomal genes were the most expressed genes at 192 hours and at 336 hours library the most expressed genes were basically globin genes. To identify the genes differentially expressed between the libraries, we considered a P value < 0.01 and fold ≥ 5 as statistically significant. In the comparison of the 0 hour and 192 hours libraries, 179 differentially expressed transcripts were identified. From these genes, we found in addition to the globin genes, an up-regulation of several genes such as GATA-1, TPSB1, GSTM3, TRIP6, PRDX2. Genes such as CSTB, CAPG, PLA2G7 and IFI30In were found to be down-regulated. Comparing the 192 hour and 336 hour libraries, 103 differentially expressed transcripts were identified. The up-regulated genes were generally genes related to hemoglobin synthesis, such as ALAS2, a gene involved in the biosynthesis of the heme group and related to sideroblastic anemias and genes related to intracellular transport such as MSCP and NUDT4 genes. The functional classification of genes was performed according to the Gene Ontology Consortium using the GOLIAS (Gene Ontology Library Analyser for SAGE), a program developed in our laboratory that uses the Gene Ontology structure to provide integration and to automate the SAGE analysis. GOLIAS reports quantitative data regarding the sequenced tags, statistics and graph charts. The results showed that the global aspects of the transcriptome were similar during the differentiation for most of the genes and that a set of genes involved in the modification of erythroid cells during differentiation. Among these, we found genes involved in signal transducer activity, transporter activity, proteic activity and transcription regulator activity.

To the best of our knowledge, this is the first report where the entire transcriptome of the red blood cell is quantitatively assessed during differentiation. The results found in this study will contribute to the comprehension of erythroid differentiation and identification of new target genes involved in some erythroid diseases.

Supported by FAPESP

[1] Velculescu VE, Zhang L, Vogelstein B, Kinzler KW (1995) Serial analysis of gene expression. Science 270:484