Signatures of non–small-cell lung cancer relapse patients: differential expression and gene network analysis

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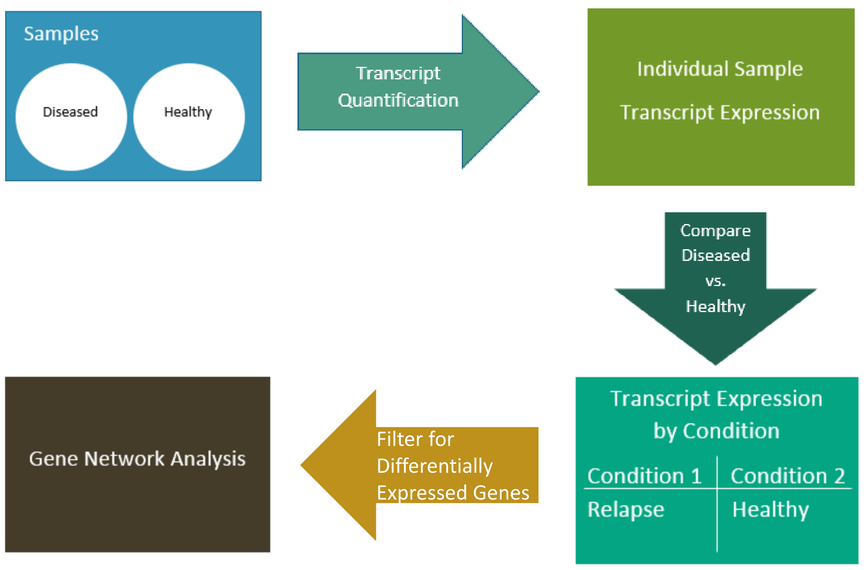
Introduction

Lung cancer is both the second most represented cancer diagnosis and the leading cause of cancer death within the United States[1]. Despite the high occurrence of non–small-cell lung cancer (NSCLC), 30 to 55% of patients relapse after curative resection, and the 5-year relative survival rate is 15 to 21%[1]. Biomarker programs help select patients who may benefit from a given drug, thereby high costs of cancer medication and cancer drug failures may be reduced[2,3]. We identified differentially expressed genes that are significantly linked to disease outcomes, which could lead to investigations of how these candidate biomarkers function to impact risk of relapse.

Methods

- NSCLC RNA samples were taken from 38 patients,
  - clinical outcomes were determined by the American College of Surgery Oncology Group [4];
  - 20 were diagnosed as disease-free, and 18 as relapse patients.
- RSEM[5] was used to assemble and quantify the abundance of transcripts, which produced gene–count-based expression.
- EBSeq[6] was used to perform median normalization and differential expression analysis with a threshold of false discovery rate (FDR) < 0.05.
- Differentially expressed genes were analyzed for over-representation of protein complexes, gene ontology terms and pathways with ConcensusPathDB[7].



Results

Empirical Bayesian methods[6] identified 122 differentially expressed genes (FDR < 0.05). Among these genes, only one protein complex-based set was over-represented, G protein-coupled receptor ligand. This included 7 of our differentially expressed genes, 4 of which have suggested implications in NSCLC prognosis: Existing research indicates that overexpression of TGF-α, IGF1, or IGF2 predicts poor survival, which is consistent with the gene expression of our relapse group[8-10]. Interestingly, while overexpression of HGF is also associated with poor survival, we saw higher expression of HGF in the disease-free patients[11-13].

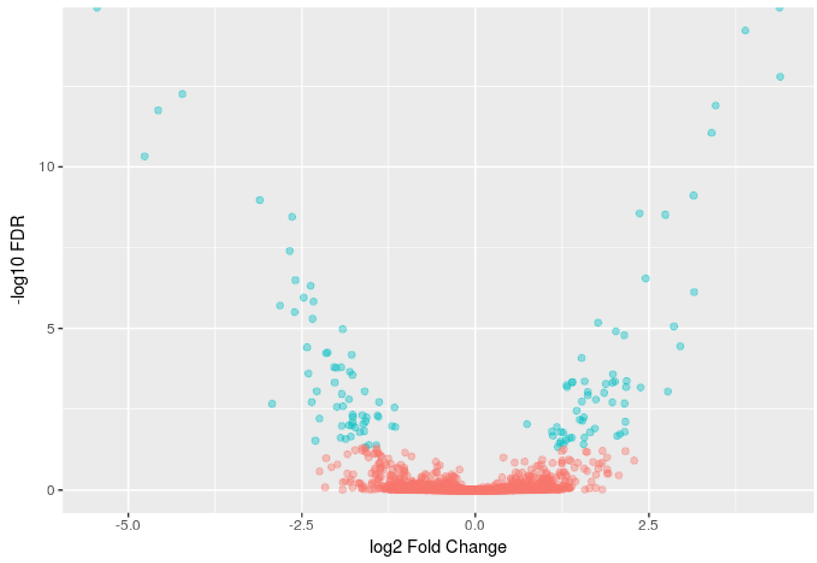


Figure 1 | Volcano plot of differentially expressed genes (blue) and similarly expressed genes (red).

Gene	PPDE	Real FC	R Mean	DF Mean
CPS1	1	21.0382203	3861.452147	183.53510
UPK1B	1	20.8719792	1640.955927	78.61052
KLK11	1	14.8335518	1476.094288	99.50118
CD70	1	11.0330937	161.379705	14.61778
PI3	1	10.5927592	2248.496323	212.25824
ADGRB1	1	8.8462140	885.679398	100.11073
KIAA0087	1	6.6737762	118.581664	17.75980
COMP	1	5.1584334	2448.075614	474.56928
SLC7A14	1	0.1605313	29.495441	183.78865
C11orf70	1	0.1568151	55.741074	355.51117
WNT11	1	0.1163370	63.640695	547.11328
SHISA3	1	0.0536560	55.093038	1026.95836
PRR4	1	0.0421482	48.838449	1158.95959
SEC14L5	1	0.0367918	9.453563	257.20964
LGALS4	1	0.0228392	28.253722	1237.50056

Table 1 | Top 15 differentially expressed genes (FDR < 0.05). Posterior probability of differential expression (PPDE); fold change (FC); relapse and disease-free normalized expression (R & DF means).

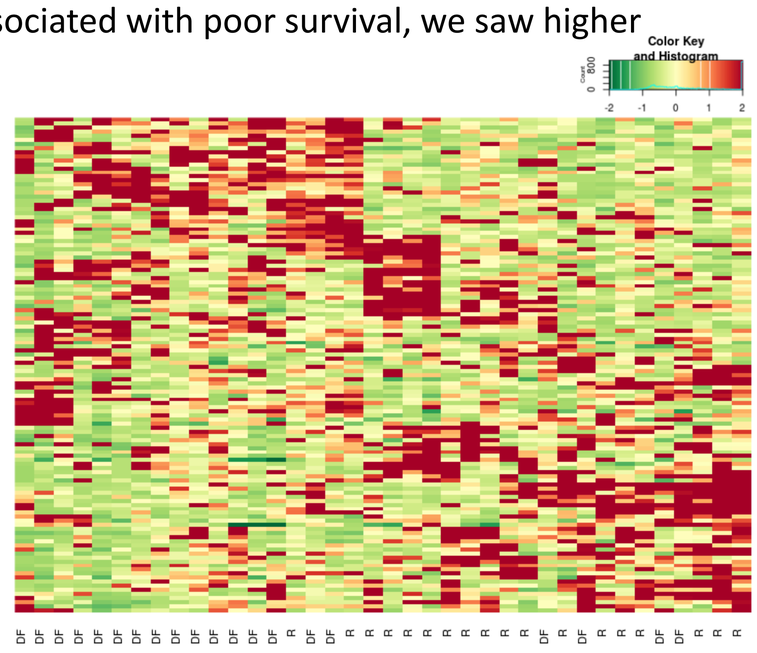


Figure 2 | Heatmap of normalized gene–count-based expression. Disease-free (DF); relapse (R). 122 differentially expressed genes on y-axis.

A

GO term	Category, level	P-value	Q-value
GO:0008284 positive regulation of cell proliferation	BP 5	4.66e-06	0.00142
GO:0019372 lipoxygenase pathway	BP 5	6.95e-05	0.0106
GO:0099503 secretory vesicle	CC 5	0.000236	0.00189
GO:0033293 monocarboxylic acid binding	MF 5	0.000267	0.00479
GO:0004252 serine-type endopeptidase activity	MF 5	0.000274	0.00479

B

Pathway	Source	P-value	Q-value
GPCR signaling-G alpha i	INOH	6.77e-05	0.00636
GPCR signaling-pertussis toxin	INOH	6.77e-05	0.00636
Prostaglandin Synthesis and Regulation	Wikipathways	8.8e-05	0.00636
GPCR signaling-cholera toxin	INOH	9.73e-05	0.00636
GPCR signaling-G alpha s Epac and ERK	INOH	0.000113	0.00636

Table 2 | **(A)** Top five level five gene ontology (GO) terms. **(B)** Top five pathways. Biological process (BP); cellular component (CC); molecular function (MF); Integrating Network Objects with Hierarchies database (INOH).

Conclusions

Identifying NSCLC patients at risk of recurrence is crucial in cancer research. Our analyses identified 122 differentially expressed genes among disease-free and relapse NSCLC patients, including known lung cancer-related genes and new candidate biomarker genes that are involved in the diverse processes related to NSCLC development. Future research in alternative splicing and the development of a predictive model based on our results could support methods of identifying individual recurrence risk.

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Acknowledgments

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