TMEM132C: EXPRESSION AND FUNCTION IN THE DEVELOPMENT AND MIGRATION OF NEURAL CREST CELLS

By

Cynthia Thienan Nguyen

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APPROVED

Dr. Martin I. Garcia-Castro

Biomedical Sciences

Dr. Richard Cardullo, Howard H Hays Jr. Chair

University Honors

ABSTRACT

Neural crest (NC) cells are embryonic cells unique to vertebrates that emigrate from the dorsal aspect of the neural tube and migrate throughout the embryo contributing to a wide range of organs and systems. NC derivatives include neurons and glia of the peripheral nervous system, melanocytes of the skin, craniofacial bone, cartilage, adipocytes, and odontoblasts amongst many others. Deviant NC cell development or deficient homeostasis can lead to many pathologies globally known as neurocristopathies, which include common birth defects, rare syndromes, and cancers. Migration of neural crest cells from the neural tube is preceded by an epithelial to mesenchymal transition (EMT), in which NC detaches from neighbors, enabling their emigration from the neural tube. Our laboratory recently identified the gene TMEM132C as a putative candidate factor involved in NC development using a human model. Further studies characterized the expression of TMEM132C in chick embryos and identified its specific expression in neural folds, where prospective NC reside and are coexpressed with Pax7, an early marker and essential transcription factor of NC development. TMEM132C belongs to the novel TMEM132 family of genes that have been associated with a number of diseases. The general TMEM132 structure consists of 5 domains with evolutionary conserved motifs associated with the control of actin cytoskeletal dynamics, that have been associated with WNT modulation, providing a link between signaling and mechanical forces at play during EMT and NC migration. I aim to, for the first time, assess the function of TMEM132C during neural crest development. To do so, I will perform loss of function studies through morpholino antisense oligonucleotides. These studies will reveal if this promising and under characterized novel candidate contributes to NC development, and may shed light on its molecular participation in cancer, opening paths for

further research to better understand if and how it could contribute to EMT and/or migration of NC cells, and beyond.

ACKNOWLEDGMENTS

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INTRODUCTION

Neural crest (NC) cells are specialized embryonic cells that are distinctive to vertebrates. They move from the dorsal neural tube to various parts of the embryo, where they differentiate into terminal derivatives and contribute to multiple tissues and organs. Depending on where the NC cells reside, they can differentiate into different cell types. For example, cranial NC cells can generate craniofacial cartilage, parasympathetic and sensory ganglia, adipocytes, etc. , but in more caudal territories like the trunk NC only generate neurons and glia of the peripheral nervous system and melanocytes, but no bone or cartilage (Charney, 2021). Deviants in NC development or failure to maintain homeostasis can result in a number of defects (i.e. Cleft palate) and diseases (i.e. Melanoma). A number of different signaling pathways such as Wnt, bone morphogenetic protein signaling, and fibroblast growth factor are responsible for the formation of NC cells. At the neural plate border, many transcription factors are expressed that would initiate the type of cell the NC cell would differentiate into. Therefore, transcription factors play a role in the migration and distinction of NC cells. The transcription factor Pax 7, one of the earliest markers, is activated by signaling pathways including FGF and WNT.

Pax 7 is expressed as early as HH4+ and is an important NC specifier. Its expression is critical for the acquisition of later markers of more mature neural crest stages like Sox9, Sox10 and others. Furthermore, during gastrulation, prior to overt expression of Pax7, distinct regions of the embryo are already poised to launch Pax7 expression and NC development (Basch, 2006).

To migrate and differentiate, NC cells must undergo an epithelial-to-mesenchymal transition (EMT). This is when epithelial cells go through different biochemical pathways to become a mesenchymal cell. Mesenchymal cells are more resistant to apoptosis, can migrate, create more extracellular matrices (ECM), and have the ability to invade. All important aspects

for a differentiating NC cell. Many different pathways activate the EMT such as transcription factors, cell-surface proteins, cytoskeletal proteins, ECM-degrading enzymes, and microRNAs (Kalluri, 2009). Once crest cells have undergone EMT, they detach from neighboring cells in the neural fold and can initiate their stereotyped migration throughout the body.

In the Martin I. Garcia-Castro lab, a novel gene called TMEM132C was identified in a transcriptomic screen in a model of human Neural Crest formation, and its expression was further confirmed both in the human model and in chick embryos using in situ hybridization. TMEM132C is a part of the TMEM132 family, known to cause many diseases and mutations such as cancer, panic disorder, and non-syndromic hearing loss (Sanchez-Pulido, 2017). The general structure consists of five general conserved domains: cohesin domain, 3 bacterial immunoglobulin-like domains (BIG-1, BIG-2, BIG-3), and a transmembrane region. The BIG domains can be found in bacteria, archaea, and eukaryotes. These domains are organized into a beta-sandwich fold, composed of nine strands. These nine strands are further organized into three beta sheets, two of which form the core of the domain. In general, these domains have functions that are associated with the cytoskeleton and their extracellular domains could simultaneously link to other cells, through the extracellular matrix or other protein-protein interactions in bacteria. It has been recently reported that TMEM132A is a regulator of the Wnt signaling pathway. Without TMEM132A, there is a decline in Wnt signaling and therefore a decline in adhesion (Li et. al, 2020). The cohesin domain can be found in animals and more diverse eukaryotes. This domain binds dockerin domains in cellulolytic bacteria, forming one of the strongest Cellulosome complexes, where cellulose is broken down (Sanchez-Pulido, 2017). However, dockerin domains have not been identified in vertebrates, and no cellulose

degradation seems necessary. Here we explore the possible participation of TMEM132C in neural crest development using the chick embryo as a model.

MATERIALS AND METHODS

Embryo Extraction

The eggs acquired from Chino Farms or AA were incubated for 18-19 hours to obtain stage HH 3-4. Once they have grown to this stage, extraction of the embryos took place. The materials required included: forceps, tweezers, scissors, cut filter paper, 2 petri dishes, 1x ringers (pH 7), kim wipes, and 5 mL syringe fitted with an 18 gauge needle. All tools and materials must be sterile before beginning. Wash with DI water then spray with 70% ethanol. Disinfect surfaces with 70% ethanol. Before extracting the avian embryo, be sure to gently shake the egg to ensure that the embryo sits at the top of the yolk. Fill the petri dishes with ringers until the entire bottom is covered. With the back of the forceps, gently tap the side of the egg near the top. Once cracks form, slip the tongs inside the crack and peel away the shell. Let the thick albumen leak out of the egg but be careful not to puncture the yolk. If you need to, use the forceps to pull the thick albumen out. Remove the shell until only half of the shell remains. Using your needle, carefully remove 5 mL of the thin albumen and set aside. Do not touch the yolk. Dump out the remaining thin albumen until only the yolk remains. Remove the shell until there is just enough to hold the yolk. With a Kim wipe, carefully blot the area around the embryo until it is no longer glossy. This will ensure that the membrane sticks to the filter paper. With the tweezers, place the filter paper over the embryo, ensuring that the embryo is within the cut out circle of the embryo. With small scissors, cut the membrane around the filter paper. Be sure to cut all the corners. Using the tweezers, lift the embryo out of the egg and place in a ringers filled petri dish dorsal side up. Do not flip the embryo. This will allow the excess yolk cells to fall off of the embryo. Repeat with the other eggs until the first petri dish is full. In the second petri dish, transfer the embryos ventral side up. Be sure there are little to no yolk cells attached to the embryo.

Electroporation Preparation and Setup

Two different morpholinos were used in this experiment: TMEM132C 20 and TMEM132C 24. Aliquot the morpholino into 2 µL into 1.5 mL eppendorf tubes. Before first use, add another 2 µL of molecular water to dilute it. Disinfect the lab bench with 70% ethanol. Tape a 15 mL tube to the top back of the microscope. This will hold your air injection tool. Attach the air injection tool to a clear, plastic pipe. Attach the pipe to the air valve. To make the glass needle, obtain a 0.1-1.1*100mm borosilicate glass capillary rod. Insert into the PC-10 manual puller. Be sure the rod is attached securely and the middle of the rod is behind the heating element. Turn it on and press the red button to heat the glass rod. It should break the rod into two pieces, each with a sharp side and a blunt side. Store the other half in a box. Load 1-2 µL of morpholino into the needle using a P20 micropipette and 20µL microloader. There should be a small neon pink dot at the tip of the needle. Using a Kim wipe, gently wipe the tip of the needle to open the needle. You should see the solution move down or there should be a small amount of morpholino on the Kim wipe. Attach the glass needle to the air injection tool and screw on tightly. Prepare the electroporator. The voltage should be 5.4 V and should be applied for 50 ms and 5 pulses. Clean the probe and be sure the tip is at about 225 degree angle. Clean the electroporation plates with DI water and spray with 70% ethanol. Wipe dry with a Kim wipe. Place the electroporation plate under the microscope and attach the wire clamp to the metal electrode on the plate. Turn the electroporator on. Fill the well with 1x Ringers and slightly break the surface of the ringers with the probe. Press the petal while looking under the microscope. You should be able to see small bubbles between the platinum sheet at the bottom of the well and the probe. To make the culture chamber, obtain a small tupperware and rinse with

DI water. Spray with 70% ethanol. Wipe dry with a paper towel. Using a folded, sterile paper towel, rip it in half and place it in an even layer on the bottom of the tupperware. Drip a small amount of DI water onto the paper towels until slightly damp. Label the lid with name, date, and time when put into the incubator.

Electroporation

Making sure the embryo is ventral side up, place the embryo into the electroporation well. The platinum sheet should be directly under the embryo and about 4mm away. Using the glass needle, inject the embryo between the vitelline membrane and the embryo. At this stage, the morpholino should be injected near the left side behind the anterior portion of the embryo. Turn on the air until you can hear a slight whistling noise. To inject, plug one of the valves of the air injection tool and slowly inject into the embryo until there is a cloud of pink. As quickly as possible, run an electrical current through the embryo by just breaking the surface of the ringers with the probe. Like the platinum sheet, the probe should be about 4 mm away from the embryo. Press the petal to apply the electrical current. Let the embryo sit undisturbed for about 1 minute. Using the syringe from extraction, place a small bead of thin albumen on the center of the culture plate. Place 4 smaller beads around the perimeter of the lid. Place the electroporated embryo into the plate, on top of the bead of thin albumen. Place the cover on and screw it on back and forth to ensure a tight seal. Place the plate inside the culture chamber and close the lid to ensure a tight seal. Place in the incubator when finished. Leave the embryos in the incubator overnight for about 24 hours.

Fix and Trim Embryos

Once the embryos have been culturing for about 24 hours, remove the culture chamber from the incubator. Using a microscope, check if the embryos have developed past HH3-4. If they have developed further, you can fix the embryos. To fix, use 100% paraformaldehyde (PFA) and 1x PBS to make a dilution of 4% PFA. Perform this in the fume hood. Remove the embryos from the culture dishes and move them to a sterile petri dish. Be sure each embryo is laying completely flat and is not touching another embryo. In the fume hood, cover the embryos completely with the PFA solution. Put the lid back on the petri dish and cover with foil. This will ensure no fluorescence degrades. Allow the embryos to fix for an hour. After an hour, rinse the embryos 3 times with 1x PBS with separate disposable pipettes and discard the solutions safely in a specified waste container. When washing the embryos, be sure the PBS just covers the embryos. Once the washes are finished, place the petri dish under the microscope to trim. Using forceps and spatulas specifically for PFA products, carefully remove the embryo from the filter paper. Use the forceps to hold down the paper while you cut around the embryo with the spatula. Using a sterile, disposable pipet, cut the tip off. Use this to move the embryos from the petri dish to the 1.5 mL eppendorf tube. Store with PBS. It was labeled “TMEM132C, what type of morpholino was used, initials, and date.”

Pax 7 Immunostaining

With the fixed embryos, rinse twice with 1x PBT (PBS, 2mg/mL of BSA and 0.1% Tween) every 10 minute wash. Repeat 3 times. Be sure to use two different disposable pipettes, one for waste liquid, one for new liquid. Incubate the embryos for 1 hour at room temperature, nutating with a non-specific antibody binding blocker (10% Heat-Activated serum -HI FBS diluted in PBT 1:10). Remove the blocker. Dilute Mouse-a-PAX7 IG1 using a 1:10 with PBT.

This is the primary antibody. Incubate the embryos in this solution overnight at 4 ℃while nutating. When removing the primary antibody, save the solution for future use. Store at 4 ℃. Rinse twice for every wash with PBT. Wash 3 times for 1 hour each at room temperature. Dilute the secondary antibody (goat anti-mouse IGG1 alexaflurophore 488) with PBT 1:2000. This is a green fluorescent dye. Incubate the embryo with the secondary antibody at 4 ℃ for 3 hours or overnight, covered in aluminum foil and nutating. Remove the secondary antibody and rinse twice every wash with PBT. Each wash should be for an hour at room temperature and nutating. Repeat 3 times. Store the embryos in a 1.5 mL eppendorf tube with 1.5 mL of PBS. Wrap in tinfoil and store at 4℃.

Mounting Embryos on Microscope Slides

To prep a microscope slide, place 2-3 layers of black electrical tape. With a box cutter, cut out a small square in the electrical tape. This is where your embryo will be held. Rinse clean with DI water and spray with 70% ethanol. Wipe dry. Place the slide under the microscope. Cut the tip off of a sterile, disposable pipette. Use it to transfer 1 embryo with a small amount of PBS from the eppendorf tube and place it in the middle of the square on the microscopy slide. Check to see if the embryo is ventral side up. Position the embryo with the head towards the left. Be sure the body of the embryo is horizontal and parallel to the microscopy slide. In the chamber, there should be no bubbles visible in the PBS and there should be an adequate amount of PBS so that it has a slight bubble. Place a pair of PFA tweezers next to the square on the microscopy slide. Rest the edge of a clean coverslip on the pair of tweezers. It should rest above the embryo. Slowly lower the tweezer's tips to the slide. Ensure no bubbles form. Slowly slip the tweezers

out from under the cover slip. There should be no bubbles. Using a Kim wipe, wipe the edges of the cover slip and allow it to soak up any excess PBS. This creates a tighter vacuum and seal.

Imaging Embryos

Using a Nikon Eclipse 80i, take images of the embryos at 4x with Brightfield, blue fluorescence light, and light green fluorescence light. Brightfield would show the contrast between the white background and the darkness of the dense tissue. Blue fluorescence light would illuminate the green fluorescence of the Pax 7. Light green fluorescence light will show the red fluorescence of the TMEM132C morpholino. For all three light settings, image the embryos at 4x, focusing on areas where there is a lot of signal. Take the images at 5 second exposure times Merge the green fluorescence and the red fluorescence images together to compare where the signals are. In the areas where there is a lot of fluorescence (red or green), use 10x to image the photos with the blue and light green fluorescence light. Take the images at 5 second exposure time. Merge the images again to compare the difference between the areas of fluorescence. To compare the signals, take an image at 4x of an electroporated embryo with no TMEM132C morpholino signaling.

FIGURES

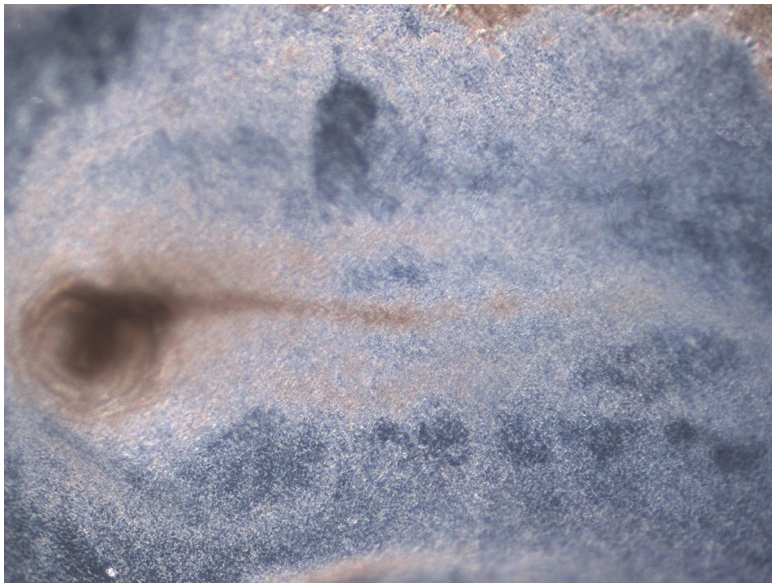

Figure 1: 4x Morpholino 20 successful embryo (HH6) brightfield

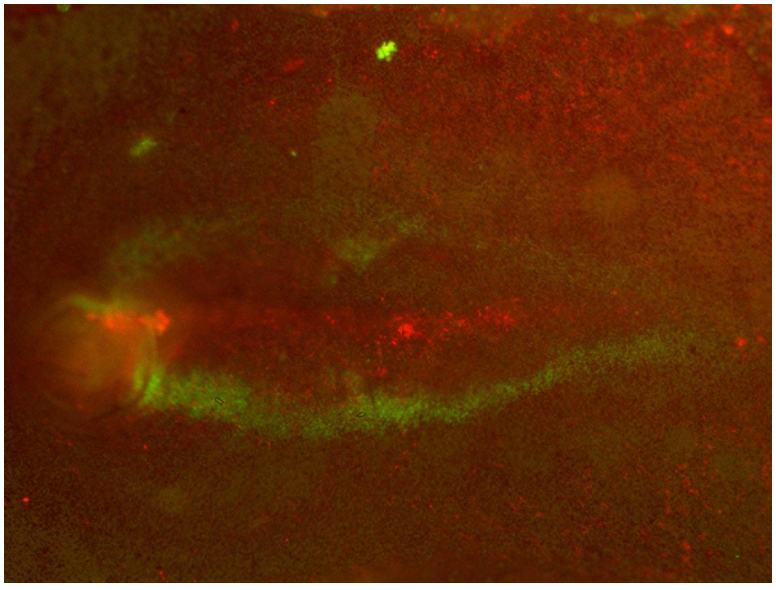

Figure 2: 4x Pax 7 and morpholino 20 taken at 5 seconds each

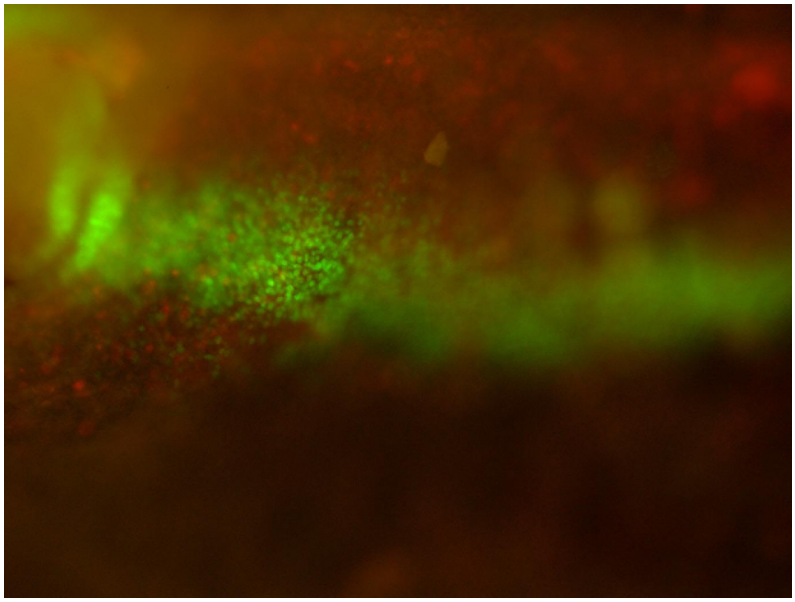

Figure 3: 10x anterior left neural fold of Pax 7 and morpholino 20

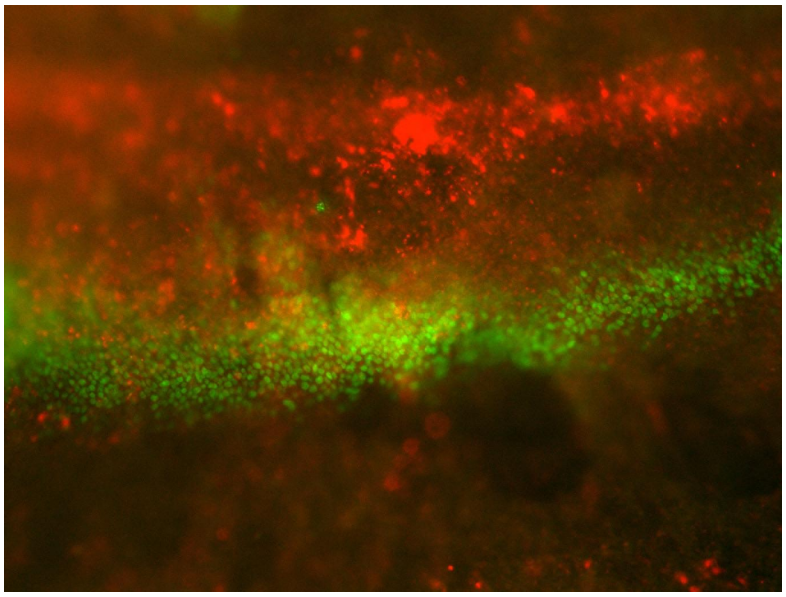

Figure 4: 10x caudal left neural fold of Pax 7 and morpholino 20

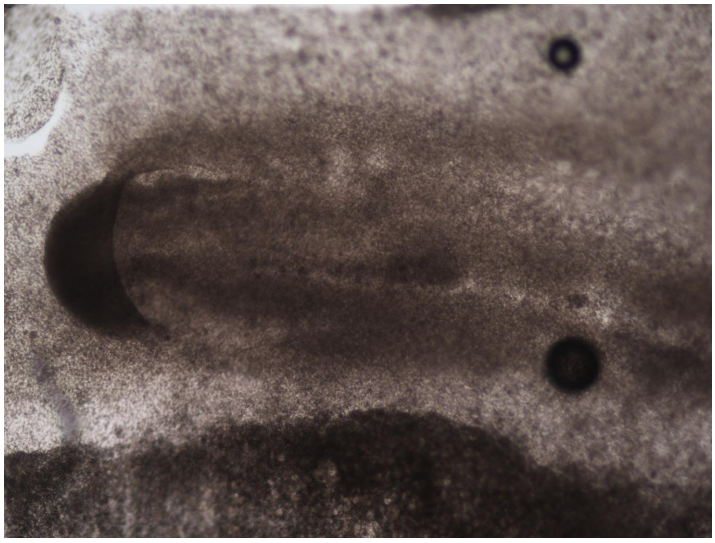

Figure 5: 4x Morpholino 20 unsuccessful embryo (HH6) brightfield

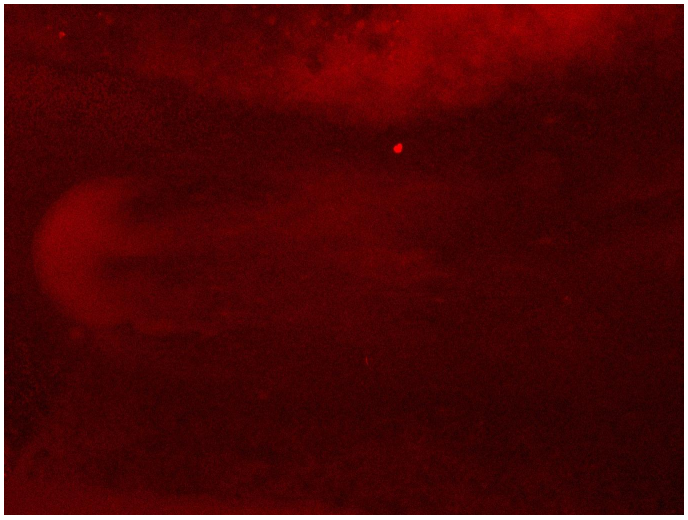

Figure 6: 4x morpholino signaling unsuccessful embryo

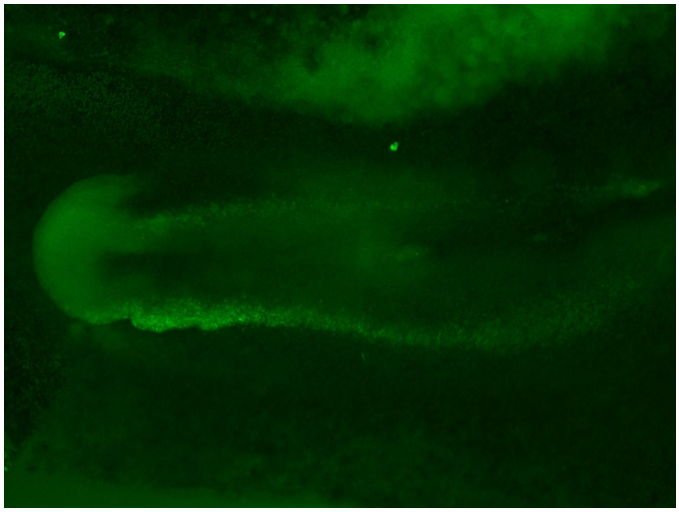

Figure 7: 4x unsuccessful embryo Pax 7

RESULTS

To assess the possible role of TMEM132C during NC development, we opted for a knock-down approach, based on translation blocking MOs. Morpholinos are short chains of subunits that bind to mRNA and prevent translation initiation and therefore, the production of proteins. For them to work, it is critical to identify a complementary sequence in the target mRNA where translation could be stopped, usually before initiation of translation. Another aspect of equal relevance in the design of MOs, is to establish that the sequence selected does not match other possible targets, other mRNAs. TMEM132C had 4 different variants, X1-4. To ensure that a unique sequence is found, each TMEM132 family domain was identified in each variant and compared. That information was sent to GeneTools to request the oligo design. The targeting oligo of interest had the sequence of CGGCCCCCGCCATGCCGAGAAGAC and would bind to the mRNA and prevent translation initiation. They also provided other sequences that could also bind to the oligo. In total, there were five other genes that the morpholino could bind to: C1QL2 X1, ST6GALNAC5 X2, RAPGEF6, TMEM131L X2, and AGK. There were two types of morpholino sent to us, 20 and 24. All of the successful embryos were treated with morpholino 20.

The optimized MO-TMEM132C sequences were obtained from GeneTools and electroporated in chick embryos stage HH4. From 64 embryos treated, we successfully got 2 embryos electroporated, which were further incubated for 24 hours. Then the embryos were fixed with 4% PF and processed for Pax7 immunostaining to assess if the downregulation of TMEM132C affects NC development.

Amongst the embryos recovered, one was carefully analyzed and is described here. The embryo grew as expected reaching stage 6 or 7, but had some apparent abnormal head fold development, and somites are not obvious.

The embryo (Figure 1) grew with a slight bend, with a truncated caudal region and reduced notochord. Transmitted light analysis using a Nikon Eclipse 80i microscope revealed small dark, black dots in some cells, specifically in the contralateral side to the cells receiving the morpholino.

Looking at the morpholino red signaling (Figure 2), there are strong, robust signals coming from the mesoderm. Strong signals can also be seen in the midbrain and hindbrain. On the neural folds of the embryo, there are individual cells that also display red fluorescence.

The Pax 7 signals can be seen in both neural folds but it is much stronger in the left neural fold, precisely where the bulk of the MO signal lies. When analyzing the coexpression of the morpholino signal and the Pax 7 signal, one could note 3 interesting points. Directly under the anterior portion, are cells in the neural fold that show lots of Pax 7 fluorescence (Figure 3). There are some holes in the Pax 7 signaling and that is where cells showed morpholino signaling. It appears as if cells that displayed red signals did not have any Pax7 signal. Moving further along the neural fold, under what appears to be a strong morpholino signal in the mesoderm, some cells display both red and green fluorescence and thus appear clearly in yellow color (Figure 4).

When comparing the growth of the embryo with successful morpholino signaling to the failed MO treated embryo, there was little to no abnormal growth (Figure 5). This embryo was also treated with the TMEM132C morpholino 20 at HH4 and incubated for 24 hours. After those 24 hours, the cultured embryo grew to stage HH7. Unlike the embryo that showed morpholino

fluorescence, the head has a normal head fold and has at least 1 pair of somites. The caudal ends also appear to be present with a long notochord.

When looking at the Brightfield image, the head, parts of the notochord, as well as the anterior portions of the neural folds, are the darkest. Some dark, black spots can be seen in individual cells and can be detected on the left neural fold.

Even though this embryo was treated with the TMEM132C morpholino (Figure 6), there is no clear signal. It is all background noise.

Pax 7 fluorescence (Figure 7) is strongest in the left neural fold, like the embryo with morpholino signaling. The cells in the right neural fold have a strong signal but the left neural fold displays more cells with the strong signal. The cells that display the most Pax 7 expression are in the left anterior portion of the neural fold.

DISCUSSION

Looking at both embryos, there are cells with black spots that can be seen when looking at the left neural fold. These dark individual cells can indicate that those cells have been burnt, due to the electroporator probe being too close to the embryo. There is damage to the contralateral side. These burnt cells might also be why both the morpholino and Pax 7 signaling are stronger and more robust in the left neural fold. The embryo also displayed anatomical abnormalities which could be due to error in electroporation or the needle poking the embryo. This might have happened because the mistake was not perceived by the user and should be replicated while looking at the microscope to ensure the probe or needle does not touch the embryo.

The embryo that was successfully treated with morpholino showed developmental abnormalities. These could be from the knockdown of TMEM132C. A normal HH6 embryo would have a head fold while this embryo remains flat and rounded. There is deterioration of the notochord at the caudal end. There were also no visible somites. This could suggest that this gene is involved in gastrulation, the formation of somites, and the lengthening of the notochord at HH6.

The strongest morpholino signal can be seen in the mesoderm or notochord in the center of the embryo, and there was an increase in Pax 7 expression. This could mean the blockage of TMEM132C in the mesoderm may lead to an increase in general Pax 7 signaling. In a study done by the lab, mesoderm was scraped off unilaterally and led to a robust Pax 7 signaling upregulation precisely in the mesoderm lacking side. This decreased expression of TMEM132C could suggest that the lack of TMEM132C may disrupt the mesoderm, leading to a more robust Pax 7 expression. This could also be seen in the left neural fold, towards the caudal region.

However, this morpholino signaling could also be in the notochord. There are some cells that display both Pax 7 and morpholino signals. This can be seen by the yellow dots in the neural fold. There are two points of interest. There are three yellow dots that could be seen in the anterior left neural fold and three yellow dots in the left neural fold towards the caudal region. It is located under the large morpholino signal in the mesoderm. However, in the anterior portion of the left neural fold, there is a lot of Pax 7 expression but there are a few gaps where there is no Pax 7 signal. Where there is no green fluorescence, there is red fluorescence, suggesting that cells that received the translation blocking MO against TMEM132C lead to a lack of Pax 7 in those individual cells. A decrease in Pax 7 signaling could lead to a decrease of NC cell production and this in turn could lead to a deficient NC development and migration.

The distribution of Pax7 in the neural folds has been well characterized and it is known to be equivalent in both sides of the embryo. The unilateral deficits in Pax7 seen could be due to technical deficits in electroporation, where as explained, cell damage can occur upon direct or closer electrode contact/display. Additionally, our analysis using whole mount imaging provides a fast and relatively easy access to phenotypes, but is ineffective in determining the precise location of the targeted tissues, as these may reside in different layers, and the depth of the tissues will obscure the true origin of the signal. Histological analysis after sectioning is an additional tool that could easily resolve this issue.

Our analysis revealed a broad, apparent stronger Pax7 signal where the majority of the MO signal lies, but this signal could be coming from a lower layer, like the mesoderm and thus TMEM132C downregulation could indirectly upregulate Pax7 through a mesodermally mediated effect. We also note that in a few cases, we observed the apparent lack of Pax7 in a few neural fold cells clearly displaying the MO against Pax7, and thus in these situations, we would argue

that perhaps Pax7 expression does directly get impacted by a downregulation of TMEM132c. Inclusion of control embryos displaying the normal, even pattern of expression of Pax7 on both neural folds should have been considered top aid in the analysis and conclusions provided.

CONCLUSION

The limited results offer a valuable experience even if the aspiration remains unstained. The conceptual portion of the experiment is of great relevance and has enriched my scientific perspective. Furthermore, I mastered a great deal of basic embryological techniques, including the *in vitro* or ex-ovo culture of chick embryos and the design of LOF tools like the MOs, which operate similar to other strategies of great value in current experimental biology and biomedical approaches like shRNA siRNA, and CRISPR. Furthermore, the method of electroporation is a sophisticated one that requires great care to avoid damaging a delicate embryo, especially the younger and more fragile stages used in this project. At HH3-4, only the primitive streak is visible, making an accurate injection a difficult one. Especially when you aim to deliver the MO in a specific layer and topologically difficult location to access. The technique also depends on the speed and accuracy of sending the electrical current. As seen by the damage done on the contralateral side, haphazard electroporation can damage the embryo and could even stunt growth. Leo Padilla, another Honors student in the lab, electroporated older embryos (HH10-12). Not only did his embryos grow in vivo, the morpholino was also injected directly into the neural tubes. Leo did not use the electroporation plate but used a two-pronged probe. This method resulted in more successful embryos. This could be due to the lack of embryo extraction which can result in embryos falling out of the filter paper and having too many yolk cells. These complications can result in a failed morpholino injection or electroporation. While electroporating, at different stages of the embryo can yield different results, it seems that doing this experiment on older embryos has a higher success rate, due to the stability of more developed embryos.

To further investigate what this novel gene's function is, more successful electroporations must be performed at all stages of development. Once there are enough embryos that have been successfully treated with the morpholino, more immunostainings should be performed with different specifiers like Pax 7. Specifiers that affect the formation and migration of NC cells should be used such as SOX8/9/10, SOX 5, Snai 1, or Snai 2.

REFERENCES

Basch, M. L., Bronner-Fraser, M., & García-Castro, M. I. (2006). Specification of the neural crest occurs during gastrulation and requires PAX7. *Nature*, *441*(7090), 218–222. https://doi.org/10.1038/nature04684

Charney, R. M., Prasad, M. S., & García-Castro, M. I. (2021). Current insights into neural crest cell development and pathologies. *Diagnosis, Management and Modeling of Neurodevelopmental Disorders*, 127–137. https://doi.org/10.1016/b978-0-12-817988-8.00011-7

Kalluri, R., & Weinberg, R. A. (2010). The basics of epithelial-mesenchymal transition. *Journal of Clinical Investigation*, *120*(5), 1786–1786. https://doi.org/10.1172/jci39104c1

Li, B., & Niswander, L. A. (2020). TMEM132A, a novel Wnt signaling pathway regulator through Wntless (WLS) interaction. *Frontiers in Cell and Developmental Biology*, *8*. https://doi.org/10.3389/fcell.2020.599890

Sanchez-Pulido, L., & Ponting, C. P. (2017). TMEM132: An ancient architecture of cohesin and immunoglobulin domains define a new family of neural adhesion molecules. *Bioinformatics*, *34*(5), 721–724. https://doi.org/10.1093/bioinformatics/btx689