REF-E014

INTEGRATED MIRNA, MRNA AND PROTEIN EXPRESSION ANALYSIS REVEALS THE ROLE OF POST-TRANSCRIPTIONAL REGULATION IN CONTROLLING CHO CELL GROWTH RATE

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CHINESE HAMSTER OVARY / GROWTH RATE / MICRORNA / MRNA / PROTEOMICS

BACKGROUND AND NOVELTY:
MicroRNAs (miRNAs) are a class of small RNA molecules involved in the post-transcriptional control of protein synthesis. Recent studies have linked miRNA expression to several industrially important traits in Chinese hamster ovary (CHO) cell culture. This study represents the first time that simultaneous analysis of miRNA, mRNA and protein abundance levels has been carried out to understand the role of miRNA in the regulation of CHO cell growth.

EXPERIMENTAL APPROACH:
qPCR, microarray and quantitative LC-MS/MS analysis were utilised for expression profiling of miRNA, mRNA and protein levels for a set of sister MAb-producing clones spanning a range of growth rates. The proteomic and mRNA data were initially analysed in isolation to identify enriched biological processes. To investigate the influence of miRNA on these processes we combined the proteomic and transcriptomic data into two groups. The first set contained proteins where evidence of translational repression was observed. The second group was a mixture of proteins and mRNAs where translational repression was less clear. TargetScan was utilised to predict targets within these two groups for anti-correlated differentially expressed (DE) miRNAs.

RESULTS AND DISCUSSION:
Gene ontology analysis of genes (n=432) and proteins (n=285) found to be DE identified biological processes driving proliferation including mRNA processing and translation. Following the integration of protein and mRNA data a number of proteins central to these processes including several hnRNPs and components of the ribosome were found to be post-transcriptionally regulated. Comparison of mRNA and protein expression with respect to the 51 DE miRNAs (35 miRNAs up and 16 miRNAs down) allowed us to identify potential miRNA targets and highlight translational repression targets which could not have been identified using a single dataset. Moreover, the use of multiple profiling datasets could allow the identification of non-seed miRNA targets and reduce false positive/negative rates.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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ASSESSMENT OF CHROMOSOMAL LENGTH VARIATION IN CHO CELLS

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BACKGROUND AND NOVELTY:
Chinese hamster ovary (CHO)-derived cells are widely used for the production of recombinant proteins. Currently, the pharmaceutical industry relies on stable cell clones for therapeutic protein production. Most of the currently used methods to analyze clonality and stability in recombinant cell lines only take into consideration the cellular phenotype. However, few rapid methods are available to study clonality and genomic stability in recombinant cell populations.

EXPERIMENTAL APPROACH:
Here we present a relatively simple, cost-effective method based on digital image processing to analyze chromosome lengths in metaphases of cells. The method was applied to CHO parental cells and derived recombinant cell lines. Chromosome spreads were prepared from growth-arrested cells and visualized with a confocal microscope after fluorescence staining. An ImageJ plugin was then used to size-sort the chromosomes.

RESULTS AND DISCUSSION:
We studied 9 different CHO derived cell lines, both parental and recombinant sublines (20 metaphase spreads for each cell line). The average chromosome number in each clonal line was not homogenous. Also, each cell line appeared to have a unique pattern of chromosome length. We believe that the analysis of chromosome length patterns of CHO lines can be useful in the characterization of a given cell population and possibly even in the identification of the parental cell line from which a subline is derived from a specific parental cell.

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