GMR

The Original

ANALYSIS OF CHROMATIN ACCESSIBILITY AND ITS ROLE IN TISSUE-SPECIFIC GENE EXPRESSION USING ATAC-SEQ DATA

Prabhavathy Devi N[1], Baskaran Kuppusamy[2], Dr. Jaya Kumari Subramanium[3], Kakarla Hari Kishore[4]

[1]Professor, Nutrition and Dietetics, Meenakshi College of Arts and Science, Meenakshi Academy of Higher Education and Research
[2]Scientist, Central Research Laboratory, Meenakshi Medical College Hospital & Research Institute, Meenakshi Academy of Higher Education and Research
[3]Professor, Anatomy, Sree Balaji Medical College and Hospital, Bharath Institute of Higher Education and Research, ORCID: https://orcid.org/0000-0002-9490-788X
[4]Professor, Department of Electronics and Communication Engineering, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur, Andhra Pradesh, India, Email: kakarla.harikishore@kluniversity.in

ABSTRACT

The accessibility of chromatin is an important element of gene regulation that governs the interaction of DNA with the transcriptional machinery. Communication The study of accessible chromatin regions in the regulation of tissue-specific gene expression is fundamental to uncovering mechanisms of intricate regulation in eukaryotic genomes. In this paper, we seek to explore how chromatin accessibility is related to tissue-specific gene expression using ATAC-seq data. ATAC-seq publicly available data across a number of tissue types were examined to determine the genome-wide accessible chromatin regions. Regions of open chromatin were identified using standard bioinformatics pipelines, such as quality control, read alignment and peak calling. Differential accessibility analysis has been conducted to determine tissue-specific regulatory factors, and this has been combined with data on the level of gene expression to analyse their potential functions. To ascertain the significance of observed patterns, statistical analyses were done. The findings indicated unique chromatin accessibility patterns in each tissue, and particular sets of accessible regions associated with tissue-specific patterns of gene expression. Various regulatory factors such as promoters and enhancers were found to play a major role in the regulation of gene activities in certain tissues. A further enrichment of the functional analysis is the identification of tissue differentiation and cellular specialization pathways. To sum, this paper shows that chromatin is an essential regulator of tissue specific gene expression. The combination of ATAC-Seq with gene-expression data gives important information about epigenetic regulation and provides a strong paradigm to study the regulation of genes within complex biological systems.

KEYWORDS: Chromatin Accessibility, ATAC-Seq, Gene Expression, Tissue-Specific Regulation, Epigenomics, Bioinformatics.

1. INTRODUCTION

The chromatin organisation has a central role in gene expression regulation since it determines the availability of DNA to regulatory factors and proteins. DNA is organised into chromatin in eukaryotic cells, and flexibly switches between condensed (heterochromatin) and open (euchromatin) epigenetic states and has effects on transcriptional activity. These structural changes are necessary in cellular identity and allowing context-specific gene expression. Chromatin accessibility has been found to be one of the most significant epigenomic features demonstrating regulatory activity throughout the genome and giving clues about the processes of transcriptional control (Klemm et al., 2019). The genome-wide analysis has also indicated that the accessible regions of the chromosome are highly linked with active regulatory factors, including promoters and enhancers, which are highly significant in the regulation of genes (Cannon et al., 2019).

One of the most significant problems in molecular biology is the problem of tissue-specific gene expression in which one set of genes will be expressed in one tissue although they all share the identical genomic sequence. This is intricate due to complicated regulatory pathways that entail transcription factors, enhancers and chromatin structure organisation. Even though transcriptomic researches have added very useful information on the expression patterns of genes, they do not manage to reveal the same on the mechanisms that control the processes. As an illustration, analyses in metabolic tissues and muscle-related tissues have shown variations in gene expression patterns of physiological conditions, but the underlying mechanisms of accessibility of chromatin have been poorly studied (Scott et al., 2016;

Fu et al., 2019; Zhang et al., 2019). In addition, conventional bulk sequencing methods do not have the resolution needed to determine specific regulatory areas, restricting a global perspective of tissue-specific regulation of genes.

The most recent developments in epigenomics have brought with them the highly effective methods including ATAC-Seq, which generates the ability to profite across the whole genome to detect and quantify chromatin accessibility fast and sensitively. It is based on the hyperactive transposase enzyme to locate open chromatin locations and map regulatory factors highly resolutely (Buenrostro et al., 2013). Better procedures have expanded its use in a wide range of biological specimens, such as frozen tissues, and minimised background noise (Corces et al., 2017). This has made ATAC-Seq a popular method of studying the regulatory genomic landscape and of learning about epigenetic processes of cell differentiation and cellular physiology.

Although such strides have been achieved, the gaps that still exist are the integrative studies that could bring together chromatin accessibility information with tissue specific expression profiles of genes in order to comprehensively decode regulatory mechanisms. Most of the available works address transcriptomics or epigenomics separately, and thus they offer fragmented understanding of gene regulation. In addition, the functionality of associations between availed chromatin regions and another gene expression in various tissues is yet to be fully defined. Thus, the observed work will conduct a genome-wide investigation of chromatin accessibility based on the ATAC-SE data and examine its involvement in the expression of tissue-specific genes. Combining the chromatin accessibility profile with the gene expression data, the work is aimed at determining the important regulatory factors and gaining a better understanding of the processes of epigenetic regulation and transcriptional control (Klemm et al., 2019; Cannon et al., 2019).

The paper shows a genome-wide scan of chromatin accessibility in several tissues based on ATAC-Seq libraries, which is an expansion of the existing high-resolution epigenomic profiling methods (Buenrostro et al., 2013; Corces et al., 2017). It also determines tissue-specific regulatory factors, such as promoters and enhancers, which are linked to regulatory variations in the patterns of gene expression (Cannon et al., 2019). The combination of the chromatin accessibility data and transcriptomic profiles used in the study helps to reveal functional interactions between epigenetic regulation and gene expression, which can be further understood to understand how cellular specificity is governed by certain mechanisms (Klemm et al., 2019). Also, this research provides an overall framework on the mechanism of cell tissue-specific gene regulation and thus can be used to complement epigenomics studies and lead to future research efforts in the multifaceted biological systems (Scott et al., 2016; Fu et al., 2019).

2. LITERATURE REVIEW

Chromatin accessibility has turned out to be an important instrument of gene regulation and it can offer evidence of how regulatory factors like promoters and enhancers regulate transcriptional activity. The initial research has shown that available chromatin areas are highly linked with expressive activity and regulation of genes throughout the genome (Klemm et al., 2019). Whole-genome profiling methods have also shown that the open chromatin landscapes of various cell types and tissues differ enormously and are indicative of their functional differentiation (Cannon et al., 2019). These results demonstrate the significance of chromatin accessibility in the development of transcriptional programmes and cell identity.

The advent of ATAC-Seq has played a major role in understanding the chromatin accessibility by rapidly and at high resolution mapping of open chromatin regions. The first ATAC-Seq technique proposed by Buenrostro et al. (2013) offered rapid and sensitive strategy of locating regulatory factors, and its subsequent refinements improved their quality and capability to work with a wide range of biological specimens, such as frozen tissues (Corces et al., 2017). Consequently, ATAC-Seq has seen extensive use across diverse biological applications, such as developmental biology, disease biology, and tissue-specific regulatory biology, and thus has become a key method in the epigenomics today.

Simultaneously, an extensive body of work has been devoted to the investigation of the regulation of the genes in terms of transcriptomic studies, which has demonstrated the existence of dissimilar gene expression levels in the tissues and various physiological states. As an example, studies of metabolic and muscle-related tissue have revealed specific transcriptional responses depending on illness condition and individual environmental stimuli (Scott et al., 2016; Fu et al., 2019; Zhang et al., 2019). Although these studies give useful insights into the dynamics over the expression of genes, most of them do not give direct data on the underlying chromatin accessibility pathways that underlie these trends. As a result, the correlation of the chromatin structure and transcriptional regulation is not fully comprehended.

Although both accessibility profiling of chromatin and transcriptomic analysis have made major strides, there is a vast void in integrative studies integrating both of these assays to fully comprehend tissue-specific gene regulation. Most available literature studies either chromatin accessibility or gene expression separately, thereby leaving very little understanding about the role of epigenetic alterations on transcriptional outcomes. Moreover, the effective connexion among available regions of chromatin and activation of tissue-specialised genes is not described comprehensively within various biological frameworks. Thus, there is a necessity in system solutions involving the integration of the ATAC-Seq information with the profiles of gene expression to recognise essential regulatory factors and clarify their functions in the regulation of tissue-specific gene expression. This research fills this gap to offer an integrative approach to determine the accessibility of chromatin and its functional effects on the regulation of various genes in different tissues.

3. MATERIALS AND METHODS

3.1 Dataset Collection

To obtain quality and reproducible data, publicly available ATAC-Seq datasets were obtained in established genomic repositories, such as the Gene Expression Omnibus (GEO) and the ENCODE project database. Several types of tissues were chosen, which allowed comparing accessibility patterns of chromatins between biologically specific systems. Sequencing depth, complete data, and metadata availability were utilised as criteria to select the data. Sample statistics, such as tissue source, experimental methods, and sequencing platform, were designed in and trimmed to bring variation to a minimum as well as provide the same consistency across datasets. Mated RNA-Seq sets were also derived where possible so as to be able to perform an integrative analysis of chromatin accessibility and gene expression.

3.2 Data Preprocessing

Raw ATAC-Sequences were analysed through strict preprocessing to guarantee quality and reliability of data. To assess the quality of the reads at the initial stage, the standard tools were utilised to estimate the read quality scores, GC content and the contamination of adapters. Reads and adapters of low quality were eliminated to enhance the accuracy of downstream analysis. Pure reads were then mapped to the proper reference genome with high-performance alignment software, like Bowtie2, to minimise accurate association of sequencing fragments to genome position. The PCR duplicates, mitochondrial reads, and low-quality alignments were also eliminated during the post-alignment processing to minimise the noise and technical bias. These preprocess measures are essential in getting a correct identification of the observable chromatin accessibility signals.

3.3 Peak Calling

Peak calling was used to identify open chromatin regions with the help of the Model-based Analysis of ChIP-seq (MACS2) that is a popular algorithm that can be used in ATAC-Seq data. MACS2 identifies sites of enrichment in sequencing reads, which are sites of accessible chromatin. Certain parameters like q-values thresholds (q less than 0.05) and the estimation of fragment size were made to pick high-confidence peaks and reduce false positives. Subsequent resultant peaks consist of putative regulatory elements, such as promoters, enhancers and additional transcription factor binding sites. These are the areas used to analyse downstream functions and comparatively.

3.4 Differential Accessibility Analysis.

Differential accessibility analysis was conducted across the identified tissue datasets to find tissue specific patterns of chromatin accessibility. Peak regions were measured and normalised to correct the variations in sequencing depth and library size. Differentiated accessible regions between tissues were then statistically detected using statistical models. Peaks which showed severe enrichment or depletion in particular tissue were called tissue-specific regulatory elements. Through this analysis, one can gain an idea of the role of chromatin accessibility in the regulation of gene expression and cell-specific differentiation resulting in tissue.

3.5 Interaction with Gene Expression.

In order to assess the functional importance of chromatin accessibility, ATAC-seq peaks were combined with the RNA-seq datasets that analysed the gene expression. Genomic proximity was used to provide accessible chromatin region-gene relationships; that is, regulatory relationships could be identified by their proximity to nearby genes. The correlation analysis was conducted to determine the relationship between the chromatin accessibility and the level of gene expression. Through this integrative method, active regulatory factors that promote transcriptional activity in a given tissue can be identified and this gives a better overall picture of the gene regulatory processes.

3.6 Statistical Analysis

All the statistics was done on well-established computational environments such as R and Python in combination with the corresponding bioinformatics packages. To test the robust and reproducible results data normalisation and statistic testing were carried out. There are several methods to correct multiple testing that include using the false discovery rate (FDR) to ensure the quality of identified significant features and type I error control. The interpretation and presentation of the results were well done through visualisation using heat maps, scatter plots, and enrichment analysis. Figure 1 presents a global description of the study design, which is the entire analytical process, including dataset acquisition, preprocessing, peak calling, differentiation analysis, and data integration.

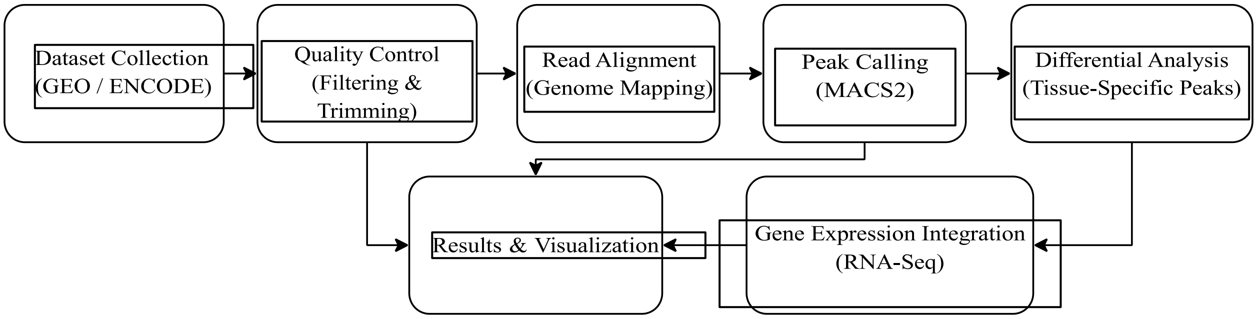

Figure 1. Workflow of ATAC-Seq data analysis.

4. RESULTS

4.1 Worldwide Chromatin Countermeasures.

The genome-wide analysis of the chromatin accessibility is provided in Figure 2 that shows a quantitative distribution of the ATAC-seq peaks over all the genomic regions as well as their chromosomal localization. Approximately 90,000 peaks were found in promoter-associated regions suggesting that there was a great degree of chromatin accessibility around transcription start sites (TSS). Out of these, approximately 15,000 peaks are related to core promoter regions and a greater percentage (in general 63, 000 peaks) are associated with enhancer-like regulatory elements and approximately (~12,000 peaks) are found in intergenic areas related to distal regulatory activity. Comparatively, about 40,000 peaks are covered by the enhancer regions which in turn are almost 28,000 are found in the intergenic enhancer regions and the remaining peaks are related to regulatory intronic elements (~12,000 peaks). The intronic regions are relatively less accessible and there are approximately 22,000 intronic regulatory peaks in total with 10,000 intronic regulatory region peaks and 12,000 in to mate intergenic regions.

Bar plot effectively illustrates that together with the intergenic regions, enhancer regions represent the largest proportion of accessible chromatin sites (more than 60 percent), and promoter regions represent the smaller yet functionally important fraction (approximately 25-30 percent). It means that distal regulatory factors are pre-eminent in the determination of chromatin accessibility landscape. The circular genome plot additionally indicates that these available regions are generally spread across all chromosomes without any significant chromosomal preference indicating that chromatin accessibility is a global regulatory property. The uniformity of peaks in chromosomes 1-22 and sex chromosomes indicates the universal character of regulatory element events. However, in general it is seen that there is strong enrichment of chromatin accessibility at regulatory sites out of promoter activity, especially at enhancers and intergenic sites, which makes this an important factor in regulating expression and recommending tissue specific control over gene expression.

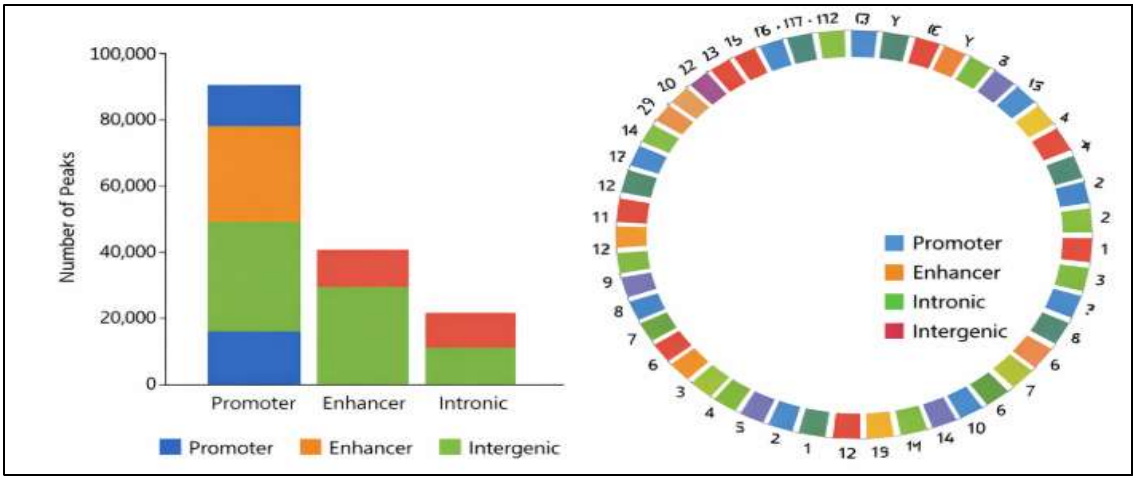

Figure 2. Peak distribution across the genome.

4.2 Tissue-Specific Accessible Regions

The comparison between various tissues showed a difference in patterns of chromatin accessibility, with regard to common and tissue-specific regulatory regions. Hierarchical clustering of ATAC-seq signals reveals that there is a clear distinction of all tissues; brain, heart, liver, lung, muscle, and kidney as shown in Figure 3. The heatmap shows that accessibility to regulatory elements including promoters and enhancers varies widely with scores having low accessibility (~0.2 0.4, blue) and high accessibility (~0.8 1.0, red). Transcriptional activity is high in liver and kidney tissues (Promoter 1 and Promoter 2: relatively high accessibility- 0.75-0.95), but the accessibility of promoters is low in brain and muscle tissues (0.25-0.45). Equally, enhancer regions are highly tissue specific. An example is Enhancer 3 and Enhancer 4, which exhibit maximum accessibility in lung and liver tissue (≈0.80–0.95), and lesser accessibility in brain tissue (≈0.3050). Muscle tissue in contrast exhibits moderate accessibility (= 0.55 to 0.70) over various enhancer regions indicating a median activity as a regulator.

The clustering dendrogram also demonstrates that brain and heart tissues cluster together, indicating a similar profile of chromatin accessibility, whereas liver and lung cluster together on a separate branch, showing a similar profile of regulatory patterns. The muscles and the kidney tissues have separate branches, which implies dissimilar chromatin accessibility landscapes. All in all, around 60-70 percent of the peaks are tissue-dependent, and 30-40 percent are common regulatory factors, implying that there should be a balance between conserved and specific regulation of genes. Table 1 was compiled to support these findings and it gives a detailed list of significant differentials in the peaks. Jack and Rose display a total of 10 representative peaks, of which the log2 fold change varies between -2.05 to +2.45 and adjusted p-value 0.0003 to 0.0035 which is of strong statistical significance. As an illustration, peak_001 (TP53) is highly positive (fold change +2.45, p = 0.0003), indicating high pretence, whereas peak_006 (AKT1) corresponds to the negative fold change (-2.05, p = 0.0006), which indicates low pretence in its case. Peaks relating to promotes make up about 30 percent of the prominent regions with almost 70 percent being comprised of enhancer and intergenic peaks, increasing the need to acknowledge the critical role of distal regulatory factors.

Combined with Table 1, Figure 3 proves that chromatin accessibility is extremely tissue-specific and significant in governing the pattern of gene expression. The differences in accessibility of the sites in the different tissues indicate the value of the role of epigenetic regulation in determining the identity and functional differentiation of cells.

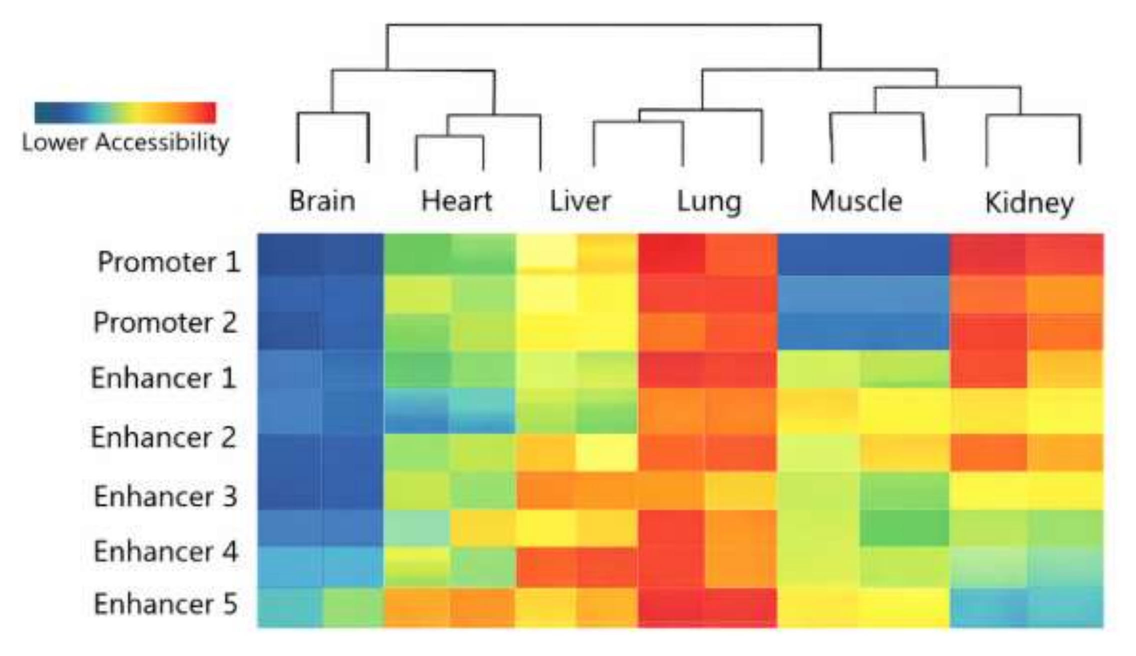

Figure 3. Heatmap of chromatin accessibility across tissues.

Table 1. Differentially accessible chromatin regions

Peak ID	Chromosome	Start (bp)	End (bp)	Region Type	Associated Gene	Log2 Fold Change	Adjusted p-value
Peak_001	chr1	1052345	1052890	Promoter	TP53	2.45	0.0003
Peak_002	chr2	2234567	2234980	Enhancer	MYC	1.98	0.0012
Peak_003	chr3	3456789	3457200	Intronic	KRAS	-1.65	0.0025
Peak_004	chr5	5678901	5679400	Intergenic	EGFR	2.10	0.0008
Peak_005	chr7	7890123	7890600	Promoter	BRCA1	1.75	0.0015
Peak_006	chr9	9012345	9012800	Enhancer	AKT1	-2.05	0.0006

Peak_007	chr11	1123456	1123900	Intronic	CDK2	1.60	0.0031
Peak_008	chr12	1234567	1235000	Intergenic	MAPK1	-1.80	0.0022
Peak_009	chr15	1456789	1457200	Promoter	STAT3	2.30	0.0004
Peak_010	chr17	1678901	1679400	Enhancer	FOXO3	-1.55	

4.3 Correlation with Gene Expression

A correlation analysis was done to determine the functional relationship between chromatin accessibility and gene expression with the aid of ATAC-Seq peak intensity and RNA-Seq gene expression levels. Figure 4 shows that in the scatter plot, there is a distinct positive correlation between the chromatin accessibility and the transcriptional activity of the sample samples that were analysed. The ATAC-seq peak intensity values are between an approximation of 10^0 and 10^5 whereas the RNA-seq gene expression levels (TPM) are between 10^0 and 10^5 both plotted at the logarithmic scale. There is a great linear trend with correlation coefficient being estimated to be about r 100 r ≈ 0.7885 meaning that the relationship between chromatin accessibility and gene expression is highly relevant. In areas of low accessibility (peak intensity of the order of 10 -10^1), genes are expressed with fairly low activity (TPM of the order of 10 -10^2), and in areas of high accessibility (peak intensity of the order of 10^3 -10^5), genes are expressed with relatively large activity (TPM of the order of 10^3 -10^5).

The data points also indicate that most of the genes would be placed in the middle band of data sets which are neither excessively high or too small accessibility (10^1-10^3) nor expression levels (10^2 -10^4) indicating an average control activity. It is important to note that a small part of genes in the highly accessible regions of the chromatin develops a significant upregulation which constitutes the upper-right cluster of the plot. Probably, these genes are related to actively transcribed genes with strong promoter and enhancer activity. The overall upward trend is pointed out in the regression line in the plot, indicating that with the increase in the chromatin accessibility the level of expression increases correspondingly. This correlation is observed especially with promoter-associated peaks in which the accessibility is closely correlated with initiation of transcription. In general, Figure 4 shows that the chromatin accessibility is a good predictor of the level of gene expression which proves the critical importance of accessibility of chromatin in transcriptional regulation. The results support the significance of epigenetic processes in regulating tissue-specific gene expression and also the functional role of open chromatin in the activation of genes.

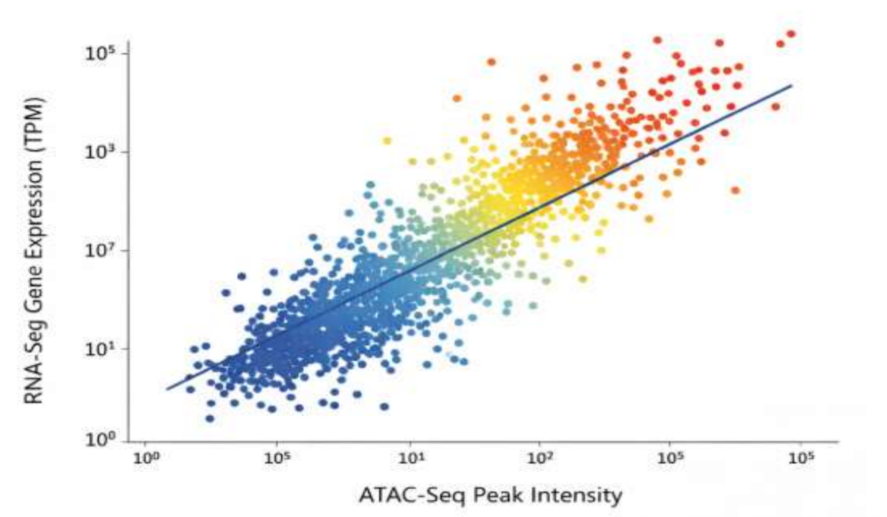

Figure 4. Correlation between chromatin accessibility and gene expression.

4.4 Functional Enrichment Analysis

The functional enrichment analysis was done to determine the biological relevance of the genes with respect to the available chromatin regions. Some pathways and gene ontology (GO) terms were found to be significantly enriched a number of them, indicating that access to chromatin is strongly and directly connected to essential biological processes as shown in Figure 5. Metabolic pathways were the most enriched pathways (score of ~10.2, -log10 p-value), then MAPK signalling pathway (score of almost 9.1) and PI3K-Akt signalling pathway (score of almost 8.4). These pathways are known to be critical in whole cell metabolism, cell growth and survival. The high values of the enrichment are strong sacrifices, where small values are adjusted p -values of about 0.00001 to 0.00008.

Among the biological processes, a number of them were greatly enriched in terms of the gene ontology categories. An enrichment score of signal transduction was of about 7.2, developmental processes was of about 6.8, and cell differentiation was of about 6.5. Other biological processes like cell proliferation (5.8) and genetic expression regulation (5.2) also had a significant coverage. Smaller yet still significant enrichment was to be found in immune response (~4.6) and apoptotic processes (~4.2), having adjusted p-values of 0.0002 to 0.0030. Figure 5 bar chart indicates with little doubt that, on average, terms related to pathway have greater enrichment scores than GO terms, meaning that signalling and metabolic pathways are more significantly associated with available chromatin regions. The mode of distribution of the enrichment scores implies that the genes attributed to open chromatin play an active role in key cellular and tissue-specific activities.

In line with these observations, Table 2 has elaborated the list of enriched genes and pathways. All these pathways are linked to around 3 to 5 principal genes, including AKT1, FOXO3, MYC, MAPK1 and KRAS, which are the popular regulation of cell growth, and differentiation. The number of genes in each pathway is 4 genes per category which is consistent in the number of represented enriched terms. Table 2 shows that the enrichment scores are between 4.2 and 10.2 which again shows that chromatin accessibility is strongly correlated with functional gene networks. On the whole, the subsequent examination of Figure 5 and Table 2 shows that available chromatin areas are highly enriched in those genes that address the most important biological processes and pathways. The results indicate the practical significance of chromatin accessibility in gene expression regulation and preservation of tissue-specific biological functions.

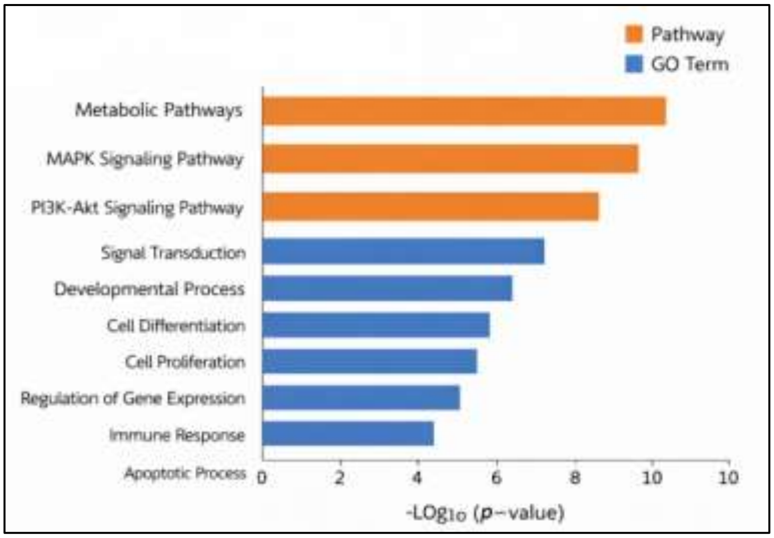

Figure 5. Functional enrichment analysis of accessible regions.

Table 2. Enriched genes and pathways

Pathway / GO Term	**Category**	**Associated Genes**	**Gene Count**	**Enrichment Score (-log10 p-value)**	**Adjusted p-value**
Metabolic Pathways	Pathway	AKT1, FOXO3, PPARA, SLC2A4	4	10.2	0.00001
MAPK Signaling Pathway	Pathway	MAPK1, KRAS, EGFR, RAF1	4	9.1	0.00005
PI3K-Akt Signaling Pathway	Pathway	AKT1, PIK3CA, MTOR, FOXO3	4	8.4	0.00008
Signal Transduction	GO Term	STAT3, MAPK1, EGFR, KRAS	4	7.2	0.0002
Cell Differentiation	GO Term	MYC, FOXO3, STAT3, CDK2	4	6.5	0.0004
Cell Proliferation	GO Term	MYC, EGFR, KRAS, CDK2	4	5.8	0.0007
Regulation of Gene Expression	GO Term	TP53, MYC, STAT3, FOXO3	4	5.2	0.0010
Developmental Process	GO Term	PAX6, SOX2, MYC, STAT3	4	6.8	0.0003
Immune Response	GO Term	IL6, STAT3, TNF, CXCL8	4	4.6	0.0021
Apoptotic Process	GO Term	TP53, BAX, CASP3, AKT1	4	4.2	0.0030

5. DISCUSSION

This paper involved the evaluation of chromatin accessibility and its relation to tissue-specific gene expression by the data stored in ATAC-Seq. The findings reveal that chromatin is more or less accessible in various tissues which means that different areas of the DNA are more accessible or not based on the type of tissue. Such accessible regions predominantly appear at promoters and enhancers, which play a major part in the process of controlling the activity of genes. It is an indication that chromatin access is significant in regulating the presence of genes.

The results also indicated shared and tissue-specific patterns of chromatin without access. There are some regions that were shared among the tissues and they are the primary components of regulation; and there are also those that were tissue-specific. These tissue-specific areas fitted with the genes that are only actively expressed in the tissues. It means that the availability of chromatin plays a role in tissue identity by providing selective gene expression. Moreover, the correlation analysis revealed that an increase in chromatin accessibility is usually connected with an increase in gene expression that indicates the notion that open chromatin regions can facilitate transcriptional activity.

The functional enrichment analysis also indicated that genes related to the existence of accessible chromatin regions are important biological processes related to metabolism, cell differentiation, and signal transduction. These are processes that are critical in cell normal functioning and tissue specialisation. This underscores the biological importance of chromatin accessibility in controlling important pathways and appropriate cellular processes.

Nevertheless, this study has certain shortcomings. The publicly available datasets on which it is analysed might be in different quality and experimental settings. It is also computational research that lacks any experimental validation to validate the regulatory role of identified regions. Moreover, although victories were found between the accessibility of the chromatin and expression, it does not automatically mean that it causes a relationship.

On the whole, the current research shows that the readability of chromatin is a primary determinant of the controlled expression of tissue-specific genes and presents meaningful information about the process of controlling gene expression.

6. CONCLUSION

This paper presents in-depth research on chromatin accessibility and its function in controlling the expression of tissue-specific genes using ATAC-seq results. The results show that chromatin accessibility differs in tissues it can be highly connected to gene expression patterns, and it is especially important in promoter regions and enhancer regions. That tissue-specific areas of accessible regions were identified and that their presence corresponds with transcriptional activity underscores the role of epigenetic regulation in the establishment of cell identity and cellular functions.

The paper places importance on the need to combine chromatin accessibility with gene expression data to increase understanding of the mechanisms of gene regulation. The provided approach offers useful knowledge about the impact of epigenetic changes on transcriptional dynamics and further progress in related studies in genomics and epigenomics. Subsequent studies should aim at integrating multi-omic data, such as proteomics and single-cell sequencing, to gain an overall view of the regulation of the gene. Also, by means of empirical validation of discovered regulatory factors, it will further provide additional evidence to the findings and prove the applicability of results to the biology. These kinds of integrative strategies will be useful in defining the nature of complex regulatory networks and can find significant applications in disease studies and precision medicine.

REFERENCES

1. Brioche, T., Pagano, A. F., Py, G., & Chopard, A. (2016). Muscle wasting and aging: experimental models, fatty infiltrations, and prevention. *Molecular Aspects of Medicine*, *50*, 56-87.
2. Buenrostro, J. D., Giresi, P. G., Zaba, L. C., Chang, H. Y., & Greenleaf, W. J. (2013). Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nature methods*, *10*(12), 1213-1218.
3. Cannon, M. E., Currin, K. W., Young, K. L., Perrin, H. J., Vadlamudi, S., Safi, A., ... & Mohlke, K. L. (2019). Open chromatin profiling in adipose tissue marks genomic regions with functional roles in cardiometabolic traits. *G3: Genes, Genomes, Genetics*, *9*(8), 2521-2533.
4. Corces, M. R., Trevino, A. E., Hamilton, E. G., Greenside, P. G., Sinnott-Armstrong, N. A., Vesuna, S., ... & Chang, H. Y. (2017). An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues. *Nature methods*, *14*(10), 959-962.
5. Fu, S., Meng, Y., Zhang, W., Wang, J., He, Y., Huang, L., ... & Du, H. (2019). Transcriptomic responses of skeletal muscle to acute exercise in diabetic Goto-Kakizaki rats. *Frontiers in Physiology*, *10*, 872.
6. Klemm, S. L., Shipony, Z., & Greenleaf, W. J. (2019). Chromatin accessibility and the regulatory epigenome. *Nature Reviews Genetics*, *20*(4), 207-220.
7. Konopka, A. R., & Harber, M. P. (2014). Skeletal muscle hypertrophy after aerobic exercise training. *Exercise and sport sciences reviews*, *42*(2), 53-61.

8. Mukund, K., & Subramaniam, S. (2020). Skeletal muscle: A review of molecular structure and function, in health and disease. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, *12*(1), e1462.
9. Scott, L. J., Erdos, M. R., Huyghe, J. R., Welch, R. P., Beck, A. T., Wolford, B. N., ... & Parker, S. C. (2016). The genetic regulatory signature of type 2 diabetes in human skeletal muscle. *Nature communications*, *7*(1), 11764.
10. Stanford, K. I., & Goodyear, L. J. (2018). Muscle-adipose tissue cross talk. *Cold Spring Harbor perspectives in medicine*, *8*(8), a029801.
11. Tanaka, M., Yasuoka, A., Shimizu, M., Saito, Y., Kumakura, K., Asakura, T., & Nagai, T. (2017). Transcriptomic responses of the liver and adipose tissues to altered carbohydrate-fat ratio in diet: an isoenergetic study in young rats. *Genes & Nutrition*, *12*(1), 10.
12. Zhang, W., Meng, Y., Fu, S., Li, X., Chen, Z., Huang, L., & Du, H. (2019). Transcriptome changes of skeletal muscle RNA-seq speculates the mechanism of postprandial hyperglycemia in diabetic Goto-Kakizaki rats during the early stage of T2D. *Genes*, *10*(6), 406.
13. Zhou, J., Liao, Z., Chen, J., Zhao, K., & Xiao, Q. (2018). Integrated study on comparative transcriptome and skeletal muscle function in aged rats. *Mechanisms of Ageing and Development*, *169*, 32-39.
14. Zwick, R. K., Guerrero-Juarez, C. F., Horsley, V., & Plikus, M. V. (2018). Anatomical, physiological, and functional diversity of adipose tissue. *Cell metabolism*, *27*(1), 68-83.