ORIGINAL ARTICLE

The Binding Occupancy of Pro-Hypertrophic Transcription Factors among Cardiac Developmental Enhancers

Rabail Zehra Raza[1*], Irfan Hussain[2], Fatima Akhtar[1], Sara Mumtaz[1]

ABSTRACT

Objective: To check which of the eight pro-hypertrophic transcription factors bind in abundance to a set of 140 functionally confirmed cardiac enhancers aiming to identify potential therapeutic targets for delaying the onset of cardiac hypertrophy.

Study Design: Bioinformatics, in-Silico Study.

Place and Duration of Study: The study was conducted at the Department of Biological Sciences, National University of Medical Sciences (NUMS), Rawalpindi, Pakistan, from January 2022 to December 2022.

Methods: For experimentally verified human and mouse noncoding regions with gene enhancer activity measured in transgenic mice, VISTA Enhancer Browser was used to obtain 140 cardiac-specific functionally confirmed enhancers. Among these enhancers, transcription factor binding sites were computationally mapped to assess transcription factor occupancy. The binding sites for all eight transcription factors were screened in the collective set of 140 cardiac enhancers via an in-house script.

Results: In this study, among a small set of eight pro-hypertrophic transcription factors, occupancy of transcription factor HAND (Heart- And Neural Crest Derivatives-Expressed Protein) was found to be maximum in the collected pool of functionally confirmed cardiac enhancers.

Conclusion: The transcription factor HAND was found to have maximum binding occupancy among a set of experimentally confirmed cardiac enhancers.

Keywords: *Cardiac Hypertrophy, Enhancers, Gene Regulation, Transcription Factors.*

How to cite this: *Raza RZ, Hussain I, Akhtar F, Mumtaz S. The Binding Occupancy of Pro-Hypertrophic Transcription Factors among Cardiac Developmental Enhancers. Life and Science. 2024; 5(1): 22-26. doi: http://doi.org/10.37185/LnS.1.1.464*

Introduction

Gene Regulation has a paramount importance in cell growth and differentiation, which, in large part, is controlled by a set of tissue-specific transcription factors.[1] Transcription factors are DNA-binding proteins that bind to particular sequences and regulate transcription.[1,2] Like many other developmental processes, transcription factors have a crucial role in cardiogenesis. In addition to controlling heart growth and development, cardiac transcription factors are attributed to play a role in managing stress to the heart tissue, which may cause cardiac hypertrophy.[1] Cardiac hypertrophy is a thickening of the heart muscles as a cellular response of cardiomyocytes to stress.[1,2] Prolonged hypertrophy resulting in thickened ventricular walls may also lead to heart failure.[3,4] Myocardial dysfunction can occur as a result of various pathological and biomechanical reasons; however, common transcriptional alterations in the final pathway of sarcomeric gene expression have been reported to occur in different types of pathologic hypertrophy, which makes it plausible to consider cardiac hypertrophy as a regulatory disorder.[1] Transcription factor occupancy among the cis-

[1]*Department of Biological Sciences*
National University of Medical Sciences, (NUMS)
Rawalpindi, Pakistan
[2]*Center of Regenerative Medicine and Stem Cell Research*
Aga Khan University, Karachi, Pakistan

Correspondence:
Dr. Rabail Zehra Raza
Assistant professor, Biological Sciences
National University of Medical Sciences, (NUMS)
Rawalpindi, Pakistan
E-mail: rabail.zehra@numspak.edu.pk

Funding Source: NIL; Conflict of Interest: NIL
Received: Aug 18, 2023; Revised: Nov 02, 2023
Accepted: Nov 08, 2023

regulatory elements known as enhancers, in a specific number and in a unique combinatorial preference with other transcription factors, happens to play a huge role in the precise spatiotemporal regulation of the genes.[5] Enhancers are short DNA sequences that make up the distal category of cis-regulatory elements and bring about the spatio-temporal expression of the gene(s).[5,6] Each enhancer has a set of transcription factor binding sites that may be ordered in a specific way along the length of the enhancer.[6] To drive gene expression, tissue-specific coordination between an enhancer and several other regulatory elements (promoters, silencers, insulators) is required.[3] With its defined repertoire of transcription factor binding sites, each enhancer can drive gene regulation in a tissue-specific manner at different developmental stages by recruiting RNA polymerase II and transcriptional machinery's component to a gene's promoter.[6] Enhancers can operate from as far as 1 mega base from their target gene(s). Several mechanisms have been proposed such as linking, looping, tracking, and transcription factor mobilization, with which enhancers can communicate with their target promoters and hence mediate the on/off mechanism of gene expression in specific tissues.[7] A single gene can be operated by multiple enhancers, each of which has a different combination of bound transcription factors. In cardio genesis, several transcription factors have been reported to express in the myocardium and ultimately control the expression of genes responsible for cardiomyocyte development.[8] Keeping the context as mentioned above in mind, this study has focused on pro-hypertrophic transcription factors and analyzed their binding sites in a pool of functionally confirmed cardiac enhancers.[9]

Methods

The In-silico analysis was carried out at the Department of Biological Sciences, National University of Medical Sciences (NUMS), Rawalpindi, Pakistan from January 2022 to December 2022 after taking waiver certificate from ethical review committee of the university held on 05th January 2022, vide exempted waiver letter no: 06/IRB&EC/NUMS/01. In order to see which of the pro-hypertrophic transcription factors display profuse binding instance with the cardiac-enhancers, we referred to the VISTA enhancer browser for the functionally confirmed repertoire of enhancers.[9] VISTA enhancer browser till date is the most comprehensive repertoire of functionally confirmed enhancers in which the enhancer sequences have been experimentally validated in-vivo.[9] The enhancers currently present in VISTA are conserved across very long evolutionary distances, such as down to zebrafish and pufferfish, whereas others are conserved across relatively shorter evolutionary distances (chicken and frog). Among the current number of enhancers present in the VISTA enhancer database with enhancer activity confirmed, 140 enhancers were shortlisted for this study with an expression in the heart tissue. Cardiac enhancer collection was conducted in two parts. In the first part, 98 enhancers were shortlisted that showed expression in the heart tissue only. In the second part, 42 enhancers were included in the analysis that showed expression in the heart as well as other tissues. A set of eight pro-hypertrophic transcription factors and their respective DNA binding sites were collected via a literature survey.[1] The binding sites for all eight transcription factors were screened in the collective set of 140 cardiac enhancers via an R script.[10] The screening and diagrammatic display of transcription factors over the enhancers was also conducted in R.[10]

Results

A stringent shortlisting standard was applied in the collection of cardiac enhancers in which functionally confirmed enhancers were shortlisted for the study.[11] This criterion avoided the chance of including any predicted noncoding regions that might not result in the enhancer activity when tested in vivo.[12] Out of the 140 enhancers shortlisted, 98 enhancers had an expression solely in the heart tissue, whereas the remaining enhancers had an expression in the heart as well as the other tissues. Transcription factor binding sites for a subset of 8 pro-hypertrophic transcription factors namely GATA family members ((A/T)GATA(A/G)), MEF-2 (CAT(A/T)GTA(G/A), Csx-Nkx-2.5 (T(C/T)AAGTG, SRF & Myocardin (CC(A/T)$_6$GG), HAND (CANNTG), TEAD (CATTCC(T/A)), and NFAT ((A/T)GGAAA(A/N)(A/T/C)N) were collected from literature.[1] The binding sites of the

aforementioned transcription factors were thoroughly examined across all 140 enhancers. This screening was divided into two parts; the first part included 98 enhancers that had a sole expression in the heart tissue, and the other part included the remaining 42 enhancers that had an expression in multiple tissues, including the heart tissue. This thorough screening in both parts resulted in an abundant presence of the HAND transcription factors (Figure 1 and Figure 2). The highest binding instance of HAND transcription factors was observed in the form of 37 binding sites in enhancer hs1963. Transcription factor binding sites of NFAT and GATA family members also had a reasonable presence among the enhancers of both the parts. Transcription factor binding sites for transcription factors TEAD and Csx-NKx-2.5 had a slight presence in all of the 140 enhancers. However, binding sites for SRF & Myocardin and MEF-2 had the scarcest presence in all of the 140 enhancers.

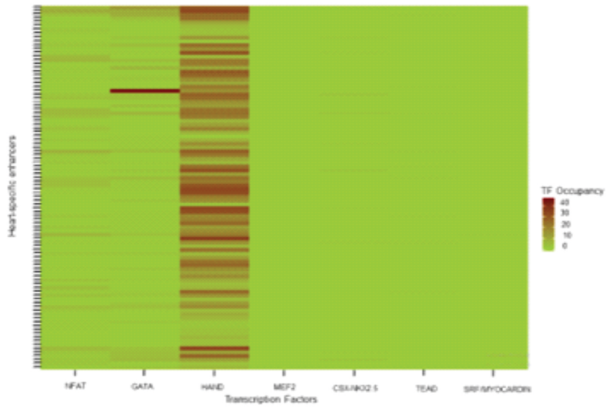

Fig 1: Heatmap for the 98 Heart-specific Enhancers (The heat map shows maximum transcription factor (TF) occupancy for the HAND protein)

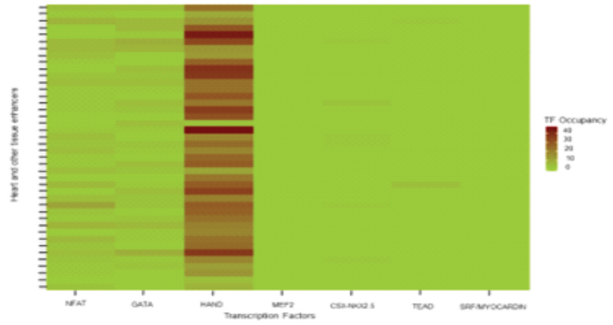

Fig 2: Heatmap for the 42 Heart and other tissue enhancers (The heat map shows the maximum transcription factor (TF) occupancy for the HAND protein)

Discussion

Gene regulation is a crucial driver of a cell's translational output. Despite having an identical genome, each cell delivers a different set of proteins. This spatiotemporal control over a gene's expression is maintained by a variety of regulatory elements. It is reported that up to 20% of the human genome has a regulatory function.[5] Besides, 80% of the SNPs associated via genome-wide association studies have also been reported in the noncoding regions, of which regulatory regions make up a significant portion.[13] Among the regulatory regions, enhancers are the most widely categorized regulatory elements. Several studies have reported transgenic mice assays employed to determine the tissue-specificity of enhancers.[14,15] Enhancers are able to recruit transcription factors in several ways. The interaction between the contiguously bound transcription factors over an enhancer can be direct or through a co-factor. The nature of this interaction between the transcription factors bound directly or indirectly over an enhancer region can largely determine the transcriptional output of an enhancer.[16] However, transcription factors can also bind to an enhancer due to easier chromatin access, and the binding event does not always result in spatiotemporal control of the genes.[16] The transcription factor binding sites over the DNA in regulatory regions such as enhancers have a huge role in organismic development.[11,17] A single base pair change in the binding site can hugely impact the binding affinity of a transcription factor. Due to a single nucleotide change in the regulatory region, a transcription factor can either bind very strongly or with less affinity to a transcription factor binding site.[18,19] Comparative analysis of enhancers between the species has also shown the lack of the binding event due to a nucleotide change.[11,20] In the case of cardiac muscle differentiation genes, a large number of transcription factors have been reported that play a maladaptive regulatory role in cardiac hypertrophy.[1] These factors have a robust activity in hypertrophic responses and therefore given the name hypertrophic transcription factors. In this study, a set of 140 enhancers was analyzed and scanned for the occurrence of eight transcription factors. The extensive binding event over all of the

140 enhancers was found to be that of transcription factor HAND. HAND proteins belong to a well-conserved class of transcription factors comprising of the basic Helix-loop-Helix structural motif.[21] Hand proteins exhibit a well-known function in many developmental processes in vertebrates, such as neurogenesis and cardiogenesis.[22] In higher vertebrates, two paralogs of the HAND proteins namely HAND1 and HAND2 are expressed in the developing heart along with the neural crest cells and lateral plate mesoderm.[22]

Conclusion

In sum, this study outlines the function of cardiac transcription factors in cardiac hypertrophy, with a focus on how they may be convergent targets for existing therapy and may present an appealing therapeutic targets to delay the onset of heart failure and sudden death. Moreover, the single nucleotide changes within the binding sites of the cardiac pro-hypertrophic transcription factors may also prove to be useful in understanding the overall evolution of the human heart anatomy in its present form.

REFERENCES

1. Kohli S, Ahuja S, Rani V. Transcription factors in heart: promising therapeutic targets in cardiac hypertrophy. Current cardiology reviews. 2011; 7: 262-71. doi: 10.2174/157340311799960618
2. Yan K, Wang K, Li P. The role of post-translational modifications in cardiac hypertrophy. Journal of cellular and molecular medicine. 2019; 23: 3795-807. doi: 10.1111/jcmm.14330
3. Frey N, Olson E. Cardiac hypertrophy: the good, the bad, and the ugly. Annual review of physiology. 2003; 65: 45-79. doi: 10.1146/annurev.physiol.65.092101.142243
4. Lu P, Ding F, Xiang YK, Hao L, Zhao M. Noncoding RNAs in cardiac hypertrophy and heart failure. Cells. 2022; 11: 777. doi: 10.3390/cells11050777
5. Pennacchio LA, Bickmore W, Dean A, Nobrega MA, Bejerano G. Enhancers: five essential questions. Nature Reviews Genetics. 2013; 14: 288-95. doi: 10.1038/nrg3458
6. Moon JM, Capra JA, Abbot P, Rokas A. Signatures of recent positive selection in enhancers across 41 human tissues. G3: Genes, Genomes, Genetics. 2019; 9: 2761-74. doi: 10.1534/g3.119.400186
7. Furlong EE, Levine M. Developmental enhancers and chromosome topology. Science. 2018; 361: 1341-5. doi: 10.1126/science.aau0320
8. Anene-Nzelu CG, Lee MC, Tan WL, Dashi A, Foo RS. Genomic enhancers in cardiac development and disease. Nature Reviews Cardiology. 2022; 19: 7-25. doi: 10.1038/s41569-021-00597-2
9. Visel A, Minovitsky S, Dubchak I, Pennacchio LA. VISTA Enhancer Browser—a database of tissue-specific human enhancers. Nucleic acids research. 2007; 35: D88-92. doi: 10.1093/nar/gkl822
10. Ihaka R, Gentleman R. R: a language for data analysis and graphics. Journal of computational and graphical statistics. 1996; 5: 299-314. doi: 10.2307/1390807
11. Zehra R, Abbasi AA. Homo sapiens-specific binding site variants within brain exclusive enhancers are subject to accelerated divergence across human population. Genome biology and evolution. 2018; 10: 956-66. doi: 10.1093/gbe/evy052
12. Kvon EZ. Using transgenic reporter assays to functionally characterize enhancers in animals. Genomics. 2015; 106: 185-92. doi: 10.1016/j.ygeno.2015.06.007
13. Hindorff LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, et al. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proceedings of the National Academy of Sciences. 2009; 106: 9362-7. doi: 10.1073/pnas.0903103106
14. Kothary R, Clapoff S, Darling S, Perry MD, Moran LA, Rossant J. Inducible expression of an hsp68-lacZ hybrid gene in transgenic mice. Development. 1989; 105: 707-14. doi: 10.1242/dev.105.4.707
15. Pennacchio LA, Ahituv N, Moses AM, Prabhakar S, Nobrega MA, Shoukry M, et al. In vivo enhancer analysis of human conserved noncoding sequences. Nature. 2006; 444: 499-502. doi: 10.1038/nature05295
16. Spitz F, Furlong EE. Transcription factors: from enhancer binding to developmental control. Nature reviews genetics. 2012; 13: 613-26. doi: 10.1038/nrg3207
17. Cao Y, Xia Y, Balowski JJ, Ou J, Song L, Safi A, et al. Identification of enhancer regulatory elements that direct epicardial gene expression during zebrafish heart regeneration. Development. 2022; 149: dev200133. doi: 10.1242/dev.200133
18. Heckmann J, Uwimpuhwe H, Ballo R, Kaur M, Bajic VB, Prince S. A functional SNP in the regulatory region of the decay-accelerating factor gene associates with extraocular muscle pareses in myasthenia gravis. Genes & Immunity. 2010; 11: 1-10. doi: 10.1038/gene.2009.61
19. Khatoon H, Raza RZ, Saleem S, Batool F, Arshad S, Abrar M,

et al. Evolutionary relevance of single nucleotide variants within the forebrain exclusive human accelerated enhancer regions. BMC Molecular and Cell Biology. 2023; 24: 1-16. doi: 10.1186/s12860-023-00474-5

20. Hussain I, Raza RZ, Ali S, Abrar M, Abbasi AA. Molecular signatures of selection on the human GLI3 associated central nervous system specific enhancers. Development Genes and Evolution. 202; 231: 21-32. doi: 10.1007/s00427-021-00672-1
21. Massari ME, Murre C. Helix-loop-helix proteins: regulators of transcription in eucaryotic organisms. Molecular and cellular biology. 2000; 20: 429-40. doi: 10.1128/MCB.20.2.429-440.2000
22. Hallier B, Hoffmann J, Roeder T, Tögel M, Meyer H, Paululat A. The bHLH transcription factor hand regulates the expression of genes critical to heart and muscle function in Drosophila melanogaster. PLoS One. 2015; 10: e0134204. doi: 10.1371/journal.pone.0134204

Authors Contribution

RZR: Idea conception, study designing, data collection, data analysis, results and interpretation, manuscript writing and proof reading

IH: Data analysis, results and interpretation, manuscript writing and proof reading

FA: Data collection, data analysis, results and interpretation, manuscript writing and proof reading

SM: Manuscript writing and proof reading