IDENTIFYING CANCER GENE SUBTYPES FROM GENE EXPRESSION BY CO-CLUSTERING ALGORITHM AND SUPPORT VECTOR MACHINE

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DEDICATION

This thesis is dedicated to my lovely grandmother,
Mrs S. SOORANAM MARIAPPAN,
who taught me the best and useful knowledge from her life experience.
Without you, none of my success would be possible. Thank you maa!

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ABSTRACT

Cancer subtype information is significant to understand tumour heterogeneity. Present methods to find cancer subtypes have focused on utilizing traditional clustering algorithms such as hierarchical clustering. Since most of these methods depend on high dimensional data, the drawback is to divide the genes into different clusters, where a gene or a condition only belongs to one cluster. A gene may contribute to more than one biological process, so a gene may belong to multiple clusters. Besides, the centroid in the objective function of network-assisted co-clustering for the identification of cancer subtypes (NCIS) dragged with outliers. So, these outliers get their cluster instead of being ignored. Hence, this research is focusing on improving the NCIS method. Enhanced NCIS (iNCIS) is basically assigned weights to genes base on a gene interaction network, and it imperatively optimizes the sum-squared residue to get co-clusters. Next, supervised infinite feature selection with multiple support vector machine (SinfFS-mSVM) is proposed to obtain significant genes from a high dimensional data by using the classes obtained from iNCIS and improve the accuracy of classification. The effectiveness of iNCIS and SinfFS-mSVM is being evaluated on a large-scale Breast Cancer (BRCA) and Glioblastoma Multiforme (GBM) from The Cancer Genome Atlas (TCGA) project. From the implementation, there are five breast cancer gene subtypes and four glioblastoma multiforme cancer gene subtypes that have been successfully identified. The weighted co-clustering approach in iNCIS provides a unique solution to integrate gene network interaction into the clustering process. The improvement of the co-clustering Rand Index and F1-measure is 54.5% and 33.9% for BRCA and 34.2% and 31.5% for GBM. Meanwhile, a significant gene subset with higher classification accuracy was selected from SinfFS-mSVM. The classification accuracy for the selected gene subset improved by 3.00% and 2.99% for BRCA and GBM, correspondingly. Furthermore, biological validation conducted on the selected genes from each subtype is to justify the validity of the results. In conclusion, the empirical study on large-scale cancer datasets using iNCIS and SinfFS-mSVM comprehensively find cancer gene subtypes and genes by achieving higher clustering and classification accuracy. Future works are needed to integrate more comprehensive gene network information and to select optimal parameters.

ABSTRAK

Maklumat subjenis barah adalah penting untuk pemahaman keheterogenan ketumbuhan. Kaedah yang sedia ada hanya akan mengenal pasti subjenis kanser dimana sebahagian besarnya ditumpukan dalam penggunaan algoritma kluster tradisional seperti kluster berhierarki. Kebanyakan kaedah ini hanya bergantung kepada data berdimensi tinggi di mana kelemahannya adalah pembahagian gen ke dalam kluster-kluster yang berbeza, di mana satu gen atau suatu syarat hanya menjadi kepunyaan satu kluster sahaja. Walaubagaimanapun, jika satu gen boleh menyumbang kepada lebih daripada satu proses biologi, maka satu gen boleh dimiliki oleh lebih daripada satu kluster. Selain itu, sentroid dalam fungsi objektif kumpulan rangkaian membantu kelompok bersama untuk pengenalpastian subjenis kanser (NCIS) diseret dengan unsur dengan nilai tersisih. Jadi, nilai tersisih ini mendapat kluster mereka yang sepatutnya diabaikan. Maka, penyelidikan ini menumpukan kepada penambahbaikkan kaedah NCIS. Penambahbaikkan NCIS (iNCIS), pada asasnya menetapkan pemberat kepada gen berdasarkan rangkaian gen dan ia berulang untuk mengoptimumkan jumlah kuasa dua lebihan bagi perolehan kluster. Seterusnya, penyeliaan pilihan rencana tidak terhingga dengan mesin vektor sokongan berbilang (SinfFS-mSVM) dicadangkan untuk memperoleh gen-gen penting dari data berdimensi tinggi dengan menggunakan kelas yang diperolehi daripada iNCIS dan seterusnya memperbaiki ketepatan pengkelas gen. Keberkesanan iNCIS and SinfFS-mSVM dinilai menerusi data barah payudara dan barah otak (GBM) daripada projek “Atlas Genom Kanser”. Melalui pelaksanaan ini, terdapat lima subjenis gen barah bagi barah payudara dan empat subjenis gen barah bagi barah otak berjaya dikenal pasti. Pendekatan klusteran bersama pemberat dalam iNCIS memberikan satu penyelesaian unik interaksi rangkaian gen ke dalam proses klusteran. Jumlah peningkatan Indeks Rand dan F1-mengukur dalam klusteran bersama masing-masing adalah 54.5% dan 33.9% bagi BRCA dan 34.2% dan 31.5% bagi GBM. Sementara itu, subset gen yang penting dipilih dengan ketepatan pengklasifikasian yang lebih tinggi. Kejituan pengklasifikasian untuk subset gen terpilih masing-masing meningkat sebanyak 3.00% dan 2.99% untuk BRCA dan GBM. Pengesahan biologi dijalankan ke atas gen-gen terpilih daripada setiap subjenis untuk menjustifikasi kesahihan keputusan. Sebagai kesimpulan, kajian empirikal ke atas kedua-dua data set barah menggunakan iNCIS dan SinfFS-mSVM secara menyeluruh berjaya mengenal pasti subjenis kanser dan gen dengan mencapai kualiti kluster bersama dan ketepatan pengklasifikasian yang lebih baik. Kerja penambahbaikkan diperlukan pada masa hadapan untuk gabungan maklumat rangkaian gen yang lebih komprehensif dan juga memilih parameter optimum.

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BLAST	-	Basic alignment search tool
BRCA	-	Breast cancer
cDNA	-	complementary Deoxyribonucleic acid
DAVID	-	Database for Annotation, Visualization, and Integrated Discovery
DNA	-	Deoxyribonucleic acid
GBM	-	Glioblastoma Multiforme
GFC	-	Gene Functional Classification
GNI	-	Gene Network Information
GO	-	Gene Ontology
HMM	-	Hidden Markov Model
iNCIS	-	improved Network assisted Co-clustering for the Identification of Cancer Subtypes
infFS	-	infinite feature selection
KEGG	-	Kyoto Encyclopedia of Genes and Genomes
MAD	-	Median Absolute Deviation
MatLab	-	Matrix Laboratory
miRNA	-	Micro Ribonucleic Acid
mRNA	-	Messenger Ribonucleic Acid
MSA	-	Multiple Sequence Alignment
MSSRCC	-	Minimize Sum-Square Residue Co-Clustering
NCI	-	National Cancer Institute
NCIS	-	Network assisted Co-clustering algorithm for the Identification of Cancer Subtypes
NetBC	-	Network Bi-Clustering
NMAD	-	Normalized median absolute deviation
PID	-	Pathway Interaction Database
RF	-	Random Forest
RI	-	Rand Index
RNA		Ribonucleic Acid
rRNA		ribosomal Ribonucleic Acid

SinfFS	-	Supervised Infinite Feature Selection
SNMTF	-	Semi-Nonnegative Matrix tri-Factorization
SVM	-	Support Vector Machine
TCGA	-	The Cancer Genome Atlas
TF	-	Transcription factors
tRNA		Transfer Ribonucleic Acid

CHAPTER 1

INTRODUCTION

1.1 Background

Cells are the basic structure of living organisms. The human body comprised trillions of cells (Lodish *et al.*, 2008). Generally, cells undergo a development process called mitosis. During mitosis, a cell replicates all its genetic information. Deoxyribonucleic acid (DNA) present in cancerous cells multiplies and grows uncontrollably. Hence, this inconsistent cell development produces cancerous cells. The newly developed cells contain damaged DNA. Therefore, cancer diseases are known as complex and heterogeneity. The complexity of cancer disease is caused by many genes and pathways (Győrffy *et al.*, 2016; Shukla *et al.*, 2013). Generally, the same types of cancer patients hardly have regularly mutated genes. Therefore, 10%-30% of patients with the same type of cancer are only able to react for a given drug. (Kaiser, 2006; Snyder *et al.*, 2014). Various types of research are ongoing to detect the right diagnosis and prognosis for cancer. Therefore, DNA microarray gene expression technology data are used in the research. Gene expression defines the translation of genetic information from genes through messenger Ribonucleic Acid (mRNA) to proteins. Messenger RNA is also known as mRNA is complementary to one of the DNA strands to form single-stranded RNA (Gilbert *et al.*, 2016).

Over the last decades, the DNA microarray gene expression profiling has made evolution in the biological field (Liang *et al.*, 2004). Thousands of genes expression level can be measured through this technology. Whereas most of the old methods capable of measuring one or a few genes at a time (Lockhart *et al.*, 1996; Schena *et al.*, 1995). Normally, gene expression can be structured in a data matrix. The rows denote genes and columns denote conditions. The impact of microarray technology essentially in cancer and genetic field is very high. It has been shown through various research studies. On that note, scientists are actively working on the

identification of valuable biological information from gene expression data. Numerous applications are developed for disease diagnosis (Saiki *et al.*, 2008), gene function recognition (Eisen *et al.*, 1998), pharmaceutical target discovery (Corn and El-Deiry, 2007), and pathway analysis (Werner, 2008).

Biological systems are complex due to the presence of a huge number of genes. Thus, the computational method is required to analyse the genes from the expression dataset. Generated gene expression dataset is usually without any known features. The primary objective is to group the gene according to their expression under different conditions. Generally, the genes are acts as regulators that underlie phenotypic differences under the conditions such as disease stage, tissue types and developmental stages, (van Dam *et al.*, 2017). Genes present in the same groups are considered to have similarity features. However, genes in different groups are considered to have dissimilar features. This grouping method is essential in the process of cancer gene subtype identification. Biological information has been integrated as guidance in the grouping process. But only limited research studies have applied biological knowledge. For example, Gene Ontology (GO) and Gene Network Information (GNI) are used as assistance (Ashburner *et al.*, 2000).

The nature of GNI offers a rich source of evidence for implying functional associations of genes and proteins. The GNI also plays a significant role in every biochemical process. A strong analysis of this network helps to disclose the disease progression (Hwang *et al.*, 2018). The requirement of network information has been identified in recent research works. However, existing computational methods rarely use this gene network information (Grimes *et al.*, 2019; Yan *et al.*, 2017). Integrating gene network information with the clustering process assists in identifying similarities among gene expressions. This identification can describe the function of an unknown cancer gene subtype by predicting similar genes.

Clustering is one of the methods for exploration and interpretation of high dimensional microarray gene expression data (also known as one-way clustering). In this clustering method usually, genes, samples, or both, are clustered together (Andreopoulos *et al.*, 2009). Traditional clustering, for instance, k-means clustering

(Tothill *et al.*, 2008), hierarchical clustering with heat map visualization (Eisen *et al.*, 1998), and self-organizing maps (Nikkilä *et al.*, 2002) identify biologically significant groups of genes or samples successfully. Yet, these methods do not utilize the data entirely as clustering is done for genes first and then for samples or vice versa. Hence, genes that are co-expressed in another subset of samples are undetected. In addition, other limitations in the traditional clustering algorithms are struggling in determining the number of clusters, random initialization of genes, and engagements in gene subtypes (Oyelade *et al.*, 2016). The cluster number in the traditional clustering determines through numerous trial and errors. Moreover, the random initialization of genes provides uneven results. Additionally, the experiments take a long time because of multiple runs. Usually, this is because of the presence of non-significant genes (Zhou *et al.*, 2018), missing values (Vazifehdan *et al.*, 2018) in the dataset. The presence of significant and non-significant genes will increase the conflicts among genes. The reason is a gene consists of multiple functions and pathways (Andreopoulos *et al.*, 2009). When there is no prior pre-processing is done, then the genes cause different types of disease properties. This leads to uneven results in prediction and classification accuracy.

Thus, Cheng and Church (2000) have introduced a promising method to analyse gene expression which is co-clustering. In the co-clustering method, the clustering is done simultaneously for genes and samples. Co-clustering methods generally called unsupervised methods. Thus, they do not have any guidance or information to be used in exploring gene expression. Hence, semi-supervised methods are introduced. These methods integrate some critical information to guide the clustering process effectively. However, there is still a drawback in this clustering method. This is due to the applications of non-comprehensive biological knowledge with gene expression datasets. For example, incomplete gene network information, transcription factors (TF), microRNA, and metabolic network cause inaccurate results.

Besides, classification is also a well-known approach in microarray analysis. This approach is used to acquire biological information from the gene expression data especially in cancer diagnosis. In classification, the microarray data set is

divided into training data and testing data. The training data set is used to train a classifier to classify the test data to identify sample class. In recent years, many classification algorithms have been developed. For example, support vector machine (SVM) (Kang *et al.*, 2019) and random forest (Nguyen *et al.*, 2013; Nguyen and Bui, 2019). SVM uses a hyperplane with a maximized margin to split the given sample classes. Random forest is a decision tree-based ensemble learning approach. Due to robustness to sparse and noisy data, SVM has been widely used in microarray data analysis (Kang *et al.*, 2019).

Microarray gene expression data is known as high dimensional data. This is because; it contains many genes and a small number of samples. It is essential to select significant genes from high dimensional microarray data. This is to construct a strong classifier to obtain better classification performance by selecting significant genes or features (Ma and Huang, 2008). This process is known as gene selection or feature selection. Commonly, there are three types of gene selection methods available. They are filter, wrapper, and embedded methods. These methods are distinguished based on their way of incorporation. Yet each has its advantages and disadvantages (Saeys *et al.*, 2007).

1.2 Challenges for Cancer Gene Subtypes Identification

Implementation of the co-clustering algorithm offers an excellent tool for studying genes to gene relationships in the biological process. However, the fundamental challenge of cancer gene subtype identification is the cluster prediction. Hence, the first challenge of this research is to handle mRNA changes during gene expression experiments. Gene expression result is represented by positive and negative values. A positive value denotes an increase in expression and negative values denote a decrease in expression. Naturally, a gene can have one or various functions. The function of a gene depends on the occurrence of other gene products. The second challenge is data quality issues in gene expression datasets. Thus, the clustering and classification will be affected by the data quality such as missing values, irregular expression, and non-significant genes. This is because; the

relationships among genes are identified by biological information and expressional features from the data. The third challenge is the presence of high and low-level expression values in gene expression datasets. When the expression value is high, then the gene is possible to be grouped in multiple clusters. The lower expression values may be missed out from the group, which is considered necessary in certain subtype identification. Thus, handling of lower expression values from gene expression is becoming a challenging task for the researchers. On that note, this research is focusing on identifying significant genes from the presence of many genes in high dimensional data.

1.3 Current Methods for Cancer Gene Subtype Identification

Generally, there are two categories of approaches for cancer gene subtypes identification. The first is experimental (*in vivo* and *in vitro*) and the second is computational analyses (*in silico*) (more details are presented in Chapter 2). Figure 1.1 shows an overview of these two categories of approaches.

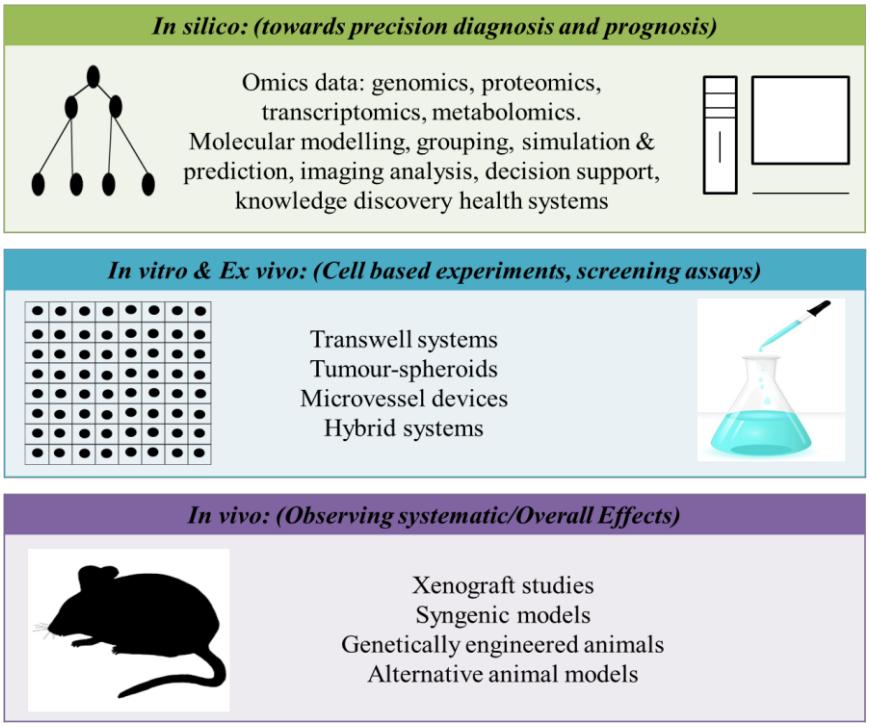


Figure 1.1 Cancer research preclinical techniques (Jean-Quartier *et al.*, 2018)

Animals are regularly used *in vivo* research studies. For instance, the rat is used for cancer diagnosis and prognosis (Yee *et al.*, 2015). This type of preclinical model can help in the identification of novel targets. However, there is no sufficient information provided on the target validation (Gould *et al.*, 2015). Furthermore, *in vitro* assists in the learning of cellular processes of tumour mechanisms (Katt *et al.*, 2016). Besides, *ex vivo* methods are utilized for drug reaction prediction in cancer patients (Vaira *et al.*, 2010). Therefore, researchers are planning to reduce the cost spent on wet laboratory experiments. This is because purchasing of biological and chemical materials for an experiment is costly. In specific, in silico approaches have been proposed for refining clinical and general biomedical studies involving laboratory work (Jean-Quartier *et al.*, 2018).

In-silico method executes through a computer or via computer simulation. Thus, *in silico* methods can be defined as computational approaches. This method incorporates biological analysis and simulation. Examples are computational validation, inference, prediction, classification, and modelling. Various cancer research is conducted by different peoples and organizations. For example, the National Cancer Institute has done the Cancer Genome Anatomy Project. The prime objective of this research is to identify cancer gene subtype from gene expression profiles and gene network information data of the patient to produce output for diagnosis and prognosis.

1.4 Problem Statement

Traditional clustering methods usually do not utilize the full benefit of the gene expression data. The clustering is done for genes first and then for samples or vice versa. Hence, groups of genes that are co-expressed or share similar characteristics only in a subset of samples are identified (Denitto *et al.*, 2017a; Huang *et al.*, 2014; Wang *et al.*, 2002). Figure 1.2 shows traditional clustering clusters a gene into a single cluster. This means a gene do not present in the other cluster. Thus, the problem in these clusters is considered low-quality clusters to be used in cancer diagnosis and prognosis. This is because a gene can involve in more

than one biological process that can be grouped into multiple clusters. In different conditions of the biological process, there are possibilities of a gene to be clustered in numerous clusters (Gasch and Eisen, 2002). The aim is to cluster genes that participate not only in single but more than one biological process into multiple clusters. For instance, in a recently published study, a network-assisted co-clustering algorithm (NCIS) for microarray analysis was proposed (Liu *et al.*, 2014). This NCIS method integrates gene network information to cluster genes and samples into biologically significant clusters concurrently. Despite the excellent performance shown in NCIS, the cancer gene subtype identification can still be improved on the objective function. The centroid in the objective function of (NCIS) dragged with outliers that get their cluster instead of being ignored. This cluster can be low quality cluster with the presence of outliers. Hence, the NCIS need to be improved to obtain better clusters to be used further in classification.

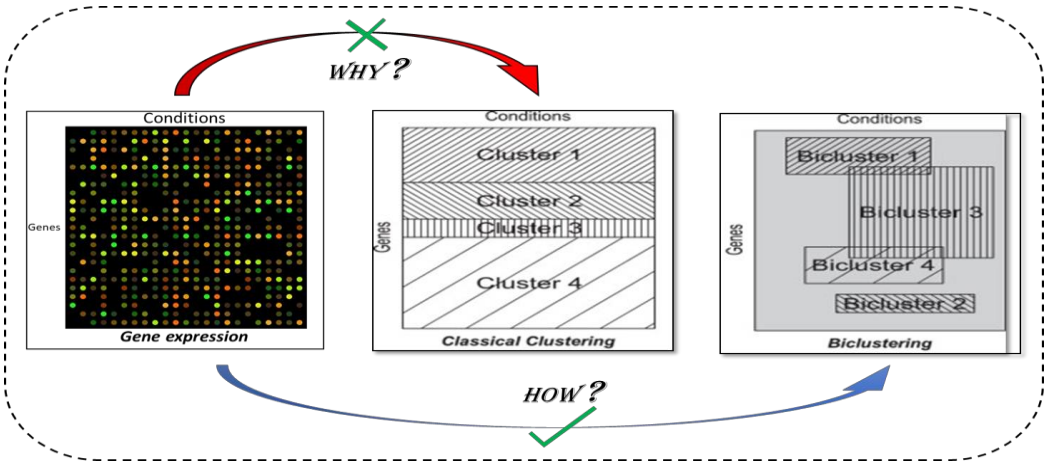
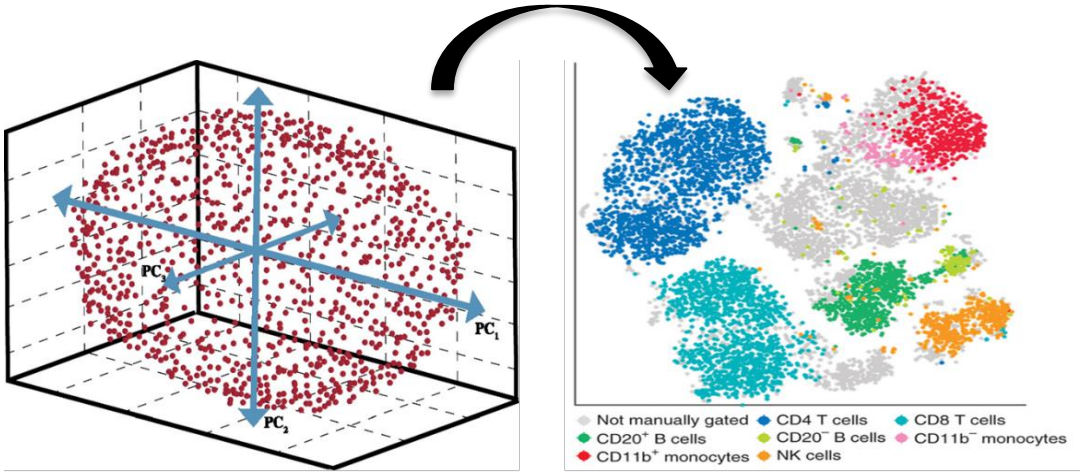


Figure 1.2 Traditional clustering and co-clustering method

The fact of high dimensional microarray gene expression data could cause the performance to drop in the analysis. High dimensional microarray data is which has hundreds to thousands of affected genes but limited number samples (Amir *et al.*, 2013; Tomasev *et al.*, 2014). The presence of non-significant genes in the classifier construction could lead to a classifier with weak discriminative power. Therefore, the cancer gene subtypes obtain will be huge and inaccurate for cancer diagnosis and prognosis. Figure 1.3 (a) shows the general view of high dimensional data for all

genes which will be classified according to suitable criteria as in Figure 1.3 (b). Therefore, the aim is to select significant genes and then classify them to help in diagnosis. The significant genes are selected by feature selection algorithm, Supervised infinite Feature Selection algorithm then classify those genes with multi-class support vector machine (SinFS-mSVM).



(a) *(b)*

Figure 1.3 High dimensional data (a) (Chester and Maecker, 2015) (b) (Amir *et al.*, 2013)

Thus, the main problem in this research is the ineffectiveness of the gene clustering due to the objective function of NCIS is dragged with outliers and selection of significant gene leads to the low performance of the results with the existence of non-significant genes in the microarray gene expression to study complex disease cancer. On that note, the hypothesis of this research is set as, if the co-clustering method is improved by minimizing sum-squared residue, then the accuracy for cancer gene classification increases. Therefore, this research proposes to deal with the above-mentioned problems based on the following research questions:

(i) How to efficiently identify cancer gene subtype co-clusters in the gene expression changes to improve clustering quality?

(ii) How to ensure to select significant gene for cancer gene subtype to improve the efficiency of identification of significant genes to improve the classification performance?

1.5 Research Goal and Objectives

The goal of this research is to propose and develop an effective improved objective function of the network-assisted co-clustering algorithm by minimizing sum-squared residue for the identification of cancer gene subtypes from high dimensional microarray data. The objectives are as follows:

(a) To propose an improved NCIS by minimizing sum-squared residue for efficient identification of cancer gene subtype in gene expression for better co-clustering performance.
(b) To propose a supervised infFS-mSVM method for multiple cancer gene subset selections besides to obtain better classification performance for selected significant gene subset.

1.6 Research Scope and Significance

This research work is primarily to address the problems in microarray gene expression data and traditional clustering. Presence of non-significant gens in gene expression data and grouping a gene into a single cluster are major issues. A single gene may fall into multiple clusters with the presence of significant genes. The existing NCIS algorithm improved due to objective function which cannot identify the outliers. Hence, this addressed by minimizing sum-squared residue to identify co-clusters of cancer gene subtype. Then, the significant genes are identified by infinite feature selection (infFS) method to be classified further. Two well-known cancer datasets are used to evaluate the effectiveness of the proposed methods. They are breast cancer (BRCA) (Network, 2012) and glioblastoma multiforme (GBM) (Verhaak *et al.*, 2010). These microarray datasets are used together with gene

network information from Reactome (Croft *et al.*, 2010), NCI (National Cancer Institute)-Nature curated Pathway Interaction Database (PID), Kyoto Encyclopaedia of Genes and Genomes (KEGG), GeneCards, and Database for Annotation, Visualization and Integrated Discovery (DAVID). The development of the proposed methods in this research is based on Network-assisted Co-clustering for the Identification of Cancer Subtypes (NCIS) in Matlab, and the performance is justified by comparing with NCIS (Liu *et al.*, 2014), MSSRCC (Cho and Dhillon, 2008), and NetBC (Yu *et al.*, 2017a). Identified genes are validated through biological literature in *Genecards* (www.genecards.com) to justify the relationship of these genes with the disease.

The significance of this research is addressed from co-clustering and classification accuracy of identified cancer gene subtypes using microarray data. The proposed method efficiently incorporates network information with gene expression across samples to produce co-clusters. Also, this method has the potential to handle high dimensional variations and integrating prior knowledge in analysing datasets. Besides this, it also expands the understanding of the cellular and molecular-level mechanisms of cancer. The obtained co-clusters assist to improve classification accuracy. Then, these genes are validated biologically for their contribution to a cancer type. This validation is to identify the drug target genes, biomarkers, tumour suppressor, etc. Additionally, the drug target of specific cancer can study from the validated genes. On top of that, it would be beneficial for the cancer genomics field, especially the medical field to enhance diagnosis and prognosis.

1.7 Thesis Organization

This thesis comprises of six chapters. The remaining chapters of the thesis are described as follows:

i. Chapter 2: This chapter provides a literature review of the research. There are fundamental concepts in molecular biology such as central dogma, genes, gene expression, microarray technology, machine learning, data mining,

clustering and classification, gene network and, review on previous research works in gene and subtypes identification using data mining approaches is also described. A comparative analysis of the selected algorithms is also presented. Finally, a description of current research trends and directions are explained.

ii. Chapter 3: This chapter presents the research methodology. It shows the main research framework described in this research. Then, microarray cancer datasets details are described. Besides, performance measurement for the verification and validation process of the research is presented.

iii. Chapter 4: This chapter illustrates details of standard NCIS and proposed improved NCIS (iNCIS) algorithms to identify cancer gene subtypes. A detailed comparison of the proposed method with the previous work is presented. Suitable parameter settings to experiment are also listed - results obtained from the research presented with previous research results. Finally, a comprehensive discussion about the results and analysis are described.

iv. Chapter 5: This chapter provides the Supervised Infinite Feature Selection (SinfFS) algorithm with a multiclass support vector machine (SVM) for further cancer gene ranking for classification. Classification accuracy and error rate for gene selection are presented. Furthermore, biological validation for selected genes and their pathways are illustrated.

v. Chapter 6: This chapter discusses the general conclusion and contribution of the research from the achieved results. Together with this, topics for future studies also have been suggested.

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