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Gene Expression of Human Lung Adenocarcinoma-Derived A549 Clones

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Abstract

For clarifying interactions between genes and proteins, the GenSensor Suite comprises of four web tools. The outcomes of GenPath reveal whether categories of biochemical, regulatory, or other gene set are over- or underrepresented in an input list when compared to a background list. GenPath includes some specific gene sets in addition to all common gene sets that can be searched. Users may include unique background lists. GenInteract creates an interaction gene list from a single input gene and then uses GenPath to evaluate it. In order to extract a gene list from a list of PubMed IDs and query it in GenPath, GenPubMed employs a PubMed query to find the list of PubMed IDs. Users of GenViewer can compare one gene set to another in GenPath. For healing harmed or irreparable lung tissue, stem cell treatment looks to hold promise. However, because the molecular mechanism underlying differentiation of alveolar epithelial cells is not entirely known, developing a straightforward and repeatable methodology for lung progenitor populations is challenging. Using the human alveolar epithelial type II cell line A549, we looked into an in vitro system to examine the control mechanisms of alveolus-specific gene expression. A549 subpopulations were cloned, and each clone was divided into five categories based on the cell morphology and marker gene expression. B7 and H12, two clones, underwent additional examination. Surfactant protein C, an ATII marker, was increased in both H12 and B7 when cultured in a serum-free environment. An ATI marker known as aquaporin 5 (AQP5) was highly elevated in B7 and H12, respectively.

Keywords: GenSensor; Lung progenitor

Introduction

Large lists of differentially expressed genes or proteins are frequently produced by transcriptome analysis, high-throughput investigations of gene expression, and proteomics technologies. Without a second level of analysis that splits them into interpretable functional categories, these lists remain incomprehensible. To do this, a number of techniques have been created, with Gene Set Analysis being the most popular (GSA). In GSA, the test list—a list of differentially expressed genes-is contrasted with gene sets, which are gene lists that have been organised into categories and made available in searchable databases [1]. It is provided, with appropriate statistical significance, which gene sets are significantly over- or under-represented by the genes in the test list. This gives the researcher a place to start when analysing functional genomics. The GSA method was first used to analyse gene sets derived from Gene Ontology categories and was then included into a wide range of tools [2]. In recent years, the technique has been expanded to encompass any gene set category that is pertinent to the experiment at hand, including KEGG pathways, chromosomal locations, cis-regulatory elements, and so on. It is beyond the scope of this work to detail every tool that has been developed recently for comparing test lists against gene sets; suffice it to say that 68 of these tools have recently been examined, and that DAVID and GSEA are the two that are most frequently used. This presentation will introduce the GenSensor Suite as a flexible, user-friendly tool with extra features that are uncommon in a regular GSA [3]. To a wet-lab researcher, getting an accurate response from a freely available, extremely user-friendly tool as soon as feasible is of utmost importance. This tool should ideally provide pathway analysis and literature searching as well. This was the impetus behind the GenSensor Suite because no such tool currently exists.

It is possible to lose your life from lung conditions including idiopathic pulmonary fibrosis and chronic obstructive pulmonary disease [4]. The most severe cases have traditionally been treated with lung transplantation. Although there are a number of concerns with lung transplantation, including histocompatibility problems and a donor shortage, these problems are not the only ones. Due to its potential as a treatment, stem cell-based regenerative medicine for the lungs is receiving a lot of interest. Alveolar epithelial type (AT) cell regeneration has recently been studied using induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs). The regulation of this embryonic process is currently unknown, and differentiation into AT cells from ESCs and iPSCs still needs to go through numerous developmental stages [5]. To better understand the molecular underpinnings of the differentiation of divergent progenitor populations in the human lung and to further the development of lung regenerative medicine, a straightforward and repeatable model system must be established. Two types of alveolar epithelial cells, type I (ATI) and type II, make up the lung alveoli, which are crucial for respiratory function (ATII). The flat cells known as ATI cells, which make about 95% of alveoli, are involved in the exchange of oxygen and carbon dioxide [6]. These cells also generate surfactant, which is made up of proteins including surfactant proteins A, B, C, and D (SPA, SPB, SPC, and SPD), and phospholipids. These cells display certain differentiation markers, such as aquaporin 5, boidal cells, and surfactant. These surfactants are necessary for the upkeep of alveoli and host defence. Both Clara cells and ATII cells can manufacture SPA, SPB, and SPD. SPC is a distinctive marker for ATII cells because it can only be generated in these cells. Lung respiratory function depends on Clara and ATII cells' cell-type-specific SPB and SPC expressions [7]. Thyroid transcription factor 1 (TTF-1) controls the expression of both genes during lung development. After injury, ATII cells can self-renew, proliferate, and differentiate into ATI cells,

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which is stem cell-like behaviour. Therefore, it is crucial to set up a straightforward, repeatable ATII cell model system and a methodology to manage the differentiation into ATI cells. To determine if A549 cells are appropriate for examining the regulation of gene expression of differentiation markers, we used a cell line obtained from human non-small cell lung cancer [8]. The epidermal growth factor receptor (EGFR) gene is amplified in A549 cells, which have been extensively researched and are known to have the K-RAS mutation (G12S). A549 cells lack the expression of certain genes, like TTF-1, yet they nevertheless share some of the characteristics of ATII cells. Although it has also been noted that A549 cells exhibit morphological variability, a variety of proliferative activities, and are not susceptible to differentiation cues [9].

Materials and Method

Implementation

The GenSensor Suite is constructed as a collection of common gateway interface (CGI) scripts that handle HTTP requests and answers using JavaScript and Perl. The Apache 2 HTTP Server is used to offer the graphical user interface (GUI), which is created using HTML, CSS style sheets, and JavaScript. Performance has been examined in PC and Apple environments with versions 7 and up of FireFox, Safari, Google Chrome, and Internet Explorer. On fast (25 Mbps) and slow (2.5 Mbps) internet connections, more than 1000 genes were evaluated in 1-3 seconds.

JavaScript must be enabled in the browser in order for some GSS features to function. All background gene lists are saved as Entrez gene identification text files. Microarray chip annotation files can be used to generate new backdrop lists, and users can upload their own unique background lists as text files. These species-specific gene sets are kept as flat files of related gene categories. They are made up of a single word serving as the category identifier, a brief summary of the category, and a list of Entrez Gene Identifiers that fall under that category. A gene may appear on more than one list. Through standard text file operations, users can add or remove gene sets.

Gene Sets

The information that is most frequently sought for in a normal biomedical or pharmaceutical sciences laboratory is reflected in the categories of our established gene set. These include gene sets for all KEGG pathways and GO terms, as well as made - to - order gene sets for gene transcription predicted targets, miRNA predicted targets, IPI Protein Various cellular Locations, disease- and drug-related cocited genes, and a gene set of tissue genetic variants based on the SymAtlas. These gene sets are currently accessible for Drosophila, human, mouse, and rat.

GenPath [10]

A test list of genes is run through a GSA approach by GenPath, which searches a chosen gene set, statistically compares the outcomes with those obtained using a background gene list, and then displays the final results. To get a complete understanding of their data, users can run GenPath as many times as necessary on the same test list after selecting the gene set from the first drop-down option on the program's home page. The background list that was used to construct the test list is then specified by the user. The set of probes on the array chip serves as the background in an experiment involving microarrays. Gene symbol, Entrez Gene ID, or both may be included in the input list.

GenInteract

If one or more KGML pathway files describe a connection between the two genes, then two genes are regarded as being paired. A list of all genes directly interacting with the query gene is constructed using the species and interaction data source that are selected. The genes in this initial list are used iteratively to identify the genes that interact with each other and create the second level interactions gene list if a depth of interaction larger than one is chosen. The background list of all the genes with interaction data and the final list of all the interacting genes are sent to GenPath, which then returns the pathway data.

Cell Cultures

Living thing non-small cell lung cancer cell line A549 was grown in Dulbecco's modified Eagle's media with 10% serum - free medium at 37°C in an incubator with 5% CO2. A549 cells were transfected for maintenance at 70% confluence, with media being changed every three days. The primordial A549 cells will now be referred to as the "parental cells," while the cloned cells will be referred to as "clones" with specific letters and numbers.

Single Cell Cloning

Human non-small cell lung cancer cell line A549 was grown in Dulbecco's modified Eagle's media with 10% serum - free medium at 37°C in an incubator with 5% CO2. A549 cells were transfected for maintenance at 70% confluence, with media being changed every three days. The original Hepg2 cells cells will now be referred to as the "parental cells," while the cloned cells will be referred to as "clones" with specific letters and numbers.

Results

The statistical overrepresentation of these terms is examined. Our programme is made to help with literature searches as well as data mining of PubMed's gene lists and other information. A text query is entered by the user together with the organism they want to work with, either as a gene ID, gene symbol, or using the standard PubMed syntax. We are aiming to include other species while currently only being able to query data on humans and mice. A list of all matched PubMed IDs is obtained by sending the query to NCBI's PubMed website. The NCBI Gene database is then used to retrieve a list of genes with connections to these PubMed IDs.

We initially looked at the morphology of the A549 parental cells during the growth phase, which lasted for 4 days after seeding. In the A549 parental cells, we found a number of different morphologies. With the use of one of the paired experimental sets, we simultaneously looked at the expression patterns of 14 differentiation marker genes. We discovered that the forkhead box genes, which include SPC, FOXJ1 and FOXA2, transformation-related protein 63, mucin-5AC (MUC5AC), a goblet cell marker, and the FOXJ1 and FOXA2 family of genes, were consistently expressed at similar amounts throughout the culture period. For endoderm cells, basal cells, ciliated cells, goblet cells, and ATII cells, these genes serve as unique markers. DCIK therapy did not cause morphological alterations in H12, although after 48 hours, cell clustering increased. Following that, we looked at how the combined therapy of MEK inhibitor and DCIK affected the gene expression in H12. As long as U0126 was being treated, SPC expression was increased. SPC expression was reduced by 48 h after the injection of DCIK, and it thereafter increased time-dependently until 96 h. At 48 hours, AQP5 remained suppressed in DCIK, and at 72 hours, it was still 0.7-fold. At 48 hours, TTF-1 expression increased 1.3-fold and persisted there for the next 96 hours. Up to 4.6-fold overexpression of SPB expression was seen at 72 hours, and it stayed at that level for the remaining 96 hours. SPB expression was greatly raised by 48 hours.

Discussion

Gene sets can be browsed and selected individually using GenViewer. The list of genes from any gene set can then be searched through in GenPath using various gene sets. For instance, KEGG pathways can be used to examine certain GO terms inside a specific gene set in GO. This is especially helpful if GenPath returns a result with an unfamiliar GO keyword or KEGG pathway. Through further exploration of that category in GenViewer, the user may identify connections to different pathways that might also be listed in the GenPath repository. to develop a straightforward and repeatable in vitro system that may be utilised to examine the molecular mechanisms of lung alveolar epithelial cell development. Through the use of their morphology and the gene expression patterns of markers, we identified A549 clones and described them. Two A549 clones, B7 and H12, were further examined as potential ATII and ATI/II cell representatives in accordance with the main expression patterns of alveolar cell markers. We created various growth conditions with and without serum, DCIK, and MAPK inhibitors to see if these clones responded to the differentiation stimuli in any way. When exposed to those stimuli, B7 and H12 both responded differently and steadily, exhibiting traits of ATII and ATI/II cells.

We measured the growth rate with and without serum to better understand how tightly and reciprocally regulated cell growth and differentiation are. We found that A549 cells were highly proliferative in media containing serum, but that serum removal reduced growth rate and reciprocally elevated expression of differentiation marker genes. Furthermore, serum depletion system western blot studies failed to find either SPC or AQP5 proteins. Therefore, the cells may be primed but not fully differentiated in the current situation, indicating that further signals or environmental factors may be needed to promote the protein production.

Conclusion

For the study of user-selected gene lists, DAVID offers a simple-to-use application. The results, like our GSS tool, are arranged in tables of statistically significant gene sets. In contrast to our tool, DAVID analyses user lists to all gene set categories at once. The significant gene sets that result are then grouped into functionally linked "Annotation Clusters," which can help the user find important biological traits in such extensive result reports. The GenViewer tool in GSS can be used to "cluster" related gene sets in a similar way, although it would need to be done independently for each gene set. DAVID is one of the GSA programmes that processes data the fastest since all of its statistical analysis are built within the Java programme. Even though the sorts of gene sets that can be evaluated are somewhat restricted by GSEA, it nevertheless offers an entirely distinct approach to examining gene set data. Rank-ordered lists are compared using a nonparametric approach by GSEA. The analysis of gene data from one or more treatments in which the rank order changes and subsequent comparison of the modified genes to gene sets are made very well using this method. GSEA is excellent for comparing treatment-induced gene alterations to genesignature datasets established from earlier studies, such as those employed in connection with the Connectivity Map. In cases where gene ranking is not appropriate, this method cannot be utilised to examine gene lists. Thus, GSEA cannot perform the vast majority of the features offered by our GenInteract, GenPubMed, and GenViewer applications. By creating an interaction network inside the condensed gene list of interest, numerous methods have been developed that utilise the knowledge gained from GSA analysis and add a data-driven approach. Additionally, a number of tools for network visualisation have been made available. The totality of all these tools is astounding, but the essential point is that the laboratory researcher frequently has very little time available to understand, use properly, and make the most of the abundance of sophisticated tools that are offered. An someone who is not skilled in bioinformatics can utilise the GenSensor suite of tools since they are simple to use, well integrated, and accurate. We extracted A549 clones and identified the distinctive shape and patterns of gene expression that distinguished each one. Among them, we discovered that two A549 clones, B7 and H12, have ATII cell- and ATI/ATII cell-like characteristics and were responsive to the serum depletion stimuli, indicating that they have the flexibility in gene expression of alveolar differentiation markers. These A549 clones might serve as the foundation for a model system to investigate the molecular mechanisms underlying control of ATII differentiation.

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Conflict of Interest

The author has no known conflict of interested associated with this paper.

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