Genomic variants dysregulate cancer genes by modulating microRNA activity

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Modulators of miRNA activity[1-4] include competing endogenous RNA (ceRNA) species that can regulate the abundance of other RNAs *in trans* by competing for common miRNAs. Up- or down-regulation of ceRNAs alters the expression of their cognate targets (Fig.1A), and alterations in copy number and methylation at ceRNA loci are integrated and propagated *in trans* by ceRNA, resulting in pathophysiologically relevant dysregulation of tumor suppressor and oncogene expression.

Our analyses suggest that the expression of hundreds of tumors suppressors and oncogenes are altered by genomic variants at the loci of their ceRNA regulators in each of eight cancer types. Our analyses also suggest that ceRNA interactions are near independent of individual miRNA abundance, resulting in a near context-independent *pan-cancer ceRNA interaction* network (henceforth *PCI*). We validated the PCI (Fig1.B) using data from LINCS, and other biochemical assays (not shown), and showed that key cancer genes are mechanistically dysregulated by concerted genomic alterations at their cognate ceRNA-interacting genes in samples where their genomic loci are intact (Fig1 C-D). Conclusions from our analysis were confirmed using molecular profiles of 14,240 tumors from 129 additional cohorts (Fig1. E). Focusing on specific tumor suppressors and oncogenes, we've shown that tumor suppressors, including PTEN, RB1, and P53 are able to regulate each other on the RNA level; that the same is true for oncogenes, including HIF1A, CCND1 and HMGA2; that Genomic alterations at APC (not shown) and ESR1 ceRNA regulators were predictive of their dysregulation in colon and breast cancer tumors,[5,6] respectively, and that perturbations that target these ceRNA regulators alter target expression and mimic corresponding cell and tumor phenotypes (Fig1.F-J).

We've shown that ceRNA is not only predictive of missing genomic variability of hundreds of oncogenes and tumor suppressors in each tumor type tested, but can also help predict microRNA targets[10] as the main part of a panel providing functional evidence for miRNA regulation in primary tumors (Fig. 2).

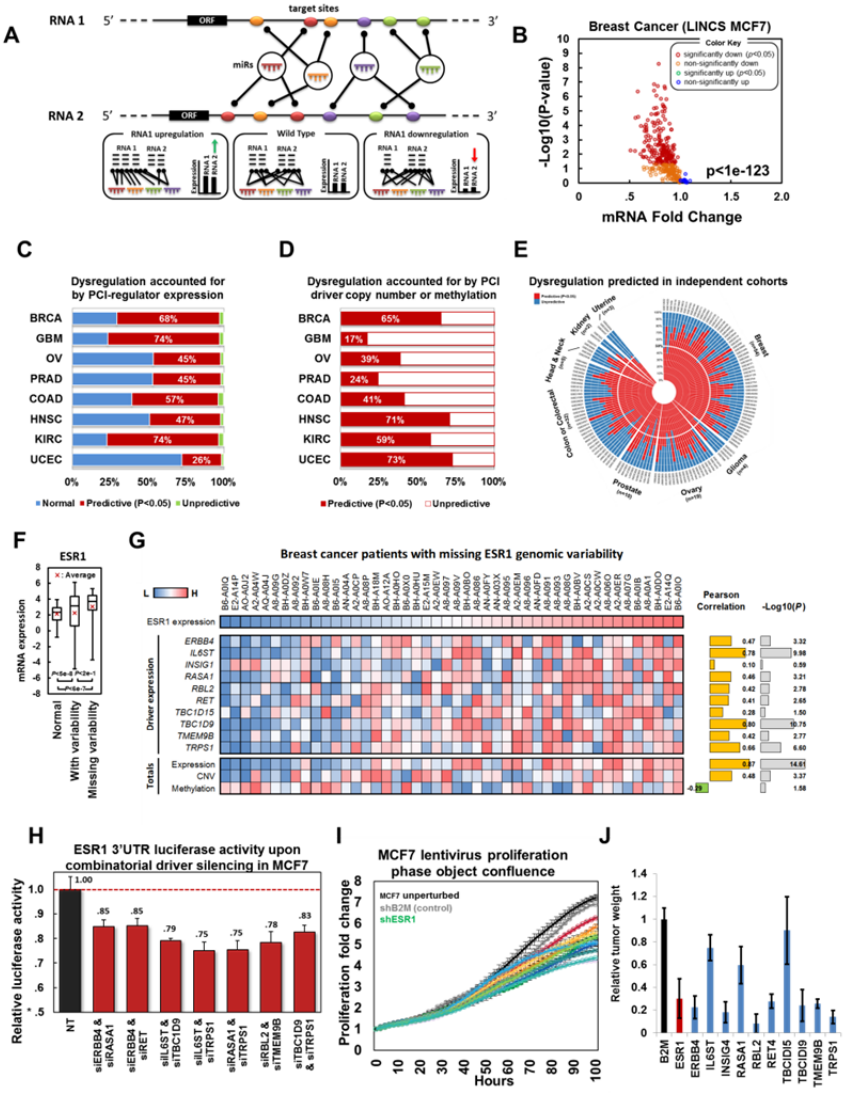

Fig1. A) RNAs regulate one another by titrating miRNA regulators. Up/down regulation of RNA 1 sequesters shared miRNAs, leading to weaker/stronger repression of RNA 2. B) ceRNA is predictive of mRNA expression changes following shRNA perturbations in LINCS. C) Dysregulation of cancer genes with no in-cis alterations in tumors (red) was predicted by ceRNA-regulator expression and (D) copy number or methylation status. E) The proportion of cancer-gene ceRNA targets whose expression profiles are significantly (p<0.05; red) predicted by ceRNA in each of 129 independent cohorts. F) ESR1 expression is dysregulated both when its locus is abnormal (with variability) and intact (missing genomic variability). G) Average ceRNA-regulator expression, CNV and methylation were predictive of ESR1 expression in tumors where its locus is intact. H) Silencing ESR1 ceRNA that are co-altered in these tumors down regulated ESR1 expression and (I) altered MCF7 cell growth and (J) tumor formation in mouse xenografts.

Altogether, our results suggest that the ceRNA regulation and PCI in particular, are key resources for cancer genomic studies and that its further study may elucidate critical pathophysiological mechanisms.

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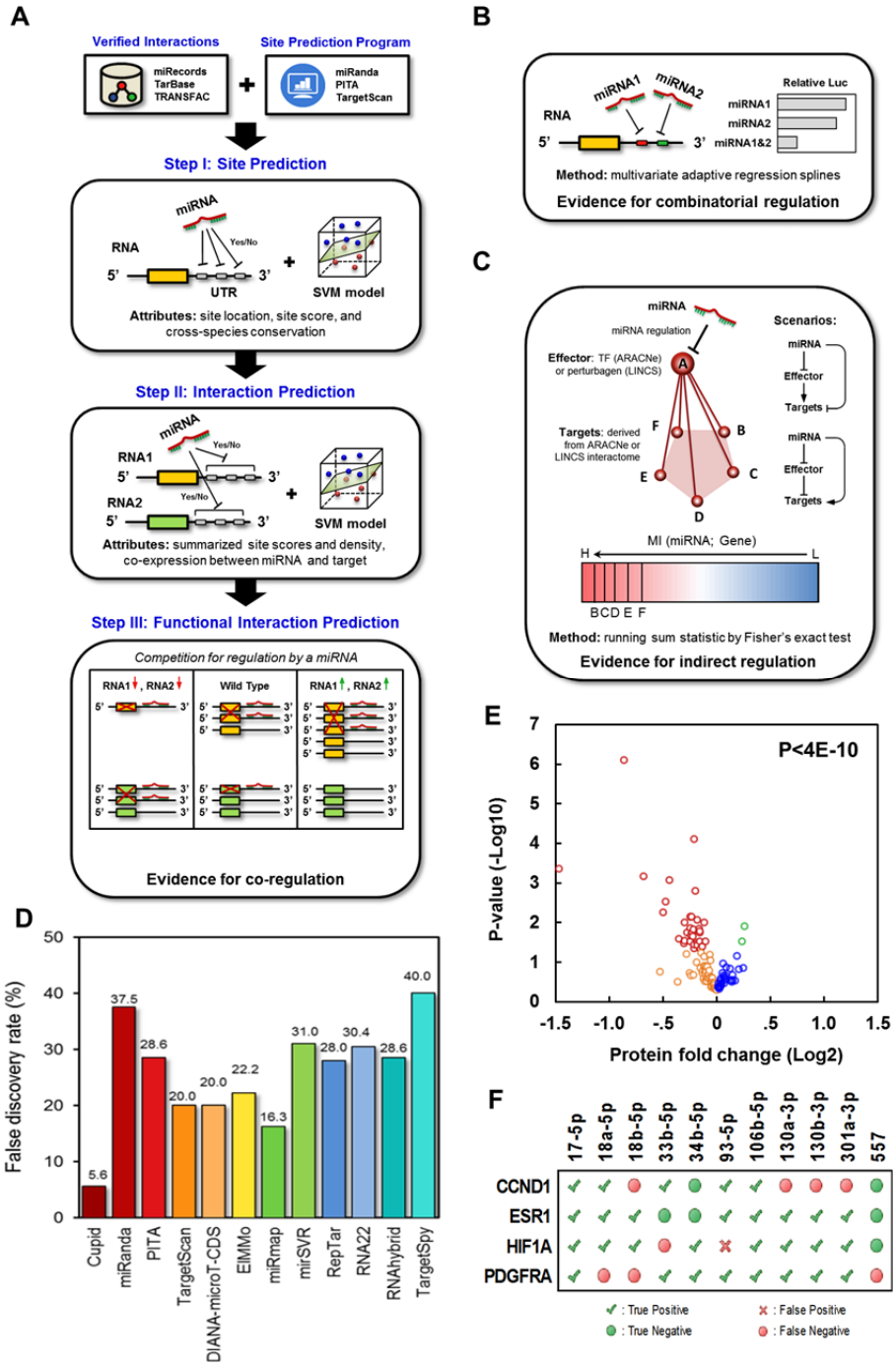

Fig2. A) Cupid first reevaluates sites predicted by TargetScan,[7] miRanda[8] and PITA,[9] selecting and rescoring each candidate site (Step I). Sites are used to select and score miRNA-target interactions (Step II), which are then examined for evidence for mediating ceRNA interactions (Step III). In addition, to support interaction prediction, we consider (B) evidence for combinatorial regulation between miRNAs and (C) evidence for indirect regulation by miRNAs through effectors. (D) Cupid predictions had a lower false discovery rate when evaluated using miRNA mimic transfection followed by protein expression profiling. (E) P-values and average protein-expression fold changes after transfection of Cupid-predicted miRNA regulators. In total, considering expression estimates made with 117 antibodies, 34 reported significant down regulation ($p<0.05$, in red), 51 reported down regulation (orange), 30 reported up regulation (blue), and 2 reported significant up regulation ($p<0.05$, in green); a comprehensive significance of $p<4E-10$. (F) Predicted miRNA-target interactions and a summary of biochemical validation, depicting true positive, true negative, false positive and false negative predictions; down regulation of 3' UTR luciferase activity in response to miRNA-mimic transfection at $p<0.05$ was taken as evidence for regulation.