A genome-wide loss of function screen identifies new genes required for lung cancer cell proliferation

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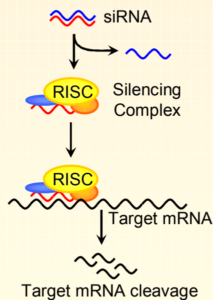
Abstract

Lung cancer is a disease with poor outcome and no efficient cure[1]. Understanding all the pathways and molecules involved in persistence of the tumoral state is essential to discover novel potential targets. RNA interference (RNAi), a natural and specific gene "knock-down" mechanism that can be triggered extraneously, provides an experimental tool to establish direct links between genes and biological functions[2]. In order to unravel previously uncharacterized genes involved in lung tumour cell proliferation, we screened a genome-wide synthetic siRNA (small interfering RNA) library in human lung cancer A549 cells. From the primary screen (22,950 genes targeted, 2 siRNAs /gene), 1624 candidate genes were re-assayed in a confirmatory screen (4 siRNAs /gene), leading to 257 hit genes. Bioinformatics analysis revealed a characteristic pattern of RNAi screens, where 72 % of genes were involved in general gene expression, protein synthesis, mitosis and metabolic functions[3]. However, some of them according to our advanced analysis could have additional specific role in cell proliferation. 16 % of the genes in the list were of unknown function or were unrelated to proliferation, and 12% consisted of uncharacterized genes (predicted ORF). These last two sets of genes provide potential novel targets for lung cancer treatment.

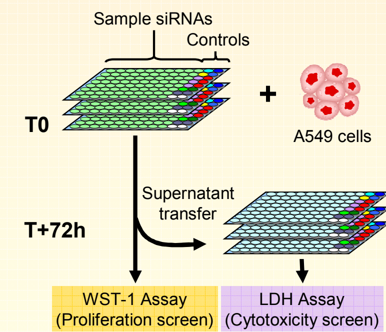
Aim

To perform a genome wide loss-of-function screen for identification of genes required for A549 cell line (human lung carcinoma) proliferation.

(A) RNAi principle



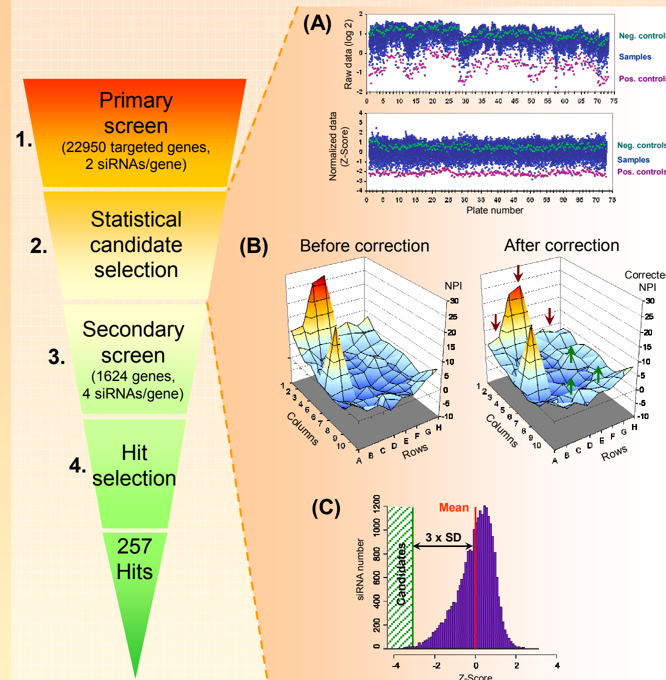
(B) Automated RNAi Assay Design



(A) siRNAs are short non coding RNAs that can induce sequence specific gene knockdown via target mRNA cleavage.

(B) siRNAs or controls were reverse transfected into A549 cells (as triplicates ; final siRNA concentration : 25 nM ; 3000 cells/well). After 72 h incubation, relative cell proliferation was assessed by WST-1 assay (Roche), and cytoxicity by LDH release measurement (Roche). Liquid-handling was performed on an 8-channel Microlab Star (Hamilton).

Primary & Secondary Screens



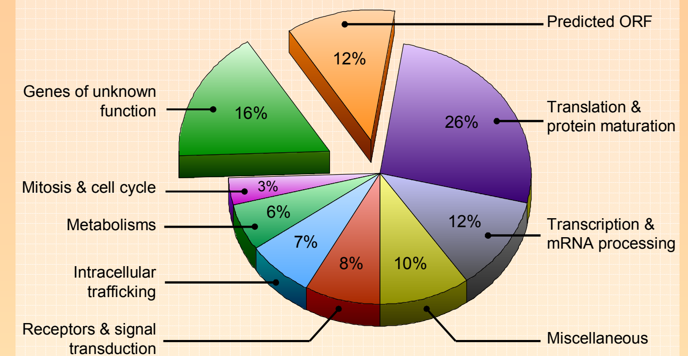
1. Primary screen : A human genome wide siRNA library (22,950 targeted genes, 2 siRNAs per gene, Qiagen) was systematically screened using the assays described above.

2. Candidate selection : (A) Raw data were rescaled according to control-based (Normalized Percent Inhibition - NPI) and sample-based normalizations (Z-Score, B-score). When needed, plate systematic errors were statistically corrected from normalized values[4] **(B)**. An siRNA was defined as active when its normalized value deviated more than 3*SD from the sample siRNAs average, in at least one of the normalization procedure **(C)**. The candidate list was constructed with genes for which at least one siRNA was found as active in any selection procedure.

3. Secondary screen : The candidate genes were then re-assayed for proliferation and cytotoxicity in a confirmatory screen using 4 siRNAs per gene.

4. Hit selection : Results were normalized (NPI) and genes were ranked, according to the balanced NPI activity of their corresponding siRNAs. By two independent bioinformatics approaches, 257 top active genes were selected.

Results



As expected, previously characterized regulators of mitosis and cytokinesis (Polo-like-kinase 1, CDC2L1-2, E2F or KIF11, our internal control,) were identified as top hits, underscoring the reliability of the screen. Data analysis showed a pattern reminiscent of RNAi screens[3,5] with a large amount of hits involved in general gene expression and metabolic processes (like RNA polymerase II and spliceosome components, elongation initiation factors and ribosomal subunits…).. 25 genes, grouped as "Misc", were functionally associated to various processes unrelated to each other. Interestingly, our screen identified among them two fusion proteins.

Bioinformatics analysis of the derived network (e.g. degrees of connectivity and centrality of the nodes, clustering coefficient, etc) suggest that some of these genes could have additional specific role in A549 cells proliferation.

Aside from them, our screen identified 72 genes of unknown function (42 functionally uncharacterized genes, and 30 ORF). This last group will be the main focus of our next step, i. e. multifunctional analysis.

Conclusions

We have successfully implemented a functional High-Throughput Screen that highlighted 257 genes required for A549 cell proliferation, from which some were previously functionally uncharacterized. Ongoing studies are focusing on the differential analysis of these genes in cancer *vs.* non cancer cell lines. Identification of genes specifically required for lung tumour cell proliferation will increase our knowledge of mechanisms underlying tumour persistence, and may open new perspectives for targeted lung cancer treatment.

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