Sains Malaysiana 47(12)(2018): 2933–2940
http://dx.doi.org/10.17576/jsm-2018-4712-01

Construction and Analysis of Protein-Protein Interaction Network to Identify the Molecular Mechanism in Laryngeal Cancer

(Pembinaan dan Analisis Jaringan Interaksi Protein-Protein untuk Mengenal Pasti Mekanisme Molekul Kanser Larinks)

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ABSTRACT

Laryngeal cancer is the most common head and neck cancer in the world and its incidence is on the rise. However, the molecular mechanism underlying laryngeal cancer pathogenesis is poorly understood. The goal of this study was to develop a protein-protein interaction (PPI) network for laryngeal cancer to predict the biological pathways that underlie the molecular complexes in the network. Genes involved in laryngeal cancer were extracted from the OMIM database and their interaction partners were identified via text and data mining using Agilent Literature Search, STRING and GeneMANIA. PPI network was then integrated and visualised using Cytoscape ver3.6.0. Molecular complexes in the network were predicted by MCODE plugin and functional enrichment analyses of the molecular complexes were performed using BiNGO. 28 laryngeal cancer-related genes were present in the OMIM database. The PPI network associated with laryngeal cancer contained 161 nodes, 661 edges and five molecular complexes. Some of the complexes were related to the biological behaviour of cancer, providing the foundation for further understanding of the mechanism of laryngeal cancer development and progression.

Keywords: Functional enrichment analysis; laryngeal cancer; protein-protein interaction network; text mining

ABSTRAK

Kanser larinks adalah kanser kepala dan leher yang paling biasa di dunia dan kejadiannya semakin meningkat. Walau bagaimanapun, mekanisme molekul yang terlibat dalam patogenesis kanser larinks masih kurang difahami. Tujuan kajian ini dijalankan adalah untuk membangunkan jaringan interaksi protein-protein (IPP) bagi kanser larinks untuk meramal tapak jalan biologi menerusi analisis kompleks molekul daripada dalam jaringan IPP yang dibina. Gen yang terlibat dalam kanser larinks telah diekstrak daripada pangkalan data OMIM dan pasangan interaksinya telah dikenal pasti melalui pencarian teks dan data menggunakan Agilent Literature Search, STRING dan GeneMANIA. Jaringan IPP kemudiannya digabung dan divisualisasikan menggunakan Cytoscape ver3.6.0. Kompleks molekul dalam jaringan diramalkan oleh plugin MCODE dan analisis pengkayaan berfungsi kompleks molekul dilakukan menggunakan BiNGO. 28 gen berkaitan dengan kanser larinks ditemui dalam pangkalan data OMIM. Jaringan IPP yang dikaitkan dengan kanser larinks mengandungi 161 nodus, 661 interaksi dan lima kompleks molekul. Beberapa kompleks didapati berkaitan dengan tingkah laku biologi kanser dan ini telah menyediakan asas untuk memahami lebih lanjut mekanisme dalam pembangunan dan perkembangan kanser larinks.

Kata kunci: Analisis pengayaan berfungsi; jaringan interaksi protein-protein; kanser larinks; pencarian teks

INTRODUCTION

The most common rare cancers of the respiratory system occur in the larynx, nasopharynx, nose, and nasal cavity. From that list, laryngeal cancer is the most common malignancy with the estimated incidence of is 156,877 worldwide, with 83,376 mortalities (Ferlay et al. 2015). In the US, incidence and mortality are 5 and 1.4 per 100,000 people, respectively and the laryngeal cancer rate is up to five times higher in males than in females (Siegel et al. 2017).

The well-established risk factors for laryngeal cancer are cigarette smoking and alcohol consumption, as they cause cancer development through multiple genetic alterations such as tumor suppressor genes silencing and oncogene activation (Mehrotra & Yadav 2006). A family history of laryngeal cancer or other head and neck squamous cell carcinoma also increases the risk of laryngeal cancer development (Coskunpinar et al. 2014). Laryngeal cancer is considered to be very challenging for both patients and clinicians. The diagnosis usually requires numerous hospital visits and misdiagnoses are quite commonly occurred. On top of that, treatment options for this cancer are limited and less effective compared to the more common cancers, as fewer clinical trials are conducted for rare cancers (Siegel et al. 2017).

Current treatments for laryngeal cancer are surgery and/or radiotherapy or the combination of surgery and chemoradiotherapy for patients at more advanced stages.

However, despite modern therapeutic advancements, the clinical outcome for laryngeal cancer patients remains the same as it was two decades ago due to treatment inadequacy and frequent recurrence (Coskunpinar et al. 2014). Thus, a better understanding of the molecular mechanisms involved in laryngeal cancer is needed to develop effective biomarkers and specific therapeutic targets.

Recently, systems biology approaches such as the application of network-based methods to understand human diseases have been developing rapidly (Ideker & Sharan 2008). This is due to the well-established notion that a disease, especially cancer, is usually a result of complex interactions and communications within a large set of proteins and other molecules. Such integrative analysis has been applied to study lung cancer (Wu et al. 2016), brain cancer (Engin et al. 2013), prostate cancer (Chen et al. 2016), and non-cancer diseases such as diabetes (Vyas et al. 2016) and polycystic ovarian syndrome (PCOS) (Mohamed-Hussein & Harun 2009). In this study, we analysed laryngeal cancer-related protein-protein interaction (PPI) networks to improve our understanding of the complexity of the molecular pathways that underlie laryngeal cancer to identify the dynamic processes involved in cancer progression.

Materials and Methods

Selecting laryngeal cancer-related genes

Genes associated with laryngeal cancer were obtained from the Online Mendelian Inheritance in Man (OMIM) database (https://www.ncbi.nlm.nih.gov/omim) using keywords 'laryngeal' AND 'cancer' and 'laryngeal' AND 'carcinoma'.

Construction of PPI network

Predicted interaction partners for each gene were extracted from the literature using Cytoscape plug-in Agilent Literature Search 3.1.1 (USA Agilent Technologies, Santa Clara, California). Two other databases were also used in this study: GeneMANIA (Zuberi et al. 2013) and STRING (Szklarczyk et al. 2017). All retrieved data were loaded into Cytoscape ver3.6.0 (Shannon et al. 2003) to construct an integrated PPI network.

Topological and functional analysis of PPI network

To identify the key nodes of the PPI network, NetworkAnalyzer tool in Cytoscape was used to calculate the simple topological parameters. The PPI network was then analysed for molecular complexes using the Molecular Complex Detection (MCODE) plug-in (Bader & Hogue 2003) with degree cutoff = 3. This analysis extracted the densely connected regions in the PPI network, assuming that they may represent molecular complexes. The list of proteins in each molecular complex were then analysed using BiNGO (Maere et al. 2005), which is another plug-in in Cytoscape, to identify the Gene Ontology (GO) terms enriched in laryngeal cancer.

The data flow diagram given in Figure 1 summarises the steps and bioinformatics tools used in this study.

Results and Discussion

Construction and analysis of laryngeal cancer PPI network

The OMIM database retrieval process identified 28 genes that were associated with laryngeal cancer: *ADH1B*, *BCL10*,

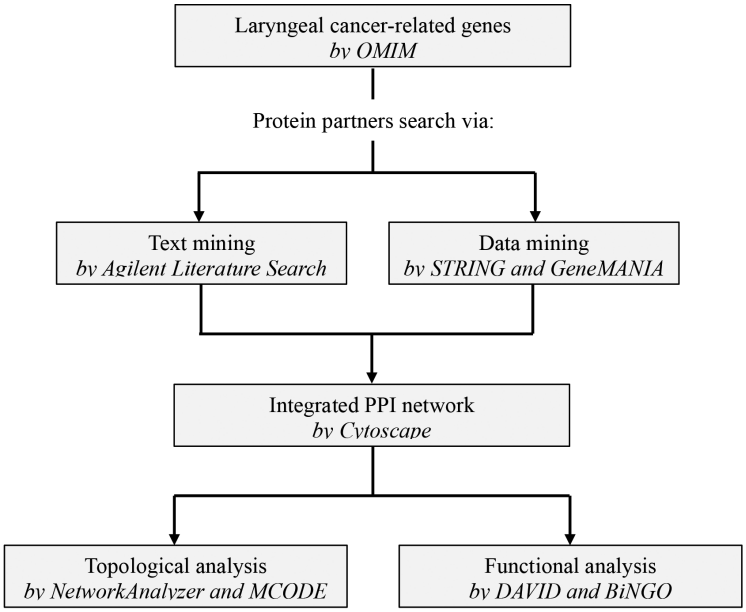

FIGURE 1. Flowchart depicting the steps and tools used in the construction and analysis of laryngeal cancer PPI network

BRCA2, *C1R*, *CASP3*, *EDS8*, *GGNBP2*, *GJB2*, *HPV6AI1*, *HPVC1*, *ICK*, *ING1*, *KRT14*, *KRT5*, *LNCR1*, *MGP*, *MLH1*, *MSH2*, *MYCT1*, *PIK3R2*, *PLG*, *PTEN*, *RAF1*, *RET*, *RSPO1*, *SDHD*, *TNFRSF10B*, and *TP53*. Based on these genes, three separate PPI network were constructed and produced: 111 nodes and 220 edges via text mining through Agilent Literature Search; 74 nodes and 517 edges through GeneMANIA; and 28 nodes and 98 edges through STRING (Figure 2). The diamond nodes represent laryngeal cancer-related proteins extracted from OMIM and the circles represent the proteins obtained from the three PPI databases.

These separate PPI network were then merged in Cytoscape to produce an integrated PPI network of 161 nodes and 661 edges (Figure 3). The different scale of color from green to yellow to red denotes the number of connections for each node (range 1 to 56). Therefore, the more reddish nodes are considered to have the highest connectivity and are regarded as hub nodes.

Apart from detecting hub nodes through node degree calculation, the measurement of betweenness centrality (BC) to identify bottleneck nodes is also important. The BC measures the total number of shortest paths going through a certain node and the nodes with high betweenness; known as bottleneck nodes; act like a bridge in between highly interconnected network clusters (Yu et al. 2007). In this study, hub nodes with large BC value are considered as the key genes (Table 1). KEGG pathway analysis using DAVID functional annotation tools indicated that all six key genes are involved in cancer pathway ($p = 5.87 \times 10^{-7}$).

The PPI network was further analysed using the MCODE plugin to identify subnetworks that might represent molecular complexes. This analysis is usually performed to investigate new molecular functional groups or to discover unknown protein functions. According to the principle of 'guilt by association', whenever a protein interacts with another protein, it is part of the same biological process and is located in the same cellular compartment. Therefore, proteins of the same molecular complex generally have the same function. In this study, the MCODE algorithm analysis identified five subnetworks

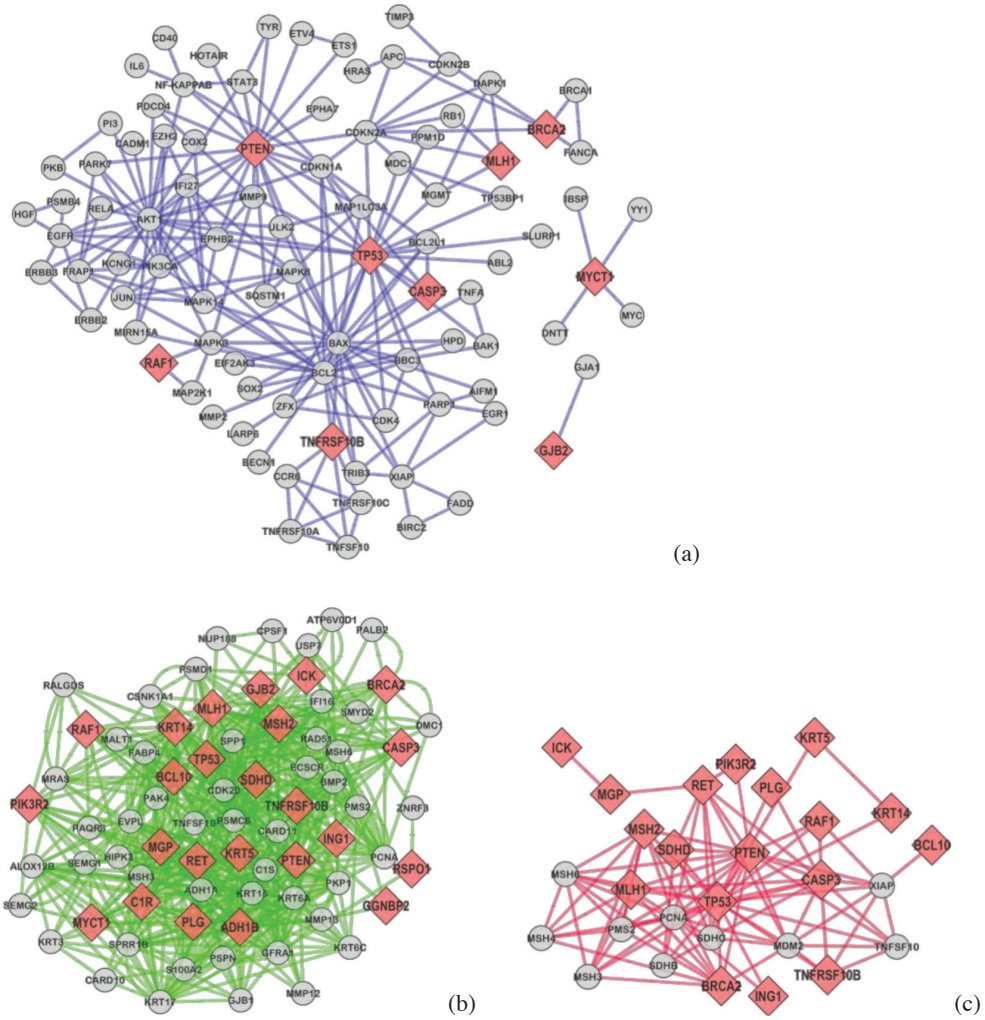

FIGURE 2. The laryngeal cancer-related proteins retrieved from OMIM produced the following three PPI networks: (a) the PPI network constructed from Agilent Literature Search produced 111 nodes and 220 edges; (b) the PPI network constructed from GeneMANIA produced 74 nodes and 517 edges; (c) the PPI network constructed from STRING produced 28 nodes and 98 edges

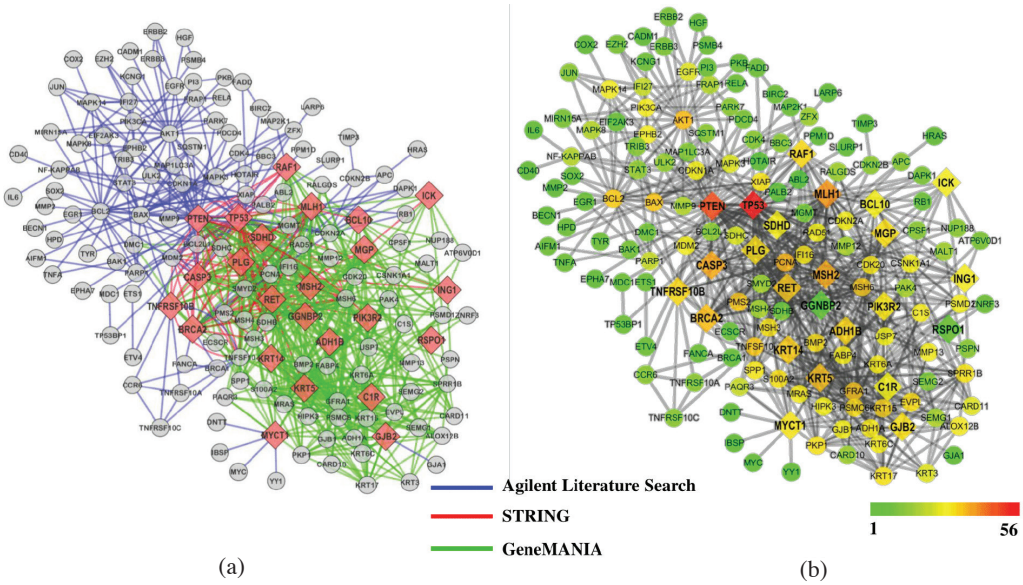

FIGURE 3. Integrated PPI network of laryngeal cancer-related proteins: (a) integrated PPI network constructed from three databases (Agilent Literature Search, STRING, and GeneMANIA); (b) PPI network differentiated with the number of connections for each node

TABLE 1. Hub and bottleneck nodes in PPI network

Parameters	Gene Name
Hub nodes	*TP53, PTEN, MLH1, CASP3, MSH2, PCNA, AKT1, BRCA2, KRT5, BAX*
Bottleneck nodes	*PTEN, AKT1, BCL2, BRCA2, BAX, MYCT1, XIAP, MLH1, TNFSF10, CASP3*
(Hub + Bottleneck) nodes	*PTEN, MLH1, CASP3, AKT1, BRCA2, BAX*

that might represent functional molecular complexes (Figure 4).

GO TERMS ENRICHED IN THE PPI NETWORK

The list of proteins for each molecular complex was submitted to BiNGO to obtain information regarding GO terms (biological process, molecular function and cellular component) that are overrepresented in all of the complexes. The GO analysis suggested that proteins in complexes 1, 2, 3, 4 and 5 were enriched in ectoderm development, mismatch repair, release of cytochrome *c* from mitochondria, apoptosis, and glycogen cell development involved in embryonic placenta development, respectively (Table 2). The latter is not significant in this study hence will not be discussed further.

Ectoderm development is the most significant node in complex 1, which involves nine proteins (KRT17, KRT15, KRT14, ALOX12B, KRT5, EVPL, KRT6C, SPRR1B, and KRT6A). Of these, KRT14 and KRT5 are laryngeal cancer-related proteins extracted from OMIM. KRT14, KRT6A, and KRT5 are members of the keratin protein family and are co-expressed during the differentiation process of simple and stratified epithelial tissues (Zhang et al. 2014). Furthermore, Jeřábková et al. (2010) reported that mutations that occur in these keratin proteins were associated with skin diseases. Zhang et al. (2014) used next generation sequencing to study laryngeal squamous cell carcinoma patients and found that KRT14, KRT6A, and KRT5 were part of nine mitochondrial genes (*SFN, CTB-63M22.1, HSPB1, KRT5, KRT6A, KRT14, IGHA1, IGLC2*, and *IGLC3*) that were significantly mutated compared to those with healthy tissues.

Another highly significant biological process node, mismatch repair, involves seven proteins (MSH6, PCNA, MSH2, MSH3, MSH4, PMS2 and MLH1) that contribute to maintaining genetic stability (Harfe & Jinks-Robertson 2000). MLH1 and MSH2 are the laryngeal cancer-related proteins extracted from OMIM. DNA mismatch repair refers to the process that corrects mismatches produced during DNA replication that had escaped proofreading (Kunkel & Erie 2005). *MLH1* is one of the mutated genes in cancer that is known to encode the proteins that are involved in mismatch repair. Sasiadek et al. (2006) used immunostaining to assess the expression of MLH1 and other related proteins and found that both CCND1 and MLH1 were functionally connected in chromosomal imbalances specifically in larynx cancer. The other two players involved in the DNA mismatch repair pathway are MSH2 and MSH3; they recognise DNA damage induced by carcinogens (Kunkel & Erie 2005).

Release of cytochrome *c* from mitochondria is the most significant biological process in complex 3 and

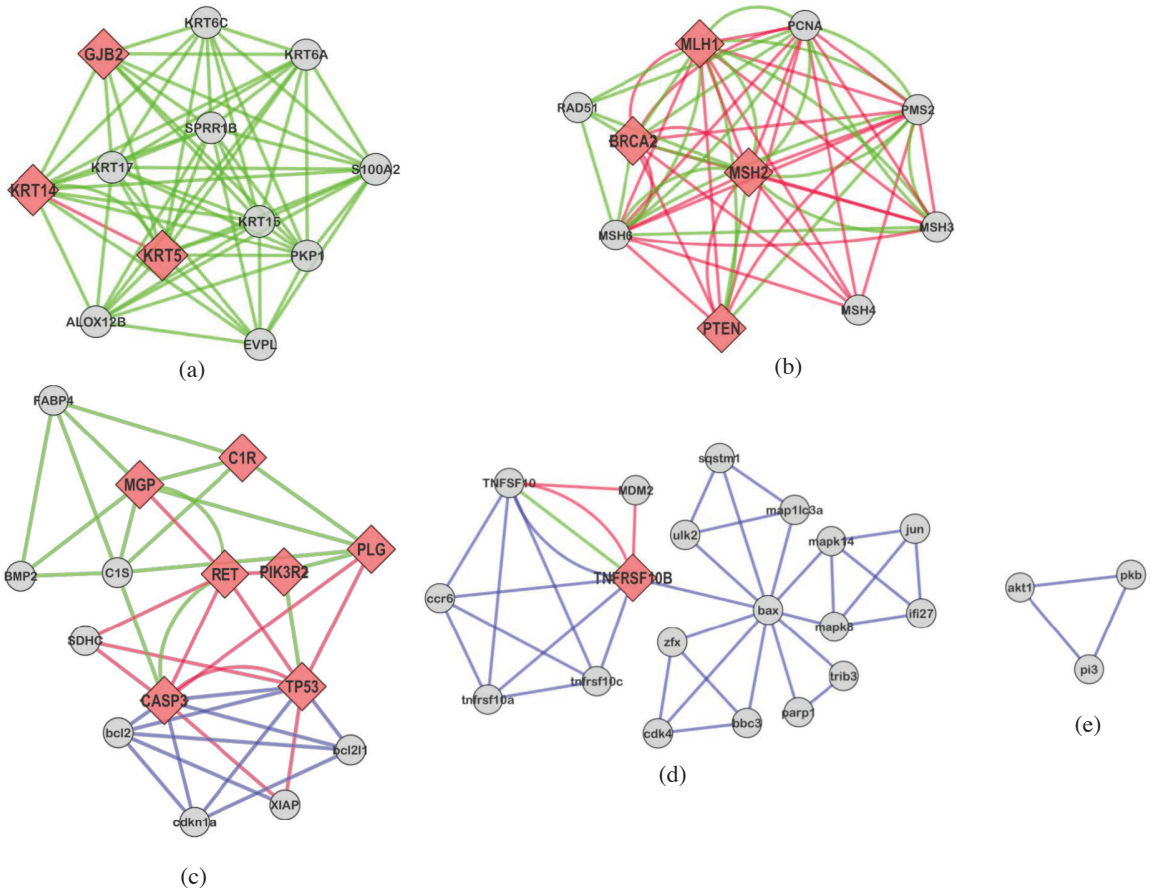

FIGURE 4. Molecular complexes obtained from the MCODE algorithm analysis: (a) complex 1 (score: 11.091, 12 nodes, and 62 edges); (b) complex 2 (score: 8.444, 10 nodes, and 60 edges); (c) complex 3 (score: 5.286, 15 nodes, and 40 edges); (d) complex 4 (score: 4, 19 nodes, and 38 edges); (e) complex 5 (score: 3, 3 nodes, and 3 edges)

involves CASP3, BCL2, TP53, and BCL2L1. CASP3, and TP53 are the laryngeal cancer-related proteins extracted from OMIM, whereas BCL2, and BCL2L1 were added from the Agilent Literature Search. BCL2 family proteins are known to regulate the permeabilisation of the mitochondrial outer membrane as well as the release of cytochrome *c* (Wei et al. 2001). This process is a key initiative step in the apoptotic process (Gogvadze et al. 2006), which is linked to complex 4 in this study.

Regulation of apoptosis was overrepresented in complex 4. This biological process is involved in cancer development and progression and is regulated via activation of tumor necrosis factor receptor super-family (TNFRSF) by tumor necrosis factor related apoptosis induce ligand (TRAIL). Several papers have studied the different levels of TRAIL and TRAIL receptor in laryngeal cancer (Verim et al. 2014; Yoldas et al. 2011). TRAIL is known as a key effector molecule in cell signalling pathway that would trigger apoptosis in cancer cells. However, the most recent study that compared TRAIL receptors in patients with laryngeal squamous cell carcinoma and benign laryngeal pathologies concluded that TRAIL receptors are not suitable to be used as biomarkers (Erkul et al. 2016). The association of other genes that encode TNFRSF such as *TNFRSF10B* would shed light on understanding the mechanism of laryngeal cancer. Previous study found that mutation of *TNFRSF10B* contribute to the progression of head and neck tumors where it functions in metastasis, migration and invasion of tumor cell tissues (Schabath et al. 2013; Tahir et al. 2013).

CONCLUSION

In this post-genomic era, massive amounts of biomolecule data for many diseases are accessible at many public databases. However, translating this information into actionable knowledge about disease pathogenesis for disease prevention, diagnosis, and treatment remains a great challenge. In this study, we established a PPI network of laryngeal cancer that is associated with three significant biological processes that can be related to laryngeal cancer: Ectoderm development, mismatch repair, and release of cytochrome *c* from mitochondria. Each biological process was described and the proteins involved were identified. These findings will provide a basis for understanding molecular pathogenesis for the development of better strategies in laryngeal cancer prevention, diagnosis and therapy.

TABLE 2. GO terms related to the molecular complexes of the laryngeal cancer PPI network

Category	GO term	Genes	*P* value	FDR[a]
Complex 1				
Biological process	Ectoderm development	*KRT17, KRT15, KRT14, ALOX12B, KRT5, EVPL, KRT6C, SPRR1B, KRT6A*	4.1418×10^{-15}	4.7217×10^{-13}
Molecular function	Structural constituent of cytoskeleton	*KRT17, KRT15, KRT14, KRT5, KRT6C, KRT6A*	9.6569×10^{-12}	2.8971×10^{-10}
Cellular component	Intermediate filament	*KRT17, KRT15, KRT14, PKP1, KRT5, KRT6C, KRT6A*	5.6985×10^{-12}	1.8075×10^{-10}
Complex 2				
Biological process	Mismatch repair	*MSH6, PCNA, MSH2, MSH3, MSH4, PMS2, MLH1*	8.3997×10^{-19}	4.3174×10^{-16}
Molecular function	Mismatched DNA binding	*PCNA, MSH2, MSH4, PMS2, MLH1*	6.8782×10^{-14}	5.9152×10^{-12}
Cellular component	Mismatch repair complex	*MSH6, MSH2, MSH3, MLH1*	3.5026×10^{-13}	2.2417×10^{-11}
Complex 3				
Biological process	Release of cytochrome *c* from mitochondria	*CASP3, BCL2, TP53, BCL2L1*	4.6355×10^{-9}	4.3666×10^{-6}
Molecular function	Enzyme regulator activity	*CDKN1A, BMP2, CASP3, XIAP, PIK3R2, TP53, BCL2L1*	7.0332×10^{-6}	5.7583×10^{-4}
Cellular component	Cytosol	*CDKN1A, FABP4, CASP3, BCL2, XIAP, PIK3R2, TP53, BCL2L1*	6.5564×10^{-6}	6.1630×10^{-4}
Complex 4				
Biological process	Apoptosis	*JUN, MAPK8, IFI27, TNFRSF10C, TNFSF10, TNFRSF10B, BAX, TNFRSF10A, TRIB3, SQSTM1, BBC3*	8.5201×10^{-12}	2.7183×10^{-9}
Molecular function	TRAIL binding	*TNFRSF10B, TNFRSF10A*	1.4340×10^{-6}	1.8355×10^{-4}
Cellular component	Nucleoplasm	*JUN, MAPK8, PARP1, CDK4, MDM2, MAPK14, SQSTM1*	3.4067×10^{-5}	1.5933×10^{-3}
Complex 5				
Biological process	Glycogen cell development involved in embryonic placenta development	*PKB, AKT1*	2.9319×10^{-8}	1.4777×10^{-5}
Molecular function	Nitric-oxide synthase regulator activity	*PKB, AKT1*	3.7729×10^{-7}	1.5846×10^{-5}
Cellular component	Lamellipodium	*PKB, AKT1*	7.4047×10^{-5}	4.2947×10^{-3}

[a] FDR: false discovery rate

ACKNOWLEDGEMENTS

This publication is based on work supported by the Government of Malaysia under grant FRGS/1/2014/SG05/USM/02/1. The PhD and SLAB scholarships to SH are funded by the Ministry of Higher Education (MOHE), Malaysia.

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Received: 30 May 2018
Accepted: 13 September 2018