Large-scale microarray analysis of protein and mRNA level changes in HL-60 cells.

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Conclusion:

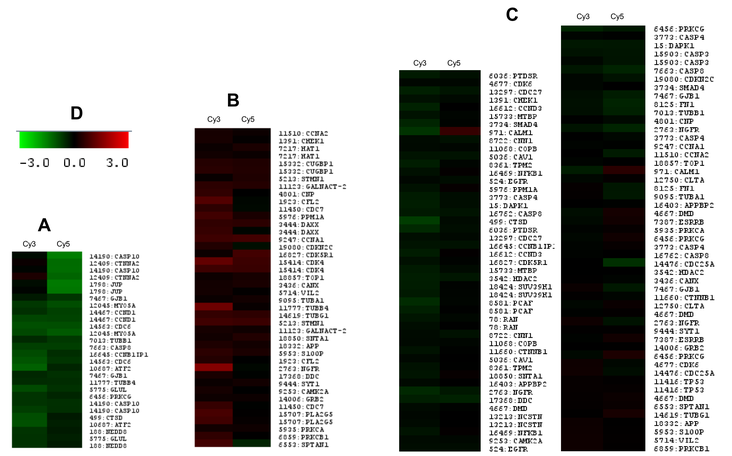
Using microarrays we have found, that level of several proteins was either up- or down-regulated after cell differentiation. In some cases there was significant correlation with appropriate genes. However, we confirmed there is not always a direct correlation between the mRNA level and the expression of the protein.

Introduction:

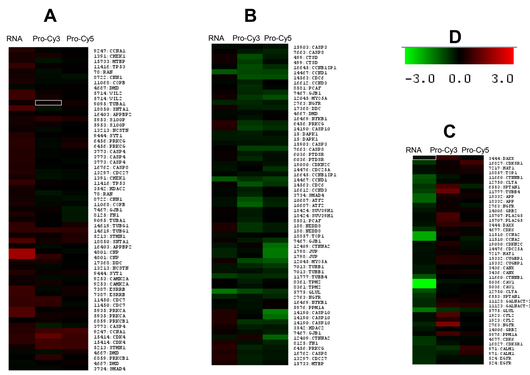
In this study we are comparing methods for large-scale microarray analysis of protein and RNA level changes in HL-60 cells, responding to differentiation stimuli. We tried to find out a correlation between levels of mRNA and the expression of certain proteins. We also established gene activity changes and therefore enhanced or decreased protein expression levels after cell differentiation.

Tools:

Panorama Ab Microarray Cell Signaling slides were used for protein expression analysis. This system contains 224 antibodies spotted on nitrocellulose-coated glass slides. mRNA levels were assessed by UHN Single-spotted array containing 19,008 both characterized and unknown human ESTs. For some protein targets, the results obtained by the Panorama Ab Microarray slides were verified by Western Immunoblotting.



List of proteins with altered expression before respective after in vitro HL-60 cells differentiation into granulocytes. Proteins are represented by individual rows in duplicates. Each column represents expression type in Cy3 respective Cy5 channel. Used coloring is: green – down regulation, red – up regulation, intensity represents the amount of change (log2 of expression ratios) **(D).** Down regulation during cell differentiation has been measured for 28 proteins **(A)**, up regulation for 42 proteins **(B)**, 98 proteins showed no significant differences **(C)**.



Comparison of proteins and appropriate genes with altered expression before respective after in vitro HL-60 cells differentiation into granulocytes. Proteins and genes are represented by individual rows in duplicates. Each column represents expression type in genes in Cy3 channel and proteins in Cy3 and Cy3 channels. Used coloring is: green – down regulation, red – up regulation, intensity represents the amount of change (log2 of expression ratios) **(D).** Down regulation for both genes and proteins during cell differentiation has been measured for 61 genes and proteins **(A)**, up regulation for 63 proteins and genes **(B)**. In 44 cases proteins showed no correlation with appropriate genes **(C)**.

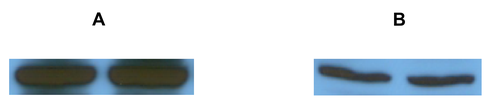
Samples:

Two samples were compared:

3. HL-60 cells
4. HL-60 cells stimulated to differentiate into granulocytes by 5 days exposition to retinoic acid

Results:

We have determined groups of proteins, which in the course of differentiation show different expression profiles in granulocytes. Down-regulation has been measured for 28 proteins, among them for beta-tubulin or caspase 10. Up-regulation was observed in 42 proteins, for instance cyclin dependent kinase 4 (CDK4) or death-associated protein (DAXX). For several proteins have been established correlation with mRNA: beta-tubulin (down-regulated), cyclin dependent kinase 4 (CDK4) (up-regulated). However, in many cases there was no correlation between mRNA levels and proteins: e.g. Phospholipase A2 (PLA2G5).



Immunoblotting confirmation of array results. The same amount of protein from HL-60 cells before **(A)** respective after **(B)** differentiation into granulocytes. Blots were incubated with Anti-beta-Tubulin antibody.

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