

# A Network-Based, Genetic-Marker Evaluation for Lewis Lung Carcinoma

Xiangyao Lian<sup>1</sup>, Dexiang Yang<sup>2</sup>, Shaolong Cao<sup>3\*</sup>

<sup>1</sup> Department of Oncology, the Affiliated Hospital of Chengde Medical University, Chengde, Hebei 067000, P.R. China;

<sup>2</sup> Department of Respiratory, People's Hospital of Tongling, Tongling, Anhui 244000, P.R. China;

<sup>3</sup> Department of Bioinformatics and Computational Biology, MD Anderson Cancer Center, University of Texas, Houston, TX 77054, USA.

**\*Corresponding Author:** Shaolong Cao, Department of Bioinformatics and Computational Biology, University of Texas, MD Anderson Cancer Center, Houston, TX 77054, USA. Email: scao@mdanderson.org; Tel: 504-453-5259.

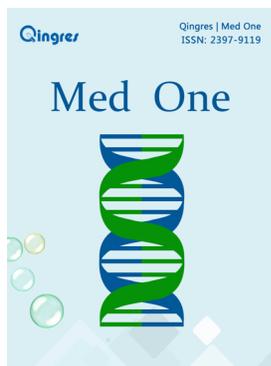
## ABSTRACT

**Background:** Lung cancer is the most common human cancer. There are numerous studies that have sought to discover genetic impact factors associated with it. The study reported on in this article is a systematic search for the genetic networks underlying the Lewis lung carcinoma (LLC) model.

**Methods:** LLC-Gene relation data were extracted from the ResNet 11 Mammalian database, containing 175 LLC candidate genes (nodes). Pathway Enrichment Analysis, Sub-Network Enrichment Analysis, Network Connectivity Analysis, and Network Metrics Analysis were conducted to study network attributes and select the top nodes (genes). LLC-Drug and Drug-Gene relation data examined LLC-Gene relations at the small molecule level.

**Results:** A total of 166 of 175 genes that were enriched in 134 LLC candidate pathways ( $p < 1e-07$ ), demonstrated strong gene-gene interactions. Metrics analysis revealed 4 genes, IL6, TNF, VEGFA, and HIF1A, as top LLC candidates as measured by replication frequency, network centrality, and functional diversity. Additionally, 169 of 175 LLC candidate genes demonstrated strong interactions with 213 of 253 LLC effective drugs which supports an LLC-gene relationship.

**Conclusion:** The results suggest that LLC genetic causes link to a genetic network composed of a large number of genes. This network, together with literature and enrichment metrics provide a foundation for further biological/genetic studies in the field.



<http://mo.qingres.com>

 OPEN ACCESS

DOI: 10.20900/mo.20160024

Received: July 22, 2016

Accepted: September 24, 2016

Published: October 25, 2016

**Copyright:** ©2016 Cain *et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Key Words:** Lewis lung carcinoma; ResNet database; Pathway Enrichment Analysis; Sub-Network Enrichment Analysis; Network Connectivity Analysis

## 1 INTRODUCTION

Lewis lung carcinoma is a tumor named after its 1951 discoverer, Dr. Margaret R. Lewis, of the Wistar Institute. Worldwide lung cancer is the most common human cancer measured by incidence and mortality<sup>[1]</sup>. LLC cells were utilized to illuminate underlying mechanisms of drug-mediated antitumor activity<sup>[2]</sup>. Recently, an increasing number of articles have been published. They report over a hundred genes relate to the disease.

Significantly decreased HIF1A expression levels has been suggested by many independent studies to associate with LLC<sup>[3]</sup>. The IL-6 family is highly up-regulated in many cancers and considered as one of the most important cytokine families during tumorigenesis and metastasis<sup>[4]</sup>. Modulating the balance between VEGFA and TSP-1 can transiently improve anti-tumor efficacy in LLC<sup>[5]</sup>.

We have found no systematic evaluation of the quality and strength of these reported genes as a functional network/group that has been used to study the underlying biological processes of LLC. This study looked to discover any comprehensive genetic networks underlying disease pathogenesis. It was hypothesized that candidate LLC genes functionally link to each other, and play roles as a network of multiple pathways influencing LLC pathogenesis.

## 2 MATERIALS AND METHODS

The study structure is as follows: 1) a disease-gene relation data analysis to identify LLC candidate genes; 2) a node enrichment analysis to study LLC pathogenic significance and identify LLC candidate pathways; 3) a gene-gene interaction (GGI) analysis to test functional associations between the genes; 4) a metrics analysis to generate node attributes and identify top nodes; and, 5) an LLC-drug-gene relation data analysis to study relationships at the small molecule level.

### 2.1 Acquisition of LLC-gene relation data

LLC-gene relation data were acquired from Pathway

Studio, Mammalian database updated August 1, 2016. Identified genes were used as the candidate network nodes. ResNet® Mammalian database is a part of PS ResNet Databases, a group of real-time updated network databases that includes curated signaling, cellular process and metabolic pathways, ontologies and annotations, as well as molecular interactions and functional relationships extracted from the 35M+ references covering the entire PubMed abstract and Elsevier full text journals. The databases can be used for data mining and pathway building. The full ResNet Databases include Plant database, and Targeted database. ResNet® Mammalian database is updated weekly and contains information for more than 6,600,000 functional relationships in humans, rats, and mice, which are linked to original literature sources. Entities in the database include: 1) 142,110 proteins; 2) 106,732 small molecules; 3) 8863 cell processes; 4) 15,911 diseases; 5) 5,038 functional classes; 6) 4,387 Clinical parameters; 7) 559 complex; and, 8) 767 cells. <http://pathwaystudio.gousinfo.com/ResNetDatabase.html>.

### 2.2 Identification of LLC candidate pathways

A pathway enrichment analysis (PEA) was performed to identify candidate LLC pathways in order better understand any underlying functional profiles and any pathogenic significance of the genes. In PEA, a Fisher-exact test is employed to test the hypothesis that a gene set with same number of genes could reach the same, or a higher, overlap with the tested pathways. A PEA was performed against a pathway database containing more than 2,000 manually-curated PS-genetic pathways. The various pathways included: cell processes, expression target, immunological, inflammation, metabolic, nociception, signaling, and toxicity, pathways. In addition, results were compared with the entire GO database and the pathway studio ontology database.

A sub-network enrichment analysis (SNEA) was conducted<sup>[6]</sup>. SNEA is similar PEA in that a given gene set is compared to the sub-networks pre-defined within the Pathway Studio ResNet Database. A SNEA was performed, using all LLC-candidate genes against diseases related sub-networks, in order to identify diseases sharing a genetic basis with LLC.

### 2.3 Gene-Gene Interaction analysis

Literature and pathway-based GGIs were conducted

to study associations between AD candidate genes. The literature-based GGI (LGGI) was performed using Pathway Studio, which identified connectivities between given genes/proteins. Edge weight from an LGGI is the number of references underlying a reported gene-gene relation. The pathway-based GGI (PGGI) analysis was conducted using identified candidate pathways. Edge weight is the number of pathways that include both nodes/genes.

### 2.4 Metrics analysis

There are 4 attributes for each node, including 2 literature-based metric scores (RScore and AScore), and 2 enrichment-based metric scores (PScore and SScore) proposed for the gene-gene network built through these steps.

These metrics are proposed based on the reasoning that if a gene is linked to LLC with high probability if it meets the following conditions: (1) it has frequently been associated with LLC (high RScore), in independent studies; (2) it plays roles within multiple pathways associated with LLC (high PScore); and, (3) it demonstrates strong functional linkages to many other genes associated with LLC (high SScore). An AScore, i.e., age score, is proposed to represent the history of each LLC-gene relation and the discovery novel genes (e.g., AScore = 1 for the genes identified in this year, 2016).

### 2.5 Two literature metrics

The reference number underlying a gene-disease relationship as gene reference score (Rscore) Shown here in Eq. (1).

$$RScore = \text{The number of references underlying a relationship} \quad (1)$$

The earliest publication age of a gene-disease relationship is the gene age score (Ascore). Shown here in Eq. (2).

$$AScore = \max_{1 \leq i \leq n} ArticlePubAge_i \quad (2)$$

where  $n$  is the total number of references supporting a gene-disease relation, and

$$ArticlePubAge = Current\ date - Publication\ date + 1 \quad (3)$$

### 2.6 Two enrichment metrics

Given that a disease is associated with a set of genetic pathways  $\mathcal{R}$ , pathway score (PScore) is defined as the number of pathways containing the  $k$ th gene.

$$PScore_k = \text{The number of path ways from } \mathcal{R} \text{ including the } k\text{th gene} \quad (4)$$

Node network significance score (SScore) is defined as the normalized centrality of the node within a network. Shown in Eq. (5).

$$SScore = \frac{N \times C_D^{W_\alpha}(i)}{\sum_j C_D^{W_\alpha}(j)} \quad (5)$$

where  $N$  is the total number of nodes within a network;  $C_D^{W_\alpha}(i)$  is the generalized centrality of the  $i$ th node<sup>[7]</sup>. Defined in Eq. (6).

$$C_D^{W_\alpha}(i) = deg_i \times \left( \frac{strength_i}{deg_i} \right)^\alpha \quad (6)$$

$deg$  is the Freeman formalized node degree centrality<sup>[8]</sup>. Defined in Eq. (7).

$$C_D(i) = deg_i = \sum_j x_{ij} \quad (7)$$

where  $N$  is the total number of nodes,  $i$  is the focal node, and  $j$  represents all other nodes;  $x$  is the adjacency matrix, in which the cell is 1 if node  $i$  and  $j$  are connected, or 0 if not. Note: For network edges built by PNCA,  $C_D \in [0, N]$ .

Strength in Eq. (6) is node strength<sup>[9]</sup>, defined as the sum of weights of node direct ties, i.e.:

$$C_D^w(i) = strength_i = \sum_j w_{ij} \quad (8)$$

where  $w$  is the weighted adjacency matrix. The cell is greater than 0 if the node  $i$  is connected to node  $j$ , and its value represents tie weight. For network edges built using PNCA,  $C_D \in [0, N * M]$ , where  $M$  is the total number of candidate pathways.

In Eq. (5), when  $0 < \alpha < 1$ , high degree and strong ties are both favorably measured, when  $\alpha$  values are greater than 1, lower degrees and stronger ties are favorably measured<sup>[7]</sup>. In this study,

$\alpha = 0.5$ , such that node degree and node strength are equally evaluated.

### 2.7 Validation at small molecule level

It was hypothesized that significant LLC candidate gene regulation contributes to disease treatment, and that these candidate genes might present upstream regulation relations with drugs that are effective in treating LLC. LLC-Drug and Drug-Gene relation data were extracted and analyzed from Mammalian database to test potential relationships between LLC candidate genes and effective LLC medications.

All medications within the relation data sets have been shown as effective in treating LLC and all genes have been identified as LLC-candidate genes using LLC-Gene relation data. Related supporting data appear in Supplementary Table S6a-S6e.

## 3 RESULTS

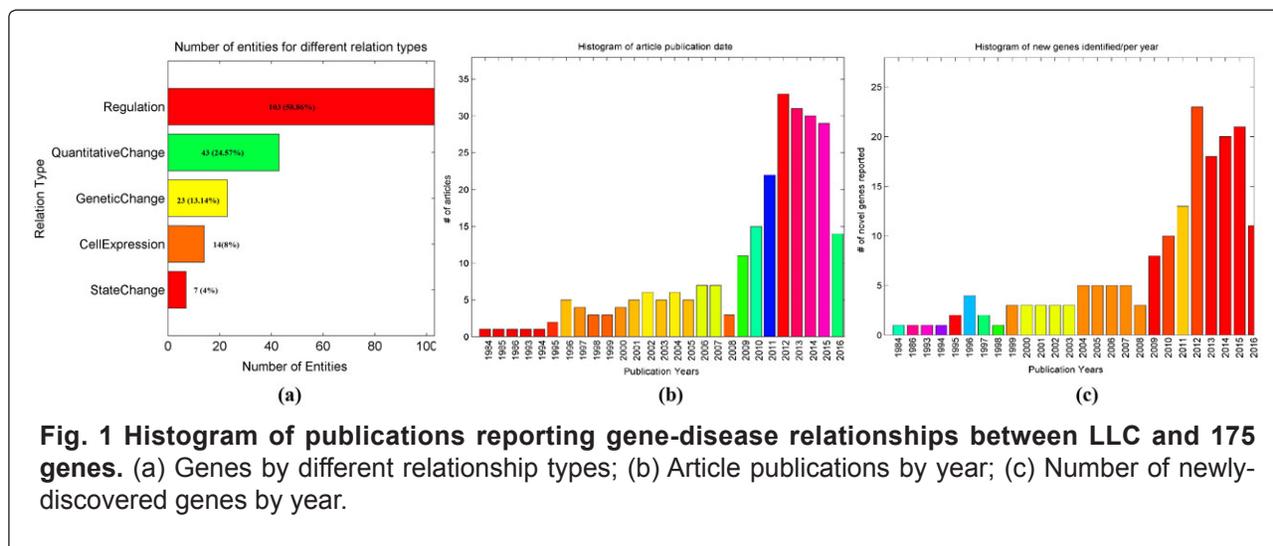
### 3.1 LLC-gene relation data with literature metrics

LLC-gene relation data revealed 175 LLC candidate genes, supported by 256 references. The full gene

list, and related information, including metric scores and related pathways appear in Supplementary Table S1a. The 256 supporting references appear in Supplementary Table S1b, including AD-gene relation types, reference titles, and sentences where an AD-gene relationship was identified. Genes with 'm\_\*' and 'r\_\*' represent mice and rat genes respectively.

A total of 175 genes were associated, in some fashion, with LLC. The ranking by change type is as follows: 103 (58.86 %) with a regulation relationship; 23 (13.14 %) genetic; 43 (24.57 %) quantitative; and, 7 (4.00 %) with state. A total of 15 (8.57 %) were reported to have multiple relationships with the disease. A total of 160 (91.43 %) presented 1 type of relationship to LLC, while 15 (8.57 %) presented 2 (Fig. 1 (a)). ('Relations: Definitions and Annotations' section at <http://pathwaystudio.gousinfo.com/ResNetDatabase.html>.)

The publication date distributions for the underlying 256 articles supporting a gene-LLC relationship appear in Fig. 1 (b). Novel genes reported in each year appear in Fig. 1 (c). As of the date of this article these articles have a 6.3 year average publication age of only 6.3 years, indicating that most were recently published. An analysis showed that publication date distributions for the articles underlying the 175 genes are similar to that presented in Fig. 1 (b).



Of these 175 genes, 11 genes were reported as newly-discovered in 2016 and have an ASocre = 1. Table 1 lists the top 11 genes with the highest

Rscore (in descending order). Complete results appear in Supplementary Table S1a.

**Table 1. Top 11 genes with reported LLC associations ranked by different scores**

|                         |  |
|-------------------------|--|
| Top Genes with AScore=1 | CD36; ACAT1; TRAF6; EIF4EBP1; IL6ST; LIF; MAPK1; MAPK3; RPS6; RPS6KB1; TNFRSF4 |
| Top Genes by RScore     | VEGFA; TNF; KDR; MMP12; HIF1A; IL6; THBS1; GJA4; IL4; PLAU; PLG                |

## 3.2 Enrichment analysis

### 3.2.1 Enrichment analysis for all 175 genes

In this section, PEA and SNEA results for all 175 genes are presented. The complete list appears in Supplementary Table S2a.

Pathway Studio was used to perform an SNEA in order to identify any pathogenic significance of the

reported genes to other disorders potentially related to LLC. The top 126 results p-value < 1e-50 (FDR corrected,  $q = 0.005$ ) appears in Supplementary Table S3a. Table 2 presents the top 10 pathways/groups enrichment analysis results and the top 10 Sub-networks enrichment analysis results by the 175 genes.

**Table 2. Top 10 results of PEA and SNEA by the 175 genes reported**

|   | Pathway/gene set Name                          | GO ID              | p-value   |
|---|--|--------------------|-----------|
|   | angiogenesis                                   | 0001525            | 1.23E-26  |
|   | inflammatory response                          | 0006954            | 3.22E-26  |
|   | response to lipopolysaccharide                 | 0032496            | 2.45E-24  |
| The first 10 pathways/<br>gene sets<br>enriched by<br>175 genes | positive regulation of cell proliferation      | 0008284            | 3.53E-23  |
|   | cytokine activity                              | 0005125            | 8.00E-22  |
|   | response to drug                               | 0042493            | 1.07E-20  |
|   | response to hypoxia                            | 0001666            | 2.27E-20  |
|   | aging  | 0016280            | 2.11E-19  |
|   | positive regulation of gene expression         | 0010628            | 5.12E-19  |
|   | positive regulation of protein phosphorylation | 0001934            | 1.12E-18  |
|   | Gene Set Seed                                  | Jaccard similarity | p-value   |
|   | atherosclerosis                                | 0.08               | 4.85E-101 |
|   | myocardial infarction                          | 0.07               | 1.07E-94  |
|   | chronic obstructive pulmonary disease          | 0.08               | 1.90E-92  |
| The top 10<br>Sub-networks<br>enriched by<br>175 genes          | preeclampsia                                   | 0.06               | 1.96E-87  |
|   | stroke   | 0.07               | 5.53E-83  |
|   | rheumatoid arthritis                           | 0.05               | 1.15E-82  |
|   | hyperglycemia                                  | 0.07               | 1.28E-82  |
|   | obesity  | 0.06               | 8.65E-82  |
|   | pneumonia                                      | 0.06               | 1.86E-81  |
|   | inflammatory bowel disease                     | 0.07               | 8.33E-81  |

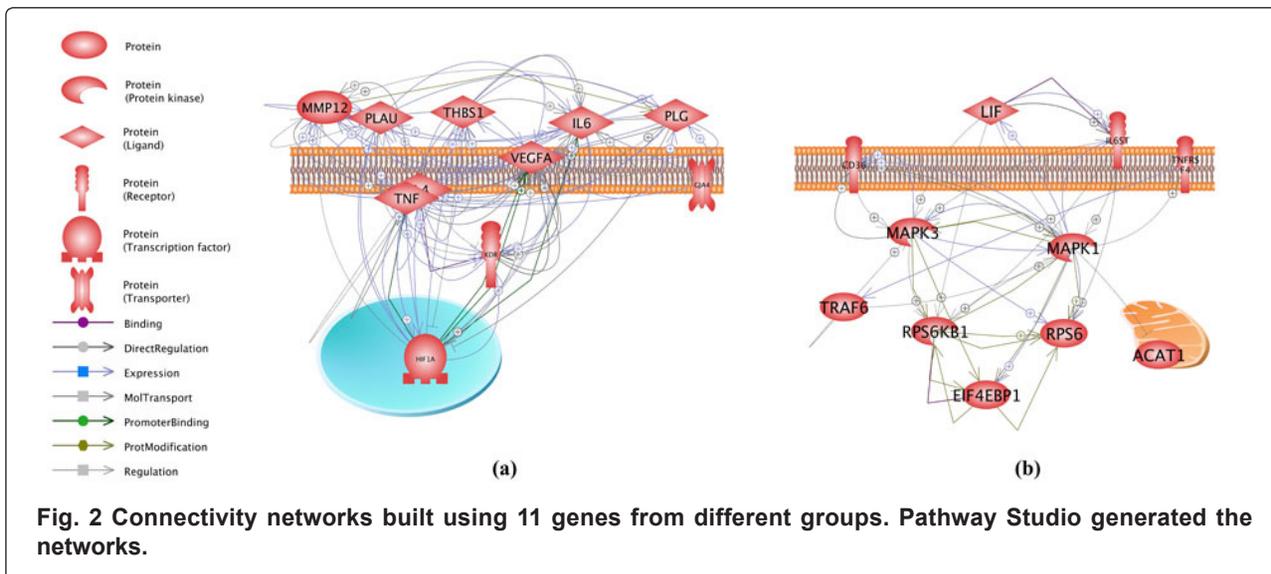
Each pathway/gene set p-value was calculated ( $q = 0.005$  for FDR correction) using one-tailed Fisher-exact test against the hypothesis that the gene set tested was not associated with the corresponding pathway/gene set.

### 3.2.2 Gene-Gene interaction

LGGI and PGGI were performed to generate two network types having the identical nodes but different weighted edges. LGGI is literature-based while PGGI is pathway-based. LGGI was used to test possible gene-gene interactions with literature reports. Building pathway scores (PScore) and pathway-based network significance scores (SScore) was based on PGGI.

LGGI was performed on the top 11 genes having the highest Rscores and AScores (Table 1) to generate gene-gene interaction networks. There were 83 connections among the 11 RScore group genes (Fig. 2(a)). AScore group genes showed only 44 relations among 11 genes (Fig. 2(b)). This observation is consistent with PEA and SNEA, suggesting that genes with the smallest AScore are not as functionally close to each other as were those from the RScore group.

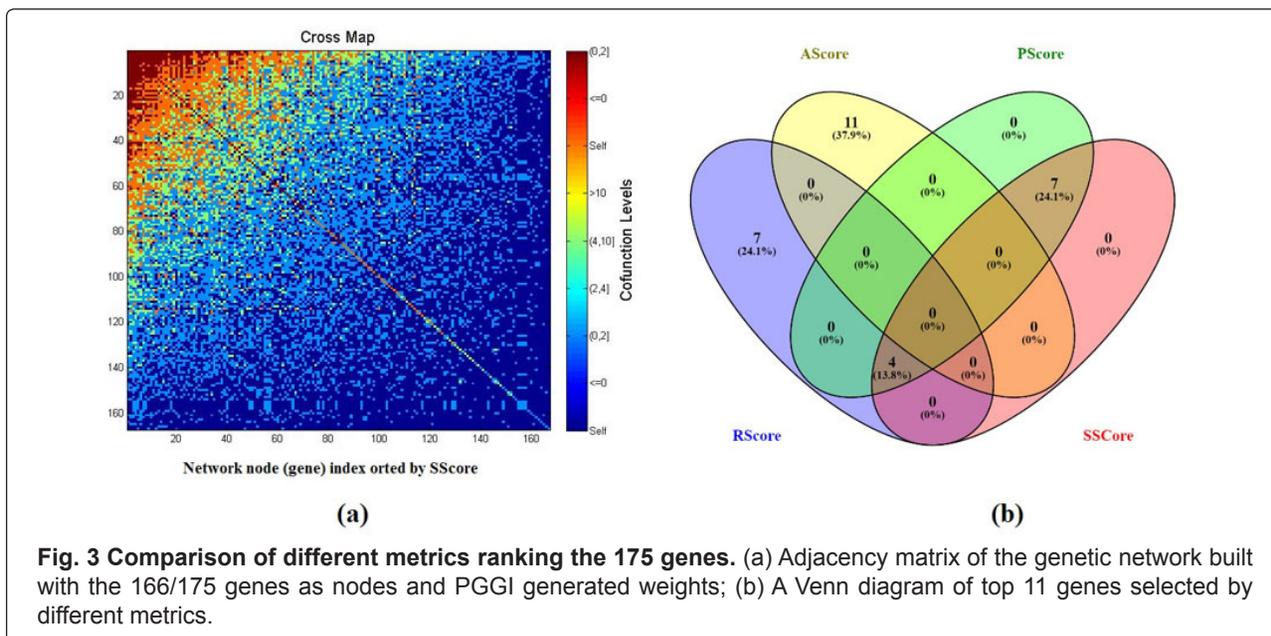
### 3.3 LGGI analysis



### 3.4 PGGI analysis

PGGI showed that, of the 175 genes (network nodes), there were 6,886 edges connecting 166 genes with 9 genes connected to no other nodes (Supplement Table S4). Average network node

strength (sum of the adjacent edge weights) was 122.28. Node strength for the 9 unconnected genes was indicated with a "0". Fig. 3(a) and Supplement Table S5 present the network adjacency matrix of the genetic network built from the 166/175 genes.



### 3.5 Enrichment metric analysis results

Two biological metric pathway scores, a (PScore) and a network significance score (SScore), for each gene using the PGGI-built network (Supplementary Table S1a). PScore values represented the number of LLC candidate pathways involved with the node. An SScore showed node significance to the network. A cross-analysis of the top 11 genes selected using different scores was conducted to study the relationship between the two enrichment metrics and the two literature metrics. A Venn diagram (Fig. 3(b)) was generated (Oliveros, 2007-2015). There was a strong overlap between PScore and SScore (11/11). These 11 genes are the ones that related to the most significantly-enriched pathways. Of these 11 genes, 4 were identified to be an SScore, Pscore, and RScore groups overlap. They include IL6, TNF, VEGFA, and HIF1A, with an RScore =  $6.25 \pm 2.63$  references, a PScore =  $50.75 \pm 10.69$  pathways, and an SScore =  $2.64 \pm 0.33$ .

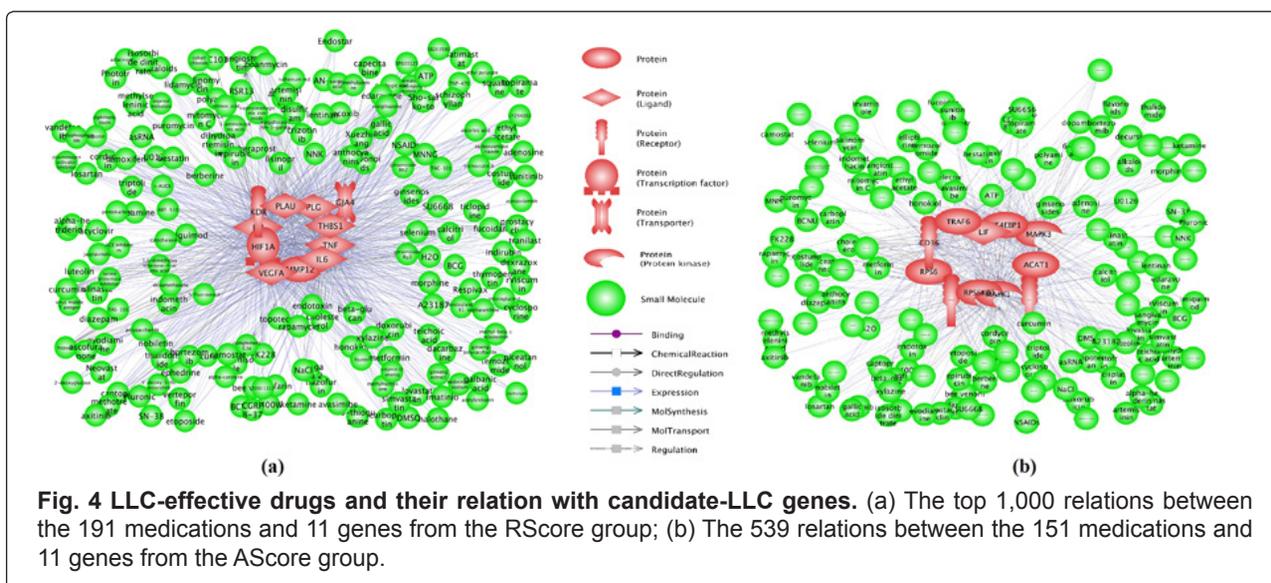
There were 7 genes in both the PScore group and the SScore group, which were not in the RScore group. They are: TGFB1, CCL5, CCL2, PDGFB, PTGS2, SERPINE1, and MMP9. These genes play roles within many significant disease pathways ( $42.43 \pm 5.97$  pathways), and demonstrate strong network centrality (SScore =  $2.38 \pm 0.18$ ). They were old (AScore:  $5.14 \pm 3.76$  years) were not frequently replicated ( $1.29 \pm 0.49$  references), our results suggest that they merit further study.

No overlap between the AScore group and any other group was identified which suggests that these novel LLC candidate genes are supported by fewer studies, and link less to LLC candidate pathways and genes. This observation is consistent with the PEA and SNEA.

### 3.6 Support from LLC drug study

A study of the LLC-Drug relation data showed there were 255 drugs proven to be effective in treating LLC and this is supported by 422 studies (see Supplementary Table S6a).

The Gene-Drug relation study showed more than 2000 relations connecting 169 out of 175 LLC candidate genes and 213 out of 255 LLC drugs, supported by more than over 20K references (Supplementary Table S6b). The 6 unrelated genes include MIR511, MIR545, IL27RA, CCDC88A, ND6, and DESI2. It was observed that there were 11 genes from the RScore group which presented over 1000 relations with 191/255 LLC drugs and supported by more than 15K references (Supplementary Table S6c). The 11 genes from the AScore group presented 539 relations with 151/255 LLC drugs which was supported by more than 5,798 references (Supplementary Table S6d). The top 1,000 Gene-Drug relations are presented in in Fig. 4.



## 4 DISCUSSION

Lung cancer risks are believed to arise from large genetic networks. In many studies, LLC cells were utilized experimentally to explain the underlying

mechanisms of drug-mediated antitumor activity of lung cancer. Results from this study revealed a complex genetic network underlying the LLC

pathogenesis. Network node (gene) and edge (gene-gene interaction) attributes were studied and presented. Small molecular level support identified from LLC-Drug and Drug-Gene relation data analysis acquired from Mammalian database was performed.

PEA results showed that most LLC-candidate genes identified included in the pathways were previously implicated with LLC (Supplementary Table S2a). This included: 2 pathways related to the immune system (with 55 unique genes); 5 pathways related to medication effects (62 unique genes); 5 pathways related to cell apoptosis (71 unique genes); 11 pathways related to cell growth and proliferation (88 unique genes); 4 pathways related to protein phosphorylation (37 unique genes); 5 pathways related to protein kinase (38 unique genes); and one ontology term related to aging (26 unique genes)<sup>[10-17]</sup>. Recognizing the possibility of false positives from the separate studies, we hypothesize that the majority of these literature-reported genes, especially those identified from significantly enriched pathways, should be functionally linked to LLC.

LGGI analysis showed that many of the frequently reported genes related to LLC were functionally associated with one another (Fig 2). This analysis was supported by thousands of references. PGGI results confirmed the observation and showed that 166/175 nodes presented strong connectivity with each other (average node degree: 41.48 edges). The results indicated that these functionally-linked genes possessed higher opportunities as true discoveries than that mere noise (false positives)<sup>[18]</sup>.

SNEA was performed to provide higher levels of confidence when interpreting experimentally-derived genetic data against the background of previously published results (Pathway Studio Web Help). SNEA results demonstrated that over 70 % of the 175 genes<sup>[19]</sup> also identified as causal genes for other lung cancer-associated disorders.

For each LLC genetic network node, 4 metric scores are proposed as node attributes to evaluate their significance in terms of: 1) publication frequency (RScore); 2) novelty (AScore); 3) number of associated LLC candidate pathways (PScore); and, 4) network centrality (SScore). Using these metrics scores, we were able to rank the genes according to different needs/significance and identify the top ones for further analysis (Supplementary Table S1a). There were 4 genes that frequently replicated (with high RScore) that also demonstrated high SScore and PScore, such as IL6, TNF, VEGFA, and HIF1A (see Fig. 3(b)). These genes have an average support of  $6.25 \pm 2.63$  references, and are connected to multiple, significantly-enriched LLC candidate pathways ( $50.75 \pm 10.69$  pathways). These genes present the highest network centralities

(SScore =  $2.64 \pm 0.33$ ), suggesting that they are important disease network nodes and are likely to pose biological significance.

There were 7 genes observed in in both the PScore group and the SScore group, but not in the RScore group. Although these genes were older, in terms of AScore ( $5.14 \pm 3.76$  years) and frequently replicated ( $1.29 \pm 0.49$  references), they played roles within multiple LLC candidate pathways ( $42.43 \pm 5.97$  pathways) and demonstrated high centrality to the whole network (SScore =  $2.38 \pm 0.18$ ). One gene, PTGS2 (SScore = 2.30), although reported 13 years ago and having only 2 references to date supported its relation with LLC, was linked to 166/175 genes and played roles within 35 significantly-enriched LLC candidate pathways. Many of these are implicated with LLC, including: angiogenesis, inflammatory response; ROS in triggering vascular inflammation; response to lipopolysaccharide; positive/negative regulation of cell proliferation; positive regulation of apoptotic process; IL1A expression targets; IL1B expression targets F2 -> STAT1/NF-kB expression targets; PAF expression targets; TLR4/AP-1 expression targets; CXCL12 expression targets; HMGB1 expression targets; TNF/AP-1 expression targets; CD40LG/NF-kB/ELK-SRF/CREB/NFATC expression targets; and, positive regulation of nitric oxide biosynthetic process<sup>[11-17, 20-28]</sup>. The observations suggest that these genes may play significant roles in LLC pathogenesis and merit further study.

An LLC-medication relation data study (Supplementary Table S6a), showed that the majority of these drugs (213/253) regulate most of the LLC candidate genes (169/173). A showing supported by over 20,000 references (Supplementary Table S6c). Eleven genes from the RScore group presented over 1,000 relations with 191/255 LLC medications, supported by more than 16,145 references (Supplementary Table S6d). The 11 genes from the AScore group presented 539 relations with 151/255 LLC medications, supported by more than 5,798 references (see Supplementary Table S6e).

These results help to understand the underlying mechanisms and biological processes of LLC and support the hypothesis that the majority of the 175 LLC gene pool plays a role in lung cancer pathogenesis. Mitomycin C has shown anti-LLC effects with by unknown mechanisms<sup>[29, 30]</sup>. This study shows that Mitomycin C presents 34 relations with 34/175 genes that were related to LLC (Supplementary Table S7). These 34 genes regulate LLC pathogenic development. The results provide supporting information for understanding the Mitomycin C anti-tumor effects.

This study has limitations that should be

considered in future work. Although the 175 gene-LLC relation is supported by 256 articles, it remains possible that some gene-LLC associations were left undiscovered. Although the metrics helps in ranking genes and selecting the top genes with specific significance, further network analysis using more complex algorithms (e.g., graph theory) and more data sets may extract additional meaningful features to identify biologically significant genes to the disease.

LLC is a complex disease whose genetic causes are linked to a network composed of a large number of genes. This study provided a comprehensive weighted genetic network with node attributes for LLC, which could be used as groundwork for further

biological/genetic studies in the area.

## CONFLICTS OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## FUNDINGS

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## REFERENCES

1. Ma X, Yu H. Global Burden of Cancer. *Yale J Biol Med.* 2006; 79(3-4): 85-94.
2. Yang C, Gagnon C, Hou X, Hardy P. Low density lipoprotein receptor mediates anti-VEGF effect of lymphocyte T-derived microparticles in Lewis lung carcinoma cells. *Cancer Biol Ther.* 2010; 10(5): 448-456.
3. Chen Y, Zhang S, Peng G, Yu J, Liu T, Meng R, Li Z, Zhao Y, Wu G. Juncendothelial NO synthase and reactive oxygen species mediated effect of simvastatin on vessel structure and function: Pleiotropic and dose-dependent effect on tumor vascular stabilization. *Int J Oncol.* 2013; 42(4): 1325-1336.
4. Taniguchi K, Taniguchi K, Taniguchi K, Karin M, Karin M. IL-6 and related cytokines as the critical lynchpins between inflammation and cancer. *Semin Immunol.* 2014; 26(1): 54-74.
5. Huang G, Chen L. Recombinant human endostatin improves anti-tumor efficacy of paclitaxel by normalizing tumor vasculature in Lewis lung carcinoma. *J Cancer Res Clin Oncol.* 2010; 136(8): 1201-1211.
6. Sivachenko AY, Yuryev, A, Daraselia, N. Molecular networks in microarray analysis. *J Bioinform Comput Biol.* 2007; 5(2B): 429-456.
7. Opsahl T, Agneessens F, Skvoretz J. Node centrality in weighted networks: Generalizing degree and shortest paths. *Social Networks.* 2010; 32(3): 245-251.
8. Freeman LC. Centrality in social networks: Conceptual clarification. *Social Networks.* 1978; 1(3): 215-239.
9. Yang S, Knoke D. Optimal connections: strength and distance in valued graphs. *Social Networks.* 2001; 23(4): 285-295.
10. Vallabhapurapu S, Karin M. Regulation and function of NF-kappaB transcription factors in the immune system. *Annu Rev Immunol.* 2009; 27: 693-733.
11. Lee EO, Lee HJ, Hwang HS, Ahn KS, Chae C, Kang KS, Lu J, Kim SH. Potent inhibition of Lewis lung cancer growth by heyneanol A from the roots of *Vitis amurensis* through apoptotic and anti-angiogenic activities. *Carcinogenesis.* 2006; 27(10): 2059-2069.
12. Thoppil RJ, Adapala RK, Cappelli HC, Kondeti V, Dudley AC, Gary Meszaros J, Paruchuri S, Thodeti CK. TRPV4 channel activation selectively inhibits tumor endothelial cell proliferation. *Sci Rep.* 2015; 5: 14257.
13. Wu X, Liu B, Ji S, Wu J, Xu C, Du Y, You X, Li B, Le J, Xu H, Duan X, Dong J. Social defeat stress promotes tumor growth and angiogenesis by upregulating vascular endothelial growth factor/extracellular signal-regulated kinase/matrix metalloproteinase signaling in a mouse model of lung carcinoma. *Mol Med Rep.* 2015; 12(1): 1405-1412.
14. Ohashi M, Sugikawa E, Nakanishi N. Inhibition of p53 protein phosphorylation by 9-hydroxyellipticine: a possible anticancer mechanism. *Jpn J Cancer Res.* 1995; 86(9): 819-827.
15. Liu P, Cheng H, Roberts TM, Zhao J. Targeting the phosphoinositide 3-kinase pathway in

- cancer. *Nat Rev Drug Discov.* 2009; 8(8): 627-644.
16. Li Y, Qi K, Zu L, Wang M, Wang Y, Zhou Q. Anti-apoptotic brain and reproductive organ-expressed proteins enhance cisplatin resistance in lung cancer cells via the protein kinase B signaling pathway. *Thorac Cancer.* 2016; 7(2): 190-198.
  17. Gozes Y, Trainin N. Enhancement of Lewis lung carcinoma in a syngeneic host by spleen cells of C57BL/6 old mice. 1977; 7(3): 159-164.
  18. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A.* 2005; 102(43): 15545-15550.
  19. Klement H, St Croix B, Milsom C, May L, Guo Q, Yu JL, Klement P, Rak J. Atherosclerosis and Vascular Aging as Modifiers of Tumor Progression, Angiogenesis, and Responsiveness to Therapy. *Am J Pathol.* 2007; 171(4): 1342-1351.
  20. Saijo Y, Tanaka M, Miki M, Usui K, Suzuki T, Maemondo M, Hong X, Tazawa R, Kikuchi T, Matsushima K, Nukiwa T. Proinflammatory cytokine IL-1 beta promotes tumor growth of Lewis lung carcinoma by induction of angiogenic factors: in vivo analysis of tumor-stromal interaction. *J Immunol.* 2002; 169(1): 469-475.
  21. Taniguchi H, Shimada Y, Sawachi K, Hirota K, Inagawa H, Kohchi C, Soma G, Makino K, Terada H. Lipopolysaccharide-activated alveolar macrophages having cytotoxicity toward lung tumor cells through cell-to-cell binding-dependent mechanism. *Anticancer Res.* 2010; 30(8): 3159-3165.
  22. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Signal.* 2014; 20(7): 1126-1167.
  23. Dondossola E, Dobroff AS, Marchiò S, Cardó-Vila M, Hosoya H, Libutti SK, Corti A, Sidman RL, Arap W, Pasqualini R. Self-targeting of TNF-releasing cancer cells in preclinical models of primary and metastatic tumors. *Proc Natl Acad Sci USA.* 2016; 113(8): 2223-2228.
  24. Li C, Li H, Jiang K, Li J, Gai X. LR4 signaling pathway in mouse Lewis lung cancer cells promotes the expression of TGF- $\beta$ 1 and IL-10 and tumor cells migration. *Biomed Mater Eng.* 2014; 24(1): 869-875.
  25. Hackler PC, Reuss S, Konger RL, Travers JB, Sahu RP. Systemic Platelet-activating Factor Receptor Activation Augments Experimental Lung Tumor Growth and Metastasis. *Cancer Growth Metastasis.* 2014; 7(7): 27-32.
  26. Sun X, Cheng G, Hao M, Zheng J, Zhou X, Zhang J, Taichman RS, Pienta KJ, Wang J. CXCL12/CXCR4/CXCR7 chemokine axis and cancer progression. *Cancer Metastasis Rev.* 2010; 29(4): 709-722.
  27. Xu X, Zhu H, Wang T, Sun Y, Ni P, Liu Y, Tian S, Amoah Barnie P, Shen H, Xu W, Xu H, Su Z. Exogenous high-mobility group box 1 inhibits apoptosis and promotes the proliferation of lewis cells via RAGE/TLR4-dependent signal pathways. *Scand J Immunol.* 2014; 79(6): 386-394.
  28. Masri F. Role of nitric oxide and its metabolites as potential markers in lung cancer. *Ann Thorac Med.* 2010; 5(3): 123-127.
  29. Lou J, Chua Y, Chew E, Gao J, Bushell M, Hagen T. Inhibition of hypoxia-inducible factor-1alpha (HIF-1alpha) protein synthesis by DNA damage inducing agents. *PLoS One.* 2010; 5(5): e10522.
  30. Duan S, Yuan G, Liu X, Ren R, Li J, Zhang W, Wu J, Xu X, Fu L, Li Y, Yang J, Zhang W, Bai R, Yi F, Suzuki K, Gao H, Esteban CR, Zhang C, Izpisua Belmonte JC, Chen Z, Wang X, Jiang T, Qu J, Tang F, Liu GH. PTEN deficiency reprogrammes human neural stem cells towards a glioblastoma stem cell-like phenotype. *Nat Commun.* 2015; 6: 10068.