Advances in Deep Learning for scRNAseq Analysis: New Horizons for Regenerative Medicine

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Abstract

A detailed survey of the use of many deep learning algorithms on scRNAseq data for regenerative medicine was published in this article. Currently, the best deep learning algorithms for scRNAseq analysis have yielded positive results, but there are still more promising ways that need to be developed to better handle technical noise, account for cell expression variability, identify MSCs, and anticipate stem cell type. To gain access to scRNAseq data, these deep learning techniques need to be paired with scRNAseq data. The ability to identify cell types and functions accurately and fast utilizing these algorithms has not yet been made possible. In the study we conducted, we reached the conclusion that further research has to be done into how to apply deep learning algorithms to interpret scRNAseq data, which may be used to better cell therapy and regenerative medicine efforts.

1 INTRODUCTION

The goal of regenerative medicine is to replace nonfunctional or dead cells in order to heal or regenerate tissues and organs, restoring normal function to damaged organs. The use of stem cell transplantation and tissue regeneration procedures in the treatment of various illnesses has expanded dramatically in recent years. 1

With the advancements in stem cell research, cell therapy, the therapeutic approach of introducing new cells into a tissue to treat illnesses, may be able to cure diseases that are resistant to standard treatments. Stem cells are self-renewing and have the ability to develop into a variety of human body cells. As a result, stem cell treatments may have an impact on the area of regenerative medicine. However, contemporary approaches for using stem cells in regenerative medicine and cell therapy are still in their infancy, and most of what is known about the subject is speculative. The regeneration mechanism of transplanted mesenchymal stem cells (MSCs) against illnesses, for example, remains unknown. Furthermore, parameters such as tissue source and separation procedure have an impact on MSC differentiation capacity. 2 and 3 There is no agreement on the conventional features of MSC differentiation potential at this time. Because of these ambiguities, we have been unable to evaluate the effectiveness and self-renewal capability of isolated MSC, implying that MSC usage will give results that are entirely different or nearly contrary to the intended value of clinical applications. It is more reliable to identify cell types in stem cells using genomics approaches, such as relying on single cell RNA sequencing (scRNAseq) data to assess and assess gene expression patterns or quantify protein content. As a result, we anticipate that deep learning approaches may be used to evaluate scRNAseq data, forecast cell types, and get a better understanding of MSC differentiation potential and direction. We can directly use stem cell subpopulations with the required differentiation direction for treatment if we can determine what type of cells they can differentiate into before using MSCs, allowing us to treat diseases more directly and achieve precision medical treatment, promoting the development of cell therapy and regenerative medicine.

Our research group has focused on musculoskeletal illness therapies, particularly tendon tissue regeneration techniques including biomaterials, stem cells, and growth factor delivery systems. We believe ourselves to be a well-established group in the field of tendon tissue engineering and regeneration, including publications on the use of stem cells and biomaterials in tendon tissue engineering. We propose that machine learning approaches such as deep learning be used to examine the scRNAseq data set and generate predictions about hidden subpopulations in the cell population. This allows researchers to accurately analyze MSCs and employ the subpopulations identified based on differentiation potential for stem cell therapy and regenerative medicine treatments.

The previously published remarks mainly describe the use of machine learning techniques on genomics data,4-9, or the use of more specialized machine learning techniques on scRNAseq data,10-12, or the use of deep learning methods on genomics data.

This review summarized the various algorithms of deep learning methods applied to scRNAseq data in this review, discussed the impact of their combination on the development of stem cell therapy and regenerative medicine, and proposed additional algorithmic suggestions for future cell therapy development.

2 scRNAseq INTRODUCTION

Previously, people analyzed the whole genome sequence information of mixed DNA samples of millions or more of cells, and the result was usually only the average value of the signals in a certain group of cells or the most dominant cells, ignoring the characteristics of a single cell or a small subset of cells. For example, if we wish to analyze a specific tissue using whole genome sequence information in the hopes of finding a small number of altered cells (perhaps early cancer cells), it will be very difficult since the features of a small number of cells would be overlooked.

However, single-cell sequencing technologies can help to overcome this issue. The Singlecell sequencing technique is more precise than typical whole genome sequencing technology, especially at the level of gene expression. Furthermore, single-cell sequencing can discover a small quantity of gene expression or other uncommon RNA. The Singlecell sequencing technique produces omnidirectional and multilayer results, which greatly aids our knowledge of cells, tissues, and human systems.

ScRNAseq revolutionized transcriptome research. Andrews and Hemberg compiled a list of single-cell isolation procedures and scRNAseq approaches that are now accessible, including quality control, dimensionality reduction, cell clustering, trajectory inference, gene regulatory network reconstruction, and so on, in 2018. 15

At the moment, scientists have proposed a number of ways to reveal the spatial structure of cells by combining single-cell transcriptomics with spatial transcriptomics. Moncada et al presented a technique for multimodal intersection analysis by combining the two omics mentioned above. 16 They discovered that using this strategy, they can map cell types, cell subgroups, and even cell states in space. Peng et al. have published a review on the use of scRNAseq and spatial transcriptomics to better understand stem cell lineage features throughout early embryonic development. 17

scRNAseq data, on the other hand, contains more noise and greater dimensions than bulk RNAseq data and is more complicated due to technological restrictions and biological considerations. As a result, many strategies for assessing bulk RNAseq data are ineffective when applied to scRNAseq data. To evaluate the scRNAseq data, users must either develop the algorithm or tweak the current method. As a result, various methods for analyzing and interpreting scRNAseq data have been developed in recent years, but the findings show that unique techniques are still needed to assure the accuracy and repeatability of the results, and deep learning will be a viable choice. From the standpoint of applying deep learning algorithms to scRNAseq data, this review will examine the influence of deep learning on stem cell treatment and regenerative medicine.

3 DEEP LEARNING TECHNOLOGIES INTRODUCTION

Machine learning is a technique for predicting unknown facts by automatically analyzing and extracting laws from data. The appeal of bioinformatics resides in their capacity to forecast models without a thorough knowledge of the model's core process, which is obviously appealing to most biologists who are not mathematicians. Machine learning technologies, such as t distributed stochastic neighbor embedding (tSNE) and high-latitude data visualizations18, provide analytic approaches for genomics, proteomics, and metabolomics, among other fields.

Extracting, converting, and loading source data; data preprocessing; feature extraction; model training; cross validation; fresh data prediction are the six processes in a typical machine learning workflow. Typically, the input data sample is assigned to x (perhaps a vector), and the output data sample is assigned to y. (maybe a single number).

The standard procedure for machine learning

Machine learning algorithms, unlike previous algorithms that need domain expertise and stringent model assumptions, can automatically discover data and anticipate model structure. As a result, machine learning techniques are well suited to data-driven science, particularly in genomics. 5 The ability of machine learning algorithms is primarily determined by how each feature is captured or calculated. 14

Artificial neural networks research gave rise to the notion of deep learning. A deep learning structure is a multilayer perceptron with numerous hidden layers. By integrating low-level features to identify dispersed feature representations of data, deep learning creates a more abstract high-level representation of attribute category or feature. Convolutional neural networks (CNN) and deep belief networks are two types of deep learning technologies (DBN).

CNN can readily extract certain local relevance characteristics using convolution kernels, is adaptable to data transformations, and has a generally positive effect on pictures. The CNN algorithm may be used to improve the accuracy of segmentation of cell pictures. 19 CNN may also be used in conjunction with scRNAseq data to infer gene relationships. 20 DBN is based on Bayesian reasoning and finds the joint probability distribution of the data to automatically retrieve the high-level information concealed in the data that is difficult to explain. Its output information gives the data a distinct personality. Based on the DBN framework, Chen et al built a Bayesian network model for the interaction between distinct histone modifications across neighboring nucleosomes. It was discovered that nucleosome communication plays a key role in signal propagation, chromatin remodeling, and transcriptional control. 21 Deep learning's application in DNA sequence data was also demonstrated in a groundbreaking study in 2015. 22 Since then, the number of studies relating to deep learning's application in genomics has risen. The use of deep learning to scRNAseq data will be discussed in this paper.

4 DISCUSSION

All genes are included in the scRNAseq findings, which are valuable for cell and tissue analysis. Machine learning approaches are now commonly utilized to evaluate scRNAseq data15, such as recognizing cells, detecting novel cell kinds, and generating pedigree trees. However, when studying various sorts of data sets, the explanations and answers provided by these approaches are not always sufficient, and there is presently no approach that can be used to analyze all distinct sorts of cells. Furthermore, the dimensionality of the collected data is increasing as a result of the in-depth investigation of the cells. Ordinary machine learning algorithms are unable to tackle difficulties created by high dimensions, and forced dimensionality reduction will result in data loss. Then deep learning entered the picture. Deep learning, for example, can analyze high-dimensional data without losing data information by using tensors (various directions in the tensor represent various data kinds).

Deep learning technology, as we all know, is very scalable and can predict unknown variables based on provided data, which is very useful in the field of single-cell genomics. For example, with an existing single cell type database of a certain organization, one may train and obtain a model, and then forecast if an unknown cell belongs to the tissue or which cell type in the tissue. This is critical for the successful implementation of stem cell treatment.

Deep learning may also be used for unsupervised learning. They learn from unlabeled scRNAseq data to provide a data-driven definition of cell kind and identity. This is useful for studying data sets where labels are difficult to come by. Unsupervised algorithms may also be utilized to combine scRNAseq data from diverse sources, according to Shaham et al.23. 25, 24 The constant expansion of similar tissue data sets, as we know from the "Human Cell Atlas Project," 26 makes this more and more significant, which is critical for the fulfillment of stem cell treatment applications. Despite the capability of scRNAseq, single-cell sequencing data is sometimes plagued by issues such as excessive deletion and noise. The transcript will be lost during the reverse transcription process due to the tiny number. As a result of this condition, "dropout" occurrences occurred. According to Kharchenko et al, just a tiny portion of each cell's transcriptome was recognized during the sequencing step,27 and these issues will result in a sparse gene expression matrix. This is a typical issue with lowly expressed genes. However, omitting these genes from the study completely isn't the most effective strategy. This method would squander much too much valuable data, such as transcription factors and surface indicators.

As a result, more effective attribution procedures are needed to recover the lost gene expression in the scRNAseq data set in order to acquire more exact gene expression measurement findings. Many approaches for attributing scRNAseq data have been proposed earlier, according to Arisdakessian et al, including MAGIC,29 ScImpute,30 SAVER,31 and DrImpute. 32 However, these approaches take a long time to execute, which makes them unsuitable for use in the ever-expanding scRNAseq data set.

Then, Arisdakessian et al presented DeepImpute, a deep neural network-based interpolation technique that employs a dropout layer and loss function to learn data patterns for interpolation. 28 This strategy minimizes complexity by breaking down issues into smaller chunks and finetuning the subneural network, or by building many subneural networks to estimate genes in a divide-and-conquer approach. In their paper, Arisdakessian et al claimed that if accuracy is measured using Pearson's correlation coefficient or mean square error, DeepImpute is more accurate on experimental data than many other scRNAseq interpolation methods (such as SAVER, MAGIC, VIPER, and DCA), and DeepImpute is faster and uses less memory than those methods. 28

Arisdakessian et al used four publicly accessible scRNAseq data sets to demonstrate the benefits of DeepImpute: two cell lines, Jurkat and 293T (10X Genomic); one mouse interfollicular epidermis data set released in GSE67602; and one mouse neuron cell data set (10X Genomic). 28 Simultaneously, they hypothesized that the DeepImpute approach may do cluster analysis or differential expression (DE) analysis on actual or simulated data sets, hence improving downstream analysis outcomes. In sum, Arisdakessian et al think that DeepImpute is suited for processing the ever-increasing number of interpolation techniques for scRNAseq data because of its accuracy, speed, and scalability.

In comparison to the DeepImpute approach, Mongia et al introduced deepMc, a deep matrix factorization-based attribution system. 33 The deepMc approach makes no assumptions about the distribution of gene expression. A matrix is used to generate a depth model and then decomposed into several matrices for further analysis using matrix decomposition or kernel specification minimization. This approach adds to the variety of interpolation problem-solving options. DeepMc does not require any hyperparameters; all it requires is a parameter that may also pass the estimated theory.This is in stark contrast to conventional deep learning models, which need a significant amount of effort to fine-tune parameters. Mongia et al. demonstrated that the deepMc approach outperforms the DeepImpute attribution technique in the majority of testing scenarios.We all know that one of the most important uses of scRNAseq is to discover distinct cell types from diverse cell populations, so the two can be compared based on the clustering accuracy obtained after imputation.DeepMc has a substantially greater accuracy than DeepImpute in terms of the Adjusted Rand Index value achieved after using kmeans clustering following various interpolation approaches. 33 Second, they evaluated the accuracy of DE analysis to compare the two approaches. They employed the receiver's operating characteristic curve's area under the curve value, and deepMc is more accurate than DeepImpute. Furthermore, starting with cell type separability (CTS).

As a result, the deepMc approach may be recommended while tackling the interpolation problem of restoring lost gene expression. Of course, the DeepImpute technique offers advantages of its own.

Data from single-cell transcriptomes can reveal previously unknown biodiversity. In practice, numerous studies are required to fully utilize the scRNAseq technique in order to determine cell lineages and real transcription signals. We must employ various strategies to adjust the batch effect in the scRNAseq data due to the combined data.

ScVI may be used to standardize and evaluate scRNAseq data since it is a completely probabilistic approach based on probability Bayesian theory and establishing conditional distributions using deep neural networks. 24 To gather information between comparable cells and genes, this technique uses deep neural networks and random optimization. It takes into account not just the data's sensitivity, but also the effects of batch processing, allowing the findings to more accurately approximate the distribution of observed expression levels. scVI delivers great accuracy in batch effect correction, cell clustering, differential expression, and other areas. 24 After using the scVI technique for clustering, it was discovered that the subgroups that were previously "hidden" owing to the clustering method's inaccuracy, scVI and other techniques (SIMLR,23 MNNs34 [Mutual Nearest Neighbor] + PCA [Principal Components Analysis]) had been annotated. 10 On the above-mentioned data set, a cell clustering analysis was done. The results reveal that scVI can capture the hierarchical structure between cell subsets more precisely and uncover cell subsets that are "hidden groups" for other approaches. Similarly, we can identify what sorts of cells the stem cells can develop into before utilizing them if we learn from the stem cell data set and then apply the learned model to the unknown group of stem cells. As a result, stem cell subsets with the needed differentiation direction may be employed for treatment directly, allowing for more direct treatment of illnesses, precision medicine, and the advancement of cell therapy and regenerative medicine.

BERMUDA (using deep autoencoders for batch effect removal) is a new approach for correcting batch effects in scRNAseq data based on transfer learning. 35 The "domain adaptive learning" approach is used to translate the original data to the new feature space (in the target domain) by picking an appropriate feature representation, ensuring that the distribution of the source domain and the target domain in the new feature space are as similar as feasible. BERMUDA can cluster the cells and remove the errors produced by the batch effect. When a clustering mistake occurs, cells from the acinar group, for example, are associated with cells from the endothelial group. The analysis that follows will be altered as well. There might be a significant disconnect, if not outright disagreement, between the connection with the real cells.

Wang et al demonstrated that BERMUDA outperforms scVI in handling batch effects on publically accessible human pancreatic datasets obtained using various scRNAseq techniques. 35 They assess BERMUDA using biological data. They tested it on datasets from the human pancreas obtained using several scRNAseq techniques that are publically available. "Muraro batch" 36 is one data set, whereas "Baron batch" is another. 37 To imitate the significant disparities in cell type distribution reported in true scRNAseq data, they utilized all of the cells from these two batches and then removed alpha and beta cells from the Baron group.

Batch effects in pancreatic cell scRNAseq data are removed. Experiment all on pancreatic data set UMAP (Uniform Manifold Approximation and Projection) representations of batch effect reduction outcomes. Dashed circles indicate identified alpha and beta cell subpopulations in the Baron batch. With permission from Genome Biology, reprinted from Wang et al35. singlecell RNA sequencing (scRNAseq)

When just batch effects are selected, the BERMUDA technique is better suited, as seen in the above comparison. scVI is, of course, a fantastic candidate for other downstream analyses as well.

To find the optimum batch corrector space, scVI mostly depends on similarities between individual cells, and these similarities do not fully use the clustering structure of distinct cell populations. To eliminate batch effects, BERMUDA employs data similarity between cell clusters. Because clustering and batch effect removal are linked, the best batch effect elimination approach should be used in conjunction with clustering. It's also envisaged that there will be a means to analyze all batches of cells at the same time. For scRNAseq analysis, Li et al presented the DESC technique, which is an unsupervised deep learning system that can iteratively learn clusterspecific gene expression representations and cluster assignments. 38 DESC will gradually reduce batch effects in iterations as long as the technical difference between batches is lower than the genuine biological difference (e.g., the difference between cell types). DESC provides a greater and more stable clustering accuracy than the scVI and BERMUDA approaches.

Furthermore, scAlign39 is an unsupervised deep learning approach for cell label sets that can combine partial, overlapping, or complete cell label sets and estimate the per-cell gene expression differential across the full data set.

ScAlign conducts unit comparison in a single data set by learning a two-way mapping (between the low-dimensional comparison space and the sorting unit). Then, in the low-dimensional comparison space, scAlign completes the grouping of units by function and type. This allows for the correct marking of fresh data sets that lack annotation labels, as well as the detection of unusual cell populations. ScAlign's neural network design helps it and allows it to increase its alignment capabilities. 39 scAlign outperforms existing comparison approaches such as scmap,40, scVI,24, and Seurat, according to Johansen and Quon. 41

We can better perform downstream analysis on the scRNAseq data, complete cell categorization, and uncover novel cell subgroups after correcting the batch effect in the scRNAseq data. When utilized in a variety of fundamental analytical tasks, such as batch correction, visualization, grouping, and differential expression, scVI has demonstrated great accuracy. 24 This enables us to better define the sorts of stem cells that will be employed in clinical trials, as well as the types of cells that they can develop into and the stem cell subgroups that have differentiation potential. It is critical that we deploy stem cell treatment in clinical settings and achieve the intended outcomes. We expect that combining deep learning and scRNAseq data can enhance cell therapy and regenerative medicine implementation, as well as affect precision medicine implementation.

Of course, the deep learning method can not only remove the batch effect in scRNAseq data or complete cell clustering, but it can also learn the data's inherent biological modules, describe the biologically meaningful control data set modules, and provide information about which modules are active for each unit.

The autoencoder, for example, after deconvolution processing,42 It also adapts well to a significant amount of "lost" data, which is valuable and crucial for processing scRNAseq data. Following deconvolution of the resultant model, Kinalis et al discovered that the autoencoder may build a relationship between the model's presentation layer and biological processes after specialized training. The autoencoder completes the signature decryption of genes or places by mapping the hidden cells in the model to well-defined modules, allowing the autoencoder to better delineate the driving mechanisms behind a specific cellular impact. 42 This will allow us to look at the stem cell population from a different perspective, allowing us to screen the subpopulations of stem cells we require and driving the development of stem cell therapy.

The algorithms listed below have aided in the advancement of stem cell treatment in some way. They don't utilize scRNAseq to cluster cells directly; instead, they look at cells from several angles, such as cell contact, quantification of certain subgroups, and tissue dependant status, or predicting the abundance of surface proteins on the scRNAseq data set.

The CNNC algorithm was proposed by Yuan and BarJoseph. 20 They analyze the scRNAseq data using the CNN method, which can conduct causal inference, illness gene prediction, function allocation, and other operations on the data. Because CNN can readily extract some local related features using the convolution kernel, which has a stronger influence on the picture, this approach turns expression data that lacks locality into an image for later processing. The technique then uses a supervised methodology to make genetic connection inferences. To use CNNC, Yuan and BarJoseph employed a significant quantity of single cell expression data and tested it on a variety of inference tasks. In terms of inferring function allocation, gene interaction, and causality inference, they found that CNNC outperforms earlier techniques (DNN, count statistics, mutual information).

Furthermore, DigitalDLSorter is a deep learning algorithm that can forecast the fraction of each cell type in a huge number of RNA sequencing data. 43-267 The immunological infiltration of colorectal cancer and breast cancer in bulk RNASeq samples is enumerated and quantified using scRNAseq data. It adjusts the composition of any cell type specified based on scRNAseq data using a deep neural network (DNN) model, allowing it to measure not just traditional cell categories like lymphocytes, but also particular subgroups and tissue-dependent states. It differs from the previous technique in that it takes into account the microenvironment's effect on the transcriptome. The physiological status of cells will be more precisely quantified and defined as a result of this. When we employ stem cell treatment, this introduces new considerations.

You may utilize a deep neural network-based transfer learning algorithm, cTPnet,44 to use existing single-cell multiomics resources to predict the surface protein abundance of scRNAseq data in addition to assessing cell information from scRNAseq data alone. The results of Zhou et al indicate that cTPnet can predict the abundance of surface proteins on the scRNAseq data set using the REAPseq and CITEseq data sets, as well as the absence of cell type data in the training data set, using the REAPseq and CITEseq data sets. 44 Furthermore, cTPnet can learn from healthy cells and then infer the immunological profile of malignant cells, which is critical for our stem cell treatment prediction.

The increased use of scRNAseq technology has given us a better knowledge of cell heterogeneity at the transcriptional level, and it has transformed the way individuals evaluate and extract meaningful information from bulk RNA. Despite the high expense of scRNAseq, we can extract a lot of useful information from scRNAseq data. This data is extensive and multilayered, allowing us to gain a thorough understanding of cells, tissues, and human bodies. Now, researchers frequently employ various approaches to reanalyze the published data set in order to verify the algorithm's correctness or to identify previously overlooked information in the published data set using a validated new technique. However, evaluating scRNAseq data is difficult in general since the data is noisy, has a high dimensionality, and has high variability, and there is no difference when evaluating it. As a result, users must select an appropriate tool for analysis based on the nature and distribution of their input data collection, as well as the study's unique aim.

Deep learning algorithms are unquestionably a suitable choice for analyzing scRNAseq data since they can handle high-dimensional data well. Because scRNAseq data includes the genome derived from the same cell, the appearance group, and transcriptome and proteome data, deep learning methods have a number of benefits. For example, endtoend learning may enhance prediction accuracy, and it can also efficiently analyze multipeak data. Multimodal modeling allows for the examination of several data sources, resulting in a more complete and precise trained model. People will be able to combine scRNAseq data with spatial transcriptomic data in the future to understand cellular information in a multidimensional fashion using deep learning. Furthermore, by abstracting many mathematical and technological elements, the deep learning framework lowers the entry barriers for constructing new models. This is due to the fact that most biomedical researchers do not have enough time to understand mathematics or computer science theory, let alone build or construct a new model.Using the proper method to evaluate the scRNAseq data can help us forecast the type of cell subpopulation or uncommon cell population with more accuracy.

We all know that stem cells, particularly MSC, have tremendous medicinal potential and might be crucial in the treatment or cure of a variety of disorders. Isolated MSCs, on the other hand, have a distinct effectiveness and self-renewal capability. If they are used directly in clinical practice, they are likely to produce discrepancies, if not outright opposing results, between expectations and actual results. However, if people can use appropriate methods to accurately predict the types of stem cell subpopulations, the cure rate will also improve when using MSC for stem cell therapy, allowing people to better use cell therapy and regenerative medicine strategies to treat various diseases and structural injuries. Humanity will gain from the use of this exact medicinal procedure. Finally, we expect that combining deep learning and scRNAseq data will enhance cell therapy and regenerative medicine implementation, as well as have an impact on precision medicine implementation.

5 Conclusion

The application of several deep learning approaches to scRNAseq data was summarized in this review. While the current deep learning approaches for scRNAseq analysis are promising, improved tools are still required to effectively deal with technical noise, account for cell expression variability, assess MSCs, and forecast stem cell type. Furthermore, using these deep learning algorithms with scRNAseq data to reliably and quickly identify the kind and function of each cell is not yet viable. We came to the conclusion that more research into how to apply deep learning algorithms to analyze scRNAseq data is necessary since it can improve cell therapy and regenerative medicine efforts.

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