EXPLORING THE EXPRESSION OF TMEM132 PROTEINS

By

Leonardo Padilla

A capstone project submitted for Graduation with University Honors

June 12, 2023

University Honors

University of California, Riverside

APPROVED
Professor Martin Garcia-Castro
Department of Biomedical Sciences

Dr. Richard Cardullo, Howard H Hays Jr. Chair
University Honors

Abstract

Neural crest cells (NC) which are unique to vertebrates, appear early in development, migrate, and differentiate into a bewildering range of derivatives. NC malfunction leads to pathologies known as Neurocristopathies including birth defects and cancers. Studying NC will improve our understanding of these pathologies, and lead to better diagnostics and therapeutics.

Our laboratory identified the expression of a novel family of single transmembrane proteins, the TMEM132 (A-E) using a model of human NC formation. The TMEM132 family remains far from being fully characterized; the few manuscripts published link it to cytoskeleton dynamics, Wnt signaling pathway modulation, NT closure and specific cancers. However, to our knowledge, no study has addressed a possible role in NC development. Here we explored the expression of TMEM132 proteins in hNC and chick embryos finding a specific expression associated with NC development and late primitive streak (TMEM132A, TMEM132C). To assess their function we implemented knock-down approaches using Morpholino oligonucleotides that block the translation of their mRNAs. The TMEM132 MOs are electroporated into early chicken embryos at gastrula and neurula stages, and their effects are established after incubation using NC markers and fluorescence microscopy. Given the known functions of TMEM132 proteins and their specific expression during NC formation we hope to unveil a significant role, perhaps associated with epithelial to mesenchymal transition or NC migration.

Acknowledgements

I would like to thank Professor Martin Garcia-Castro for his invaluable mentorship, patience, and enthusiasm that helped me complete this Honors Capstone Project. I would also like to thank Graduate Student Julio Tapia for his selflessness, taking time from his own research to assist me and my fellow undergraduates.

As an undergraduate this was my first experience conducting an independent research project and my time in the Garcia-Castro lab has only fueled me to continue to pursue my passions in healthcare. I aspire to one day be part of a team that works as diligently and cooperatively as the one I had the privilege of working with during this project.

Table of Contents

Introduction

Gastrulation is the process that involves the differentiation of early blastula cells in a developing embryo into the three germ layers, namely ectoderm, mesoderm, and endoderm. As the three germ layers are generated, the ectoderm itself can differentiate into two distinct domains: the non-neural or surface ectoderm and the neural ectoderm. The surface ectoderm will eventually give rise to, skin, and dermis, while the neural ectoderm will form the neurons and glia of the central nervous system (brain and spinal cord). The neural ectoderm, also known as the neural plate or neuroepithelium, extends almost the entire length of the vertebrate axis, from the head to the end of the trunk. During neurulation, the left and right halves of the neural plate elevate and fuse to form a neural tube. Neural Crest cells (NCC) appear at the junction of the neural plate with the surface ectoderm, a region known as the neural plate border. NCCs are involved in the formation of many important structures such as the craniofacial skeleton, the peripheral nervous system, and pigment cells. In fact, the evolution and diversity of vertebrates have been guided by NCCs as they are central to their predatory lifestyle by contributing to the jaw, denture, and cranium amongst other systems. Furthermore, abnormalities in the development or function of human NCCs can lead to a range of pathologies, broadly known as neurocristopathologies, which include rare syndromes, craniofacial birth defects, and aggressive cancers. These include but are not limited to Treacher Collins syndrome (cleft palate), Waardenburg syndrome, and Hirschsprung disease (Charney, et al. 2017).

NCCs are thought to arise via a classic induction mechanism where a cell or tissue signals a receptive cell or tissue which then responds to the signal by a change. Specifically, it has been proposed that the interaction of Neural ectoderm with non-neural ectoderm, or of Mesoderm with

overlaying ectoderm, leads to NCC formation. Several signaling pathways have been involved in these tissue interactions, and WNT, FGF, and BMP, among others, are considered to play a key role in early NCC formation. Later on, NCCs undergo an epithelial-to-mesenchymal transition (EMT) to depart from the dorsal neural tube, prior to their migration and differentiation (Charney, et al. 2017). EMT involves detachment from neighboring cells through the loss of cell-cell adhesion, and the acquisition of motility and invasive properties, and is used during development in different contexts, and is also prominent in cancer biology. A better understanding of the molecular mechanisms underlying these processes could provide broad insights into many other physiological and pathological contexts. While recent studies have made leaps in understanding the gene regulatory network of NCCs, the mechanical protagonists and their specific functions remain a largely unexplored frontier.

The study of NCCs in Gallus gallus (domestic fowl) has been particularly useful in understanding their development and function, due to the ease of experimental manipulation and the ability to observe their development directly. NCCs development in Gallus gallus and humans are remarkably similar, and given the availability and simplicity of the chick model, studies on NC in chick embryos have played a prominent role in NC biology.

Overall, NCCs are essential for the proper development of both humans and chickens, and the similarities in their roles in these organisms highlight the importance of understanding the molecular mechanisms that underlie their development and function. Understanding the developmental processes that govern NCC differentiation and migration, therefore, has the potential to aid in the development of these therapies and help improve patient outcomes.

In summary, studying the development of NCCs can provide insights into the broader process of embryonic development and the interplay between different cell types and signaling pathways. The knowledge gained from this research can inform our understanding of how other organs and tissues develop and function, and provide a foundation for future research aimed at understanding the underlying mechanisms of human biology and disease.

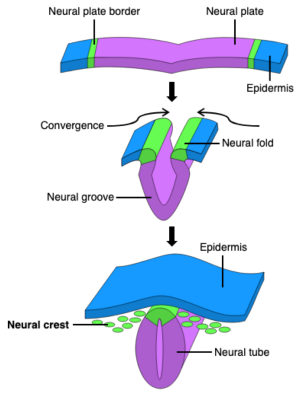

Figure 1. This image displays the development of neural crest cells and how their influence on the neural folds results in the closing of the neural tube. As development progresses the emergence of additional NCCs like Snail2 and Sox9 will become more prevalent.

Literature Review

The TMEM (transmembrane domain-containing proteins) family consists of a diverse group of proteins that span the cellular membrane at least once. These proteins play critical roles in various cellular processes, such as signal transduction, ion transport, cell adhesion, and metabolism regulation. This family was first identified in 2002, and since then, only a handful of studies have been published on the function and properties of its members. One such member,

TMEM132A is a 560 amino acid protein encoded by a gene mapping to chromosome 11. Responsible for nearly 4% of the human genomic DNA, chromosome 11 is strongly associated with the development of disease.

Confocal microscopy has shown that TMEM132A colocalizes with the ER plasma membrane, in pseudopodium and cell-cell junctions, and in some vesicle-like organelles. (Li, et al. 2020) The study also revealed that TMEM132A likely functions as a dimer or oligomer and that interaction occurs through the N-terminal domain. Co-immunoprecipitation (co-IP) experiments revealed self-association of differentially tagged FL TMEM132A constructs expressed in HEK 293T cells. Deletion of the N-terminus, but not the C-terminus, abolishes the TMEM132A self-interaction property. This finding suggests that TMEM132A likely forms homodimers or oligomers, like many other transmembrane proteins such as cell adhesion molecules and signal receptors.

An additional study examining the knockdown of the TMEM132 family used bioinformatics analysis to investigate the role of TMEM132A in gastric cancer (GC). The study found that TMEM132A expression was upregulated in GC tissues and cell lines, and had diagnostic and prognostic values in GC. Knockdown of TMEM132A inhibited cell proliferation, migration, invasion, and tumorigenesis of GC cells, at least partially, via inhibiting Wnt signaling, while overexpression of TMEM132A exerted the opposite effects. Subsequently, the Wnt signaling pathway was found to be dysregulated in approximately 50% of GC patients, thus “offering a novel therapeutic target for GC patients with poor outcomes.”(Gao, et al. 2022) The study found that TMEM132A had the potential to interact with the Wnt ligand transporting protein Wntless

(WLS), inducing the activation of the Wnt signaling pathway. Asymmetric dimethylarginine mediates GC cell migration and invasion by regulating the activation of Wnt/β-catenin signaling. The role of TMEM132A in GC is mediated, at least partially, via Wnt signaling, as signified by their conclusion, “Our results showed that TMEM132A expression was upregulated in GC tissues and cell lines... knockdown of TMEM132A restrained cell proliferation, migration, invasion, and tumorigenesis of GC cells, at least partially, via inhibiting Wnt signaling, while overexpression of TMEM132A exerted the opposite effects," the study reported.”(Gao, et al. 2022)

The formation, migration, and differentiation of NC are dependent on critical roles played by the signaling pathways of Wnt, BMP, and FGF. In the late gastrula, the formation of the neural plate border (NPB) requires the activation of Wnt and FGF signaling, as reported in several studies (Charney, et al. 2017). Pax3 and Pax7, expressed at the neural plate border (NPB) in most vertebrates, have been extensively studied. In chicks, Pax7 appears to be the earliest marker of the NC and is critical for its formation.(Basch, et al. 2006). Additionally, studies have examined the role of FGF signaling in activating Pax7 expression.(Charney, et al. 2017). A study on gene expression patterns explores the spatiotemporal distribution of the TMEM132 family of genes in mice embryos. The study found that Tmem132A was widely expressed in embryos before neural tube closure, and its absence or low expression was associated with neural tube closure defects in a KO mouse strain. Tmem132A was expressed in brain regions associated with emotional responses, including the cortex, hippocampus, and thalamus. The study suggests potential roles of the TMEM132 family genes in other aspects of nervous system function, including the cranial and spinal ganglia, and the cerebellar granule layer. The study also highlights the need for further

direct functional testing of these genes to fully understand their roles. The study concludes by acknowledging the limitations of current online databases in terms of stage selection, angle selection, annotation, and missing genes. One of the authors of the study notes, "Our gene expression analyses provide important information regarding the potential functions of the TMEM132 family members that may explain the previously observed association with human diseases and/or genetic data in model animals."(Wang, et al. 2022)

Methodology

Therefore, in an effort to better characterize and explore the TMEM protein family, I decided to examine the effects that TMEM132A had on early-stage embryo development. Electroporation delivery is the method I used in my experiments. Electroporation involves briefly applying an electric field to cells to create temporary pores in their cell membranes, allowing foreign DNA or other nucleic acids to enter the cells. Once inside the cells, these reagents enable overexpression or knockdown of specific genes of interest, helping to identify features of gene function, interactions, and regulation.

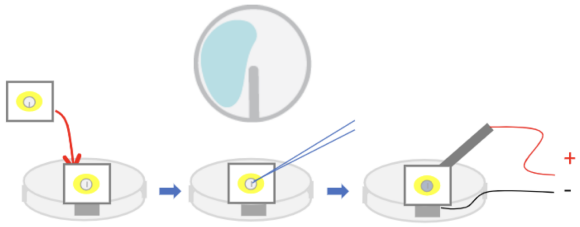

Figure 2. The process of electroporation

Through electroporation of morpholinos targeting the TMEM132A gene, researchers can downregulate the translation of TMEM132 mRNA leading to a reduced protein. This in turn may have effects during embryonic development for example, alterations in migration, proliferation, and/or differentiation. Morpholinos have been shown to be more effective and target-specific in the transient knockdown of genes in the chick embryo when compared to RNA constructs.
By manipulating the expression of TMEM132A through MOs, researchers may be able to gain insights into the mechanisms that underlie neural crest cell development, as well as potential implications for human disease.

Morpholinos are designed to bind to specific sequences of messenger RNA (mRNA) and prevent their translation, leading to the knockdown of the corresponding gene, enabling the interrogation of their function under a reduced or diminished presence of the targeted molecule.
Electroporation is a well-established technique, traditionally used to introduce nucleic acids into cells taking advantage of their electrical charge or polarity. However, electroporation also triggers membrane depolarization that leads to small vesicle intake of foreign materials into the cells regardless of their charge. MOs, which are not strongly charged use precisely this route to enter cells after electroporation. Researchers often mix MOs with plasmids which can then aid the MO intake. By designing a morpholino that targets the mRNA of the TMEM132A gene, we aim to observe if reduced TMEM132 protein leads to changes in neural crest cell biology, including their molecular makeup, EMT, migration, and/or differentiation. Importantly, this Morpholino strategy allows us to study gene function in vivo, as they are able to penetrate the tissues of developing chick embryos and affect gene expression. Additionally,

fluorescently-tagged morpholinos allow immediate, real-time can be used to visualization of the targeted or treated cells.

Materials and Methods

The Materials and Methods section of this research paper describes the experimental design used to investigate the role of TMEM132A in neural crest cell development. Poly-clonal Antibodies and morpholinos were employed in this study, including the anti-TMEM132A and anti-Pax7 antibodies. In order to investigate the roles of PAX7 and TMEM132A, FITC-labeled morpholino oligonucleotides were obtained from Gene Tools, LLC. The Pax7 antibody was a cPax7 5'UTR antisense morpholino oligonucleotide. Utilizing the National Center for Biotechnology Information (NCBI) website, I was able to identify a TMEM132A isoform precursor (Gallus Gallus). This also required the generation of primers for TMEM132A, which consisted of the following, (CCTCTCTGCATGCCTGTTCT) as a forward primer, and (TCTTGGCCTTGACTCGCAG) as a reverse primer. It was stored at -20 degrees Celsius. Fertilized chicken eggs of the Gallus Gallus species were incubated for varying intervals depending on how developed I wanted them to be. In most instances, they were incubated for nearly 30 hours at 100 degrees Fahrenheit.

Once the embryos were finished developing, I used tools including forceps and scissors to carefully extract the embryo from the yolk of the egg. In order to accomplish this, I used a cutout of a hole-punched paper to encircle the embryo within the yolk. Then using scissors I would cut the embryo out of the yolk and deposit it, while attached to the paper cutout, into a sterile petri dish filled with PBS.

Electroporation was then used to introduce the Morpholinos into the cells. The Morpholinos were loaded into a glass capillary needle, which was then inserted through the window and positioned above the neural plate. Five pulses of 5V, 30ms duration was delivered using an electroporator. Afterward, the embryos were placed into individual mini dishes, in which they were enveloped with their own albumen having been previously removed during their extraction. After incubation for an additional 24hrs, the embryos would be ready for fixing. Embryos were fixed in 4% paraformaldehyde, rinsed, washed, and either processed for immunofluorescence immediately or after cryopreservation through sucrose and gelatin treatment.
Embryos are blocked with Serum for 60m min prior to immunostaining with PAX7 and TMEM132A antibodies.

The process for Immunostaining is as follows:

1. Fix cells in freshly prepared 4% PFA for 10 minutes at room temperature
    - 4% PFA = 9mL PBS + 1mL 37% formaldehyde
2. Was 3X in PBS using Transfer Pipette
    - Pipette on side wall to prevent displacement of cells at the bottom of the well
    - For 24-well plate, use 200uL/well
3. Permeabilize cells with PBS containing 0.4% Triton X-100 for 10 min at room temperature
4. Wash 3X PBS + 0.05% Tween
    - Place on bench. Do not nutate
5. Incubate in blocking buffer (PBS + 0.05% Tween-20 + 10% HI FBS) for one hour
6. Incubate with primary antibodies in blocking buffer at 4 degrees celsius overnight
    - Spin down primary antibodies to reduce large aggregates
    - Retain primary antibody after use and mark number of uses on tube

- Primary antibody can be reused multiple time

8. Wash 3 X 20 minutes with PBS + 0.05% Tween

9. Incubate with secondary antibodies in blocking buffer for 45 minutes to 1 hour at room temperature covered.

- Spin down primary antibodies to reduce large aggregate
- Plate should be covered for all steps moving forward
- Secondary antibody is light sensitive
- Retain secondary antibody after use

10. Rinse briefly with 0.05% Tween in PBS

11. Counterstain with DAPI (10000 X dilution, Molecular Probe) for 5 minutes at room temperature (1:000) in PBS

12. Wash 3X5 minutes in 0.05% Tween-20 in PBS

13. Image within two days of staining

Once staining is complete, the samples are washed to remove any unbound antibodies and then incubated with fluorescently labeled secondary antibodies that recognize and bind to the primary antibodies. These secondary antibodies are conjugated to fluorophores that emit light at specific wavelengths when excited by light of a specific wavelength. Once the secondary antibodies have bound to the primary antibodies, the samples are again washed to remove any unbound antibodies and then imaged using a fluorescence microscope. When securing my embryos onto observation slides, I used DAPI floruomount, a mounting medium used in immunofluorescence experiments to preserve and protect the fluorescence signal of stained cells. It contains DAPI (4',6-diamidino-2-phenylindole), a fluorescent stain that binds to the minor groove of DNA, allowing visualization of the cell nucleus. This process allows for the visualization of the distribution and expression patterns of Pax7 and TMEM132A within the developing neural folds of the chicken embryo with high spatial resolution.

Analysis of Results

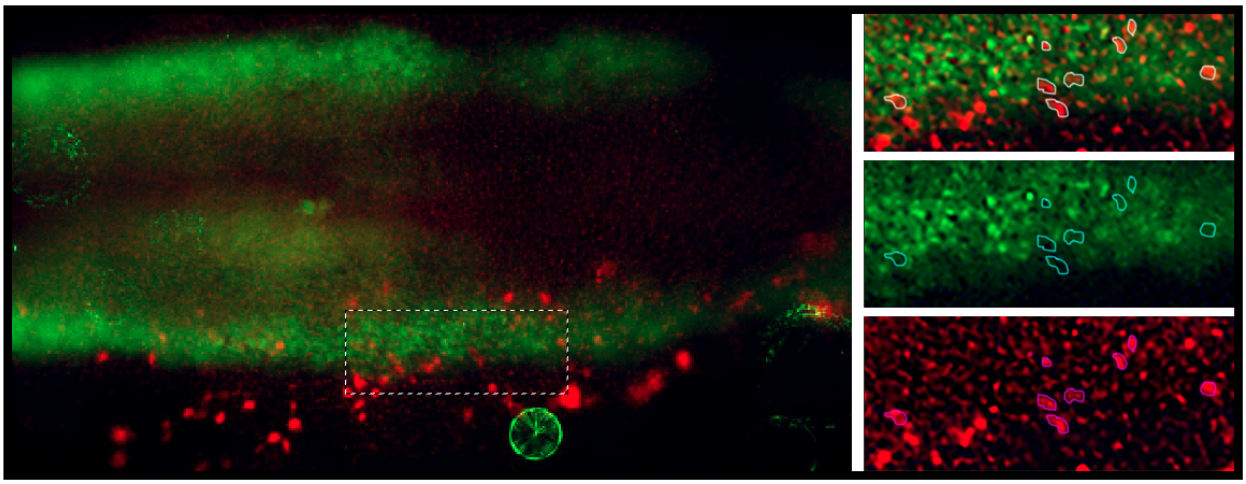

Figure 3. Displays Gallus Gallus embryo with Anterior(left) and Posterior(right) positioning as well as PAX7(green) and anti-TMEM132A-morpholino (red) expression. Dashed rectangular area on the targeted neural fold is shown in the additional panels, a few cells encircled in white, showing strong morpholino signal surrounded by multiple Pax7+ cells (top) are noted on the top merged image, and corresponding Pax7 only (middle) and morpholino only (bottom) panels allow easier identification of low to no Pax7 signal in those high morpholino signal displaying cells.

This image displays an embryo with the anterior facing left and the posterior facing right. This image visualizes two distinct protagonists within genetic expression. Primarily exhibited, PAX7 can be easily observed on the neural folds of the developing embryo in green. As evidence of true PAX7 expression, precise cells can be seen in the focused fields of the image, clearly displaying sole expression of PAX7 in green. Conversely, the red instances in the image signify the expression of the TMEM132A morpholino inserted into the embryo using the method of

electroporation. Upon examination of the complimentary images, the precise identification of cells and their expression of either Pax7 or morpholino is visible.

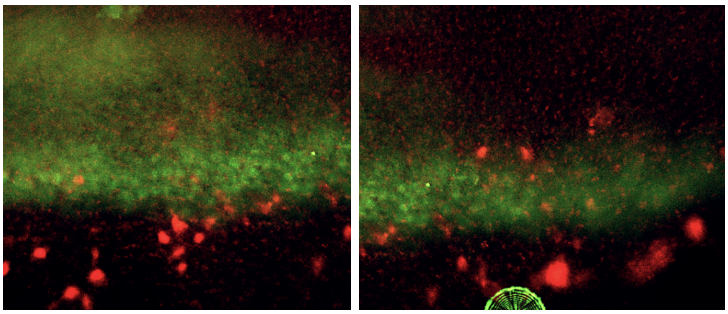

Exhibit A. & B. of Fig.3 - Both display cells with singular expression of either morpholino (red) or Pax7(green)

Purposefully, the morpholino was only introduced to the left side of the embryo, in order to gauge the validity of its effects and spread. Though this, the right side of the embryo acted as a successful control, displaying expression of Pax7, but not morpholino. Reinforcing the expectation that morpholino would only be expressed on a single side of the embryo, this image shows no expression of the TMEM132A morpholino on the right side of the embryo.

The main conclusion from the analysis of this image pertains to whether or not the TMEM132A morpholino is having a genuine and effective impact on the expression of PAX7. As a consequence of the embryo not being in full and complete focus, the right and left sides of the embryo cannot be compared in relevance to the expression of PAX7. This means that where the TMEM132A morpholino appears to have an influence on the expression of PAX7 along the periphery of the embryo, this cannot be confirmed without additional testing. However, what can

be established is that while PAX7 and the morpholino are expressed in a very confined area, their placement is exclusive, providing support for the claim that the two are expressed independently.

Focused analysis on TMEM132A-MO-targeted Neural fold cells

Upon further analysis of the image displayed in Fig.3, there is exclusivity in the observable expression between the morpholino (red) and Pax7 proteins (green). Under exhibit A. the morpholino is clearly present in separate cells than Pax7. Especially within the aforementioned region, the morpholino appears to be expressed in cells where PAX7 would be expected to be expressed. Similarly, in exhibit B. as we travel along the neural folds, there are regions that seem to display proportionately higher expression of the morpholino. While the expression of Pax7 is clearly visible on both neural folds, morpholino is only seen in the previously electroporated region.

Requirement of TMEM132 for avian NC development

As shown in Fig. 3, the electroporated side of the embryo (red signal) displays a clearly reduced Pax7 signal (green), a pattern I found in thirteen embryos. Specifically, I observed that cells with strong morpholino signal displayed little to no Pax7 signal, therefore differentiating the two from each other. While promising, these studies could be further improved in several regards. A key element missing from this experiment is the proper determination of the effectiveness of the anti-TMEM132A MOs. This could easily be achieved by performing immunostaining against TMEM132A in embryos in which one half of the embryo is electroporated and the other remains as a control. We would expect that the treated side displays a clearly reduced expression of TMEM132A. Another important missing aspect in these experiments is the evaluation of

simultaneous controls, in which embryos are electroporated with a control MO, (a mismatch, or a known irrelevant target), and processed under identical conditions (immunostaining and imaging).

To better characterize the effects of the MOs, it would be ideal to perform further analysis after sectioning the embryos to indisputably establish the tissue layers treated and affected, as well as the presence of the morpholinos in the affected cells. While the expression of PAX7 was clear and was expressed in an expected manner, the same cannot be said for the expression of the morpholino. With a lack of large amounts of external data to cross reference the results of this experiment, its internal validity will be held to a higher level of scrutiny. In summary, this data has identified the localization of the TMEM132A morpholino, after electroporation, in the open neural plate.

Future Directions

Through my experiments, I attempted the extraction of three hundred and eighty seven individual eggs. Through the trials of improper development, complications with extraction, and other extenuating circumstances, I was able to successfully incubate a total of two hundred and fifty eight embryos. I successfully electroporated seventy embryos from stages four through twelve. From all of these efforts, I recovered thirty-five embryos at approximately stage eight. It is relevant to mention that depending on multiple factors like how long they had been sitting, as well as the conditions in which they were incubated, embryos appear to progress variably in their development. From the successfully electroporated individual embryos that survived being

extracted, fixed, and stained, a total of twenty-two embryos were further analyzed for NC development by analyzing the expression of Pax7 in conjunction with the morpholino.

To increase the likelihood of obtaining more successful results in future studies investigating the role of TMEM132A in neural crest development, several additional methods could be employed. For instance, the electroporation of early chick embryos could be carried out at different developmental stages to determine if TMEM132A plays a role at different points in neural crest development. The variety in this experimentation may yield greater diversity in results, which would be useful for internal comparison. Additionally, with other methods of gene knockdown or overexpression available, such as CRISPR/Cas9 or viral transduction, efforts to corroborate the findings obtained with Morpholino knockdown would be better investigated.

To ensure better sterility and reduce the possibility of contamination, more rigorous cleaning protocols and the use of sterile tools and equipment could be implemented. Now having acquired the skills to properly clean lab benches, glassware, and the containers in which I incubate my embryos, I am certain that continued research will be conducted more efficiently. Furthermore, in situ hybridization could be performed to validate the results obtained with immunostaining for TMEM132A. Critical controls, such as using scrambled Morpholino sequences or analyzing the effect of TMEM132A knockdown on other cell types, should also be included in all experiments to ensure the specificity of the observed effects.

Conclusion

In this research project, I attempted to visualize the expression of TMEM132A as a potential indicator of its role in neural crest (NC) development. The project utilized early development chick embryos that were incubated, electroplated, and immunostained for Pax7. After fluorescent imaging, the results showed that few cells with strong TMEM132A-MO signal (red) appeared to display no or strongly reduced Pax7 signal, supporting the hypothesis that TMEM132A plays a critical role in NC development.

Further investigation through sectioning will be carried out to definitively determine if this is the case. Immunostaining for TMEM132A is also being analyzed in electroporation with the stronger and more targeted deployment of the Morpholino oligonucleotides (MOs). The identification of TMEM132A as a potential player in NC development is significant, as abnormalities in NC development can lead to a wide range of pathologies, including neurocristopathies and various craniofacial anomalies. Understanding the role of TMEM132A in NC development could provide valuable insights into the causes of these disorders and help develop potential therapies for their treatment.

Furthermore, the project's findings have the potential to contribute to the broader understanding of NC development and embryonic development as a whole. Research behind the mechanisms of Neural Crest Cells and the TMEM protein family are still in early stages of discovery, leaving room for further exploration. From Wnt signaling pathways, to cell differentiation, the processes and mechanisms involved in NC development are complex and involve the interplay of various cell types and protagonists. Studying the role of TMEM132A in NC development could provide

insights into these processes and help inform future research aimed at understanding the underlying mechanisms of human biology and disease.

Finally, it may be beneficial to explore the role of other genes or signaling pathways that are known to be involved in neural crest development and determine if they interact with or regulate TMEM132A. This could involve the use of RNA sequencing or ChIP sequencing to identify potential interacting partners of TMEM132A and illuminate the underlying molecular mechanisms of its role in neural crest development. By utilizing these methods and strategies, a more comprehensive understanding of the function of TMEM132A in neural crest development can be obtained.

In conclusion, further studies, including critical controls and analysis of immunostaining and Morpholino deployment are needed to establish if TMEM132A plays a role in NC development. The project's findings have significant implications for understanding NC development, neurocristopathies, and broader embryonic development processes.

REFERENCES

Britsch, S., Goerich, D. E., Riethmacher, D., Peirano, R. I., Rossner, M., Nave, K. A., ... & Birchmeier, C. "The Transcription Factor Sox10 Is a Key Regulator of Peripheral Glial Development." Genes & Development, vol. 15, no. 1, 2001, pp. 66-78.

Luo, T., Lee, Y. H., Saint-Jeannet, J. P., & Sargent, T. D. "Induction of Neural Crest in Xenopus by Transcription Factor AP2α." Proceedings of the National Academy of Sciences, vol. 100, no. 2, 2003, pp. 532-537.

Hu, D., Marcucio, R. S., & Helms, J. A. "A Zone of Frontonasal Ectoderm Regulates Patterning and Growth in the Face." Developmental Biology, vol. 253, no. 2, 2003, pp. 282-290.

Jiang, R., Bush, J. O., & Lidral, A. C. "Development of the Upper Lip: Morphogenetic and Molecular Mechanisms." Developmental Dynamics, vol. 235, no. 5, 2006, pp. 1152-1166.

Li, Binbin, and Lee A. Niswander. "TMEM132A, a Novel Wnt Signaling Pathway Regulator through Wntless (WLS) Interaction." Frontiers, 4 Nov. 2020, www.frontiersin.org/articles/10.3389/fcell.2020.599890/full.

Van Keymeulen, A., Mascre, G., Youseff, K. K., et al. "Epidermal Progenitors Give Rise to Merkel Cells during Embryonic Development and Adult Homeostasis." Journal of Cell Biology, vol. 187, no. 1, 2009, pp. 91-100.

Sauka-Spengler, T., & Bronner-Fraser, M. "Development and Evolution of the Migratory Neural Crest: A Gene Regulatory Perspective." Current Opinion in Genetics & Development, vol. 13, no. 4, 2006, pp. 360-366.

Mallory, S. B., Wiener, E., & Nordlund, J. J. "Waardenburg's Syndrome with Hirschsprung's Disease: A Neural Crest Defect." Pediatric Dermatology, vol. 3, no. 2, 1986, pp. 119-124.

Mossner, R., Schuhmacher, A., Wagner, M., Quednow, B. B., Frommann, I., Kuhn, K. U., Maier, W., & Rietschel, M. "Replication and Meta-Analysis of TMEM132D Gene Variants in Panic Disorder." Translational Psychiatry, vol. 1, no. 9, 2011, pp. e41-e41. doi:10.1038/tp.2011.42

Gao, Q., Chen, Y., Yue, L., Li, Z., & Wang, M. "Knockdown of TMEM132A Restrains the Malignant Phenotype of Gastric Cancer Cells via Inhibiting Wnt Signaling." Nucleosides, Nucleotides & Nucleic Acids

Basch, M. L., Bronner-Fraser, M., & García-Castro, M. I. "Specification of the Neural Crest Occurs During Gastrulation and Requires Pax7." Nature, vol. 441, no. 7090, 2006, pp. 218-222. doi:10.1038/nature04684

Charney, R. M., Prasad, M. S., & García-Castro, M. I. "Current Insights into Neural Crest Cell Development and Pathologies." Birth Defects Research, vol. 109, no. 18, 2017, pp. 890-905. doi:10.1002/bdr2.1086

Wang, Y., Herzig, G., Molano, C., & Liu, A. "Differential Expression of the Tmem132 Family Genes in the Developing Mouse Nervous System." Gene Expression Patterns, vol. 45, Sep. 2022, p. 119257. doi: 10.1016/j.gep.2022.119257. PMID: 35690356.