CHAPTER 12

DIFFERENTIALLY EXPRESSED GENE ANALYSIS IN CANCER RESEARCH BY USING BIOINFORMATIC TOOLS

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INTRODUCTION

Genome-wide screening of transcriptional changes between normal cells, cancer cells, and cells in the metastatic processes provides information about the molecular basis of various cancer type. With the help of bioinformatic analyses, personal approaches can be adopted in the diagnosis, treatment, and survival processes of various cancer types including frequently observed and rare cancers. Performing bioinformatic analyses before clinical and laboratory applications provides great advantages in terms of time and money. In this book chapter, genomic-level screening of differentially expressed genes performed by using bioinformatic tools in different cancer types that support pan-cancer genomic studies will be summarized.

1.1 The Concept of Differentially Expressed Gene Analysis

All somatic cells of an organism have the same genome, but starting from the embryonic development processes, each cell and cell group differentiates to display different functions with changes in the genome. Differentiation for different purposes is dependent on the different gene expression levels specialized for specific functions in the genome (Shiraki et al., 2014). In addition to differentiation, some genes may be expressed in a different way than they would normally be, with the effect of different stimuli in disease states. There are biochemical processes in a cell that determine which genes are actively transcribed and whether they are translated into mRNA and proteins. In addition, under certain conditions, the expression of these genes (upregulated or downregulated genes) changes under certain conditions during pathogenesis such as cancers (Fattahi et al., 2019; Moreira et al., 2008; Lin et al., 2014). In case of cancers, Differential Gene Expression (DEG) analysis of both RNA and DNA microarray data/RNA-Seq data/cDNA microarray data and determined differentially expressed genes (DEGs) are used to clarify the different gene status between healthy and disease states (primary cancers, metastasis status, etc.). To understand the difference, quantitative gene expression-based changes between control and experimental groups are compared to each other by performing statistical analysis with the help of normalized data (Fang et al., 2012; Dudoit et al., 2002; Storey et al., 2003; Bullard et al., 2010).

1.2. Approach to Cancer in terms of Differentially Expressed Gene Levels and Pan-Cancer Studies

Determination of gene expression signatures/profiles in the formation of cancers provides important information about biological phenotyping and

biological pathway-related processes. In the literature, there are many valuable studies in which individual genes or small gene clusters deviating from normal changes (such as mutation, expression profiles) are shown in different cancers.

There are more than 200 different types of cancer identified to date. In cancers, various types of genetic alterations such as somatic mutations, altered gene expression levels, epigenetic aberrations are observed (Tomczak et al., 2015).

If we focus on breast cancer, many studies have shown that breast cancer cells differ from normal cells at the gene level. Besides non-genetic factors such as physical activity, obesity, menstrual background and alcohol usage, genetic based predispositions play important role in breast cancers. Mutations can be classified into three classes such as high penetrance mutations (TP53, BRCA1-2, P53, PTEN genes, etc.), moderate penetrance variants (ATM, CHEK2, BRIP1 genes, etc.), low penetrance variants (FGFR2, TOX3, MAP3K1, COX11, NOTCH2/FCGR1B genes etc) (Antoniou et al., 2008; Birch et al., 2001; Nelen et al., 1996; Renwick et al., 2006; Meijers-Heijboer et al., 2002; Seal et al., 2006; Easton et al., 2007; Ahmed et al., 2009; Thomas et al., 2009). Furthermore, oncogene activation (such as Human epithelial receptor 2, (HER-2), c-myc, p-53) and tumor-suppressor gene inhibition (p27, Skp2, breast cancer susceptibility gene 1 and 2 (BRCA1,2), PTEN, Retinoblastoma (Rb), etc.) can be observed in breast cancer development which have been identified various valuable research studies (Osborne et al., 2004).

As can be noticed in the breast cancer studies summarized above, in addition to the determination of mutations of individual genes or the determination of gene expression levels, pan-cancer studies conducted with big data also support these studies in a wide scope. The use of patient gene expression data obtained from clinical applications gives us the opportunity to work with more heterogeneous patient groups, and this enables more effective diagnosis and treatment process planning that can be both personalized and generalized in cancer patients. For this reason, The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) projects were conducted in 2005 and 2008, respectively (https://www.genome.gov/Funded-Programs-Projects/Cancer-Genome-Atlas) (Chin et al., 2011; Tomczak et al., 2015). The Cancer Genome Atlas (TCGA) project and TCGA Pan-cancer Clinical Data Resource (TCGA-CDR) provides

genetic alterations for almost 33 cancers with 11,000 tumour gene data which can be used to determine the survival rates of patients (Liu J et al., 2018).

1.3. Differentially Expressed Gene Analysis in Cancers

Although the genetic (mutation accumulation, genome instability, gene expression levels (up-regulated or down-regulated genes), etc.) and biological mechanisms (tumour triggered inflammation, invasion and metastasis, deregulation of energy systems, escaping cell death, increasing the proliferation capacity, etc.) that occur in cancer tumorigenesis are standard, each patient's cancer status and cancer-related diseases are different from each other (Senga et al., 2021; Hanahan et al., 2022). Therefore, although there are common processes, cancer is on its way to becoming a personal disease. Starting from this point, DEGs can be determined by using big data showing patient gene changes and gene densities. Thus, using multiple data and bioinformatics tools and making gene-based screening provides us with more comprehensive data in a short time. Gene expression data at the genome scale provides a profile of differently expressed genes that can distinguish between different biological states. This abnormally differentiated gene profile can be used successfully to assess prognosis, chemotherapy status, and drug sensitivity in a tumour sample (Stevenson et al., 2012).

There are studies aiming to investigate the DEGs relevant to prognosis of various cancers. To achieve this, integrated bioinformatics analyses are used with the help of different computer-based and online bioinformatic tools.

1.3.1. Bioinformatic Based Tools used in Patient Gene Data Collection for Differentially Expressed Gene Analyses

To determine DEGs, gene intensity data and microarray data can be downloaded from publicly free databases such as Gene Expression Omnibus (GEO) Database developed by National Center for Biotechnology Information (NCBI) and The Cancer Genome Atlas (TCGA) developed by the National Human Genome Research Institute (NHGRI) and the National Cancer Institute (NCI). In this book chapter, we will summarize the NCBI GEO database.

The GEO project was initiated in response to the need to store multiple gene expression data. For example, with the help of storing and grouping the data obtained from gene expression experiments (such as microarray or RNA-Seq experiments) specific to cancers, it is possible to

compare cancer to normal with datasets including gene expression data of related genes (Edgar et al., 2002; Barrett et al., 2012).

Publicly available gene data (in here GEO data) can be analysed by using statistical platforms such as R software program. R-based GEO data analysis can be achieved by using web application such as GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) (Barrett et al., 2012). Furthermore, it can also be used via downloading free R software which is also widely used in statistical computing area (https://www.r-project.org/). There are packages such as BioConductor, repository for bioinformatics software, which can be used to compare at least two groups (such as samples and controls) to determine which genes are differentially expressed. BioConductor is a project and there are developed BioConductor packages (such as Limma for microarray and RNA-Seq data) which can be used by using R statistical programming language (https://www.bioconductor.org/) (Ritchie et al., 2015). Furthermore, there is a BioConductor package named as RankProd which can be used to detect DEGs in meta-analysis (Hong et al., 2006)

1.3.2. Determination of the Biological Relevance and Gene Enrichment Analysis

Upon determination DEGs (up-regulated or down-regulated genes in cancer patients), these DEGs can be enriched in different biological processes. To make biological sense of the data obtained from the analyses made by means of bioinformatic tools in which hundreds or even thousands of gene expression data are used, it is necessary to use bioinformatic tools and to make sense of these complex processes. For this purpose, bioinformatic tools providing gene-annotation enrichment analysis service have been developed. Thanks to these tools, it is possible for researchers to identify the biological processes, functions, and pathways most suitable for their studies. Some of these bioinformatic-based tools and free databases are available and these databases that can be used without requiring an ethical permission procedure. For instance, The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a type of analysis that uses information from genome and gene data. Eventually, signalling pathways that can interact at the functional and molecular level are identified (Kanehisa, 2002). KEGG pathway analysis can be achieved by using web-tools specified for this purpose. For instance, Gene Ontology enRIchment anaLysis and visuaLizAtion tool (Gorilla) (http://cbl-gorilla.cs.technion.ac.il/) and ShinyGO v0.741 (http://bioinformatics.sdstate.edu/go74/) tools are frequently used to predict

enriched GO terms (DEGs) and relate these comprehensive gene lists to particular biological process, function or component. The Database for Annotation, Visualization and Integrated Discovery (DAVID) is another web-based tool which is used by researchers to understand the biological relation of high number of differentially expressed genes (https://david.ncifcrf.gov/). Furthermore, KOBAS web-based analysis database is a tool where KEGG analysis can be performed by using DEGs lists (http://bioinfo.org/kobas). It has two modules named as "annotation module" and the "enrichment module" and these modules are used to annotate the GO terms and relate the biological pathways respectively (http://bioinfo.org/kobas). Onto-tools is known as a toolkit to be used as “Onto-Express”, “Onto-Translate”, ” “Onto-Design”, “Onto-Compare” and (Draghici et al., 2003). The Signalling Gateway Molecule Pages (SGMP) is a database where the interaction between the analysed proteins and signal transmission pathways can be determined (Dinasarapu et al., 2011). The STRING database is publicly available to predict and identify the interacting genes and determine the protein/protein interaction (PPI). For this reason, the database contains the data of 14094 organisms and 67.6 million proteins and can exert more than 20 thousand interactions (https://string-db.org/). Furthermore, there are other web-based tools such as MAPPFinder, GoMiner, EASE, GeneMerge and FuncAssociate, GENEONTOLOGY, PANTHER Classification System which can be efficiently used in bioinformatic analyses (Huang et al., 2009).

1.3.3. Drug Sensitivity and Drug Resistance Prediction via DEG Analysis in Cancers

DEG analysis can be conducted to determine the drug sensitivity and resistance in human diseases. For that reason, Connectivity Map (CMAP) database, which was funded by NIH LINCS (Library of Integrated Cellular Signatures) project, has been developed and it supplies broad range data also for cancer studies (https://www.broadinstitute.org/connectivity-map-cmap) (Lamb et al., 2006; Nevins et al., 2007). In this project, mostly cell line-based and patient data-based studies have been conducted to determine gene expression patterns upon the interaction of small molecules and certain drugs such as estrogen receptor agonists and antagonists, HDAC Inhibitors, Phenothiazine, Gedunin, Sirolimus, etc. The most frequently used cell lines are breast cancer cell line (MCF7), leukaemia cell line (HL60), melanoma cell line (SKMEL5) and prostate cancer cell line (PC3) in this project (Lamb et

al., 2006). In addition, Lee Y.S. et al. has been identified acquired gefitinib resistance (AGR) related hub-DEGs through network analysis (meta-analysis) in lung cancer and epidermoid carcinoma by using PC9 and A431 cell lines, respectively (Lee et al., 2015).

1.3.4. Determination of Hub-Genes and Survival Prediction via DEG Analysis in Cancers

Hub-genes can be predicted via DEG analysis in different cancers such as papillary thyroid cancers, breast cancers, lung cancers, cervical cancers, hepatocellular carcinoma, kidney cancer (Sun et al., 2021; Xiao et al., 2018; Xue et al., 2020). Furthermore, DEG analysis can also be conducted to predict survival related hub-genes in certain cancers. For instance, Zhu et al. (2019) has been shown that autophagy pathway related 16 DEGs are involved in survival process in multiple myeloma (MM) patients. They propose that autophagy related gene prognostic model can be considered as a basis of anticancer therapies in MM patients (Zhu et al., 2019). Similarly, autophagy based DEG signature with 3 autophagy-related genes (SQSTM1, BIRC5, and FOXO1) and its effect on survival rates has been identified in hepatocellular carcinoma patient groups (Lin et al., 2018). In another cancer type named as gastrointestinal pan-adenocarcinomas, alternative splicing pattern of multiple genes has been predicted and their impact on prognosis and survival of these cancer patients have been identified (Lin et al., 2018). Besides, podocan, which is a regulatory protein in extracellular matrix (ECM), encoding gene PODN has been considered as a biomarker for osteosarcoma patients in both diagnosis and prognosis processes. Furthermore, including PODN, the most significant 5 genes (PODN, OLFML2B, ACTA2, COL6A3, FAP, and COL6A1) have been determined as significantly and differentially expressed genes and they are related with the survival of these osteosarcoma patients (Yao F., et al. 2021). In gastric cancers, Wang et al. (2015) has tried to predict differentially expressed miRNA patterns as a biomarker via meta-analysis and they have determined the effects of these miRNAs on both survival and treatment responses (Wang et al., 2015).

1.3.5. Metastasis Status Prediction via DEG Analysis in Cancers

DEGs analysis can also be done to predict the metastasis status of cancers. For instance, Qi et al. (2019) has predicted that there are more than 1000 differently expressed and methylated genes (677 genes upregulated-hypomethylated, 361 downregulated-hypermethylated) which are related with certain pathways linked to tumorigenesis and metastasis in breast cancer

patients (Qi et al., 2019). Similar study has been conducted by using aggressive breast cancer cell line gene expression data and Chen et al. (2015) has predict the metastasis related DEGs (such as PTX3, SNAI2, IL-8/6, etc.) and related biological processes (such as tyrosine metabolism, calcium signalling pathway, etc.) (Chen et al., 2015). On the other hand, colorectal cancer originated liver metastasis related DEGs have been predicted by Liu et al. (2021) by using gene expression data of cancer patients and they have determined that cell adhesion molecules are the molecules which should be focused on, and peroxisome proliferator activated receptor (PPAR) signalling pathway is the key biological process in their study (Liu et al., 2021).

CONCLUCSION

Gene expression profiles and DEG analyses that allow hub-gene identification will become much more relevant once prospective and clinical laboratory-based studies are performed and data are validated. This will assist clinicians to routinely use microarrays to better diagnose and predict cancers and enhance the prognosis of cancer patients.

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