MODULATION OF THE NOTCH SIGNALING PATHWAY IN 3D STEM CELL-DERIVED CULTURE OF INNER EAR ORGANOIDS

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Hearing loss and vestibular dysfunction are inner ear disease states that arise from an array of diverse etiologies that interfere with mechanosensory hair cell function, including: congenital syndromes, noise-induced trauma, ototoxic drugs, and aging. The investigation of normal inner ear development and the pathological aberrations that cause inner ear disease has been previously advanced through formation of an easily generated, scalable, accurate *in vitro* model system that readily facilitates experimental applications. This model utilizes a 3D floating cell culture protocol which guides differentiation of stem cell aggregates into inner ear organoids, which are vesicles containing a sensory epithelium with functioning mechanosensory hair cells. Inner ear organoid formation enables studying the effects of modulating the signaling pathways that guide developing inner ear structure and function. The Notch signaling pathway heavily influences the formation of the inner ear through two major mechanisms: lateral induction of sensory progenitor cells and lateral inhibition to determine which of those progenitors differentiate into mechanosensory hair cells. The effects of inhibiting Notch signaling within the inner ear organoid system were explored through application of the ɣ-secretase inhibitor MDL28170 (MDL) at a concentration of 25μM on day 8 of organoid culture. Aggregates were harvested on day 32, fixed, sectioned, and stained according to a standard immunohistochemistry protocol. Sections were stained for the mechanosensory hair cell markers Myosin7a (Myo7a) and Sox2. MDL-treated aggregates demonstrated

statistically significant reductions in the total number of vesicles and the number of vesicles containing hair cells compared to control aggregates. In contrast to control aggregates which demonstrated two distinct organoid variants (protruding and embedded), MDL-treated aggregates only formed the embedded variant. Differences in the expression pattern of Sox2, which is also a marker of stemness and neural progenitor cells were also noted between the two conditions. MDL-treated aggregates demonstrated regions of ‘ectopic’ Sox2 expression whereas Sox2 expression in control aggregates was consistently expressed within Myo7a+ regions.

Eri Hashino PhD, Chair

TABLE OF CONTENTS

Introduction/Background

The mammalian inner ear is an intricate structure containing two sensory systems—the cochlea, which detects sound, and the vestibular apparatus, which detects positional information. The basic cellular unit of both the auditory and vestibular systems is the mechanosensory hair cell which transduces mechanical stimuli from sound or positional changes to the brain.[1] To enable that function, the inner ear also includes neural components and a diverse group of supporting cells. Importantly, mature mammalian hair cells are unable to regenerate when damaged; therefore, inner ear pathology may elicit irreversible hearing and/or equilibrium deficits. Hearing loss affects approximately 1.1 billion individuals across the globe and there is a wide range of processes that alter hair cell function including: genetics, medication toxicity, noise-induced trauma, and aging.[2]

The investigation of normal inner ear development and pathological aberrations that cause hearing loss or vestibular dysfunction would be enhanced through development of an easily generated, scalable, *in vitro* model system that readily facilitates experimental applications. Development of an accurate *in vitro* model may facilitate: 1) high-throughput drug screening for therapeutic or ototoxic compounds, 2) modelling of inner ear disease states, and 3) investigation of various cell, gene, and pharmacologic therapies for hearing loss and vestibular dysfunction.[3]

Through *in vitro* application of proteins and small molecules at precise time points, embryonic stem cells (ESCs) can be driven to differentiate into three-dimensional (3D) inner ear organoids.[4,5] During the differentiation process are correlates to specific stages and components of inner ear development including the nonneural ectoderm, otic

placode, and otocyst. The 3D inner ear organoids contain vesicles lined by an epithelium containing all compnents of the inner ear: hair cells, supporting cells, and sensory neurons.[4,5] These sensory organoids may form in one of two variants—protruding organoids which project from the surface of the aggregate and organoids which remain embedded within the surface of the aggregate.

Organogenesis is increasingly being studied in the context of 3D floating cell culture which facilitates self-organization of cells into complex organ-like structures,[6-9] as opposed to traditional monolayer culture which restricts 3D arrangement of cells.[10] 3D culture more closely models physiologic embryogenesis and allows increased freedom for morphologic changes (e.g. invagination and vesicle formation) and the spatial relationships and intricate signaling gradients within developing tissue.[10,11] The complex interactions of the various signaling pathways which play out during embryogenesis are better replicated in three dimensions; during inner ear development, one such signaling paradigm that is particularly influential is the Notch pathway.[12,13] Notch signaling contributes to important developmental phenomena including cell fate specification, proliferation, and boundary formation.[14,15]

Notch signaling mediates two important processes during inner ear development: 1) definition of prosensory domains which ultimately form the sensory epithelia and 2) establishment of the mosaic pattern of mechanosensory and supporting cells within those epithelia.[12,16] These two processes are respectively mediated through the mechanisms of lateral induction and lateral inhibition. Different Notch ligands at different time points in development characterize the two processes.

Specification and expansion of the prosensory domain defined by the otic placode is mediated by lateral induction.[16-19] This region contains the sensory progenitor cells which go on to become either mechanosensory hair cells or supporting cells within the sensory epithelium. Studies in both chick and mouse demonstrate expression of the Notch ligand Jag1 before and during emergence of sensory progenitor cells.[18,20,21] Furthermore, Jag1 is a recognized marker of otic prosensory cells and Notch activation elicits further Jag1 upregulation.[13,19,21,22] Expression of Jag1 activates the Notch receptor in adjacent cells and programs them to become sensory progenitors as well.[20] Indeed, loss of Jag1 function causes attenuation of sensory progenitor regions within the mouse embryo.[22] Conversely, overexpression of the activated Notch receptor induces ectopic regions of mechanosensory hair cells and supporting cells within the inner ear, further suggesting an important role for the Notch pathway in otic specification.[22] Conditional deletion of Jag1 has been shown to downregulate the prosensory marker Sox2 and results in malformation of the cochlea with reductions in the number of hair cells and supporting cells.[17,23] Jag1 is known to be expressed in the vesicles which arise during 3D inner ear organoid formation.[4]

Following specification of sensory progenitor cells, the well-characterized Notch mechanism of lateral inhibition takes place.[18,24] Lateral inhibition describes a process whereby expression of specific ligands by a cell destined for a particular fate prevents neighboring cells from pursuing the same fate.[25] In the developing inner ear, differentiating hair cells express Notch ligands Delta1 and/or Jag2 and thereby activate the Notch pathway in adjacent cells. Activation by either of these ligands causes upregulation of effectors Hes1 and Hes5 and subsequent attenuation of Atoh1

expression—because Atoh1 is required for sensory hair cell differentiation, Atoh1 inhibition in cells adjacent to future hair cells causes the mosaic patterning of the otic sensory epithelium.[20,26,27] Consistent with this concept of lateral inhibition, mice lacking Delta1 or Jag2 demonstrate supernumerary hair cell formation.[20,28]

In sum, Notch-mediated lateral signaling among neighboring cells initially expands the number of sensory progenitor cells through lateral induction and then limits which sensory progenitors can differentiate into mechanosensory hair cells. Therefore, attenuation of Notch-mediated lateral induction may be expected to reduce the yield of inner ear organoids within the 3D culture system. Conversely, attenuation of Notch-mediated lateral inhibition may augment the yield of inner ear organoids.

Activation of a Notch receptor causes enzymatic cleavage of the intracellular domain of the receptor. This cleavage is mediated via the ɣ-secretase enzyme, so Notch signaling may be blocked by ɣ-secretase inhibitors such as DAPT and MDL28170 (MDL).[29] Given the established and influential role of Notch signaling in both sensory specification and hair cell differentiation both *in vivo* and *in vitro*, the effects of modulating this signaling within the inner ear organoid system were investigated.

Research Design and Results

The research design encompassed the otic differentiation protocol described by Koehler and Hashino using Atoh1-eGFP mouse embryonic stem cells (ESCs) maintained in leukemia inhibitory factor (LIF) medium to prevent spontaneous differentiation.[4,5] In this protocol, ESCs are dissociated and arranged in floating aggregates of approximately 3,000 cells per each well of a 96-well U-bottom plate. Through the application of specific media, extracellular matrix components, small molecules and proteins at precise time points over the next four days followed by transfer to a 24-well plate on day 8 for self-guided organization, aggregates undergo differentiation and develop to contain vesicles with functioning vestibular hair cells (termed "inner ear organoids").[4,5] In the present experiment, samples were allocated to treatment either with control media or media infiltrated with a ɣ-secretase inhibitor at a specific time point.

Of note, the majority of aggregates treated on day 8 with the inhibitor DAPT at a concentration of 10μM demonstrated disaggregation with presence of floating cellular debris. Based on this observation, and that control specimens maintained under otherwise identical conditions did not demonstrated cellular death, treatment with DAPT at this concentration was deemed toxic to the aggregates.

The following data was generated from an experiment in which approximately half of the wells in a 96-well plate were designated for "control" aggregates and treated according to the standard otic differentiation protocol. The remaining aggregates were treated with MDL on day 8 at a final concentration of 25μM. This concentration was determined based on the best available data from prior explant studies, differentiation attempts, and absence of grossly toxic effects on aggregates.[29] Control and MDL-treated

aggregates were then maintained under identical conditions with media changes occurring every two days and underwent routine imaging under light microscopy to monitor their progression. Under light microscopy, control aggregates demonstrated higher proportions of Atoh1+ vesicles (Figure 1). On day 32, all aggregates were collected and fixed in paraformaldehyde solution prior to being embedded in cryomedia. Aggregates were then sectioned via cryostat in their entirety and mounted onto slides for immunohistochemical staining. Following incubation of slides with a blocking buffer, staining was performed utilizing primary antibodies against the hair cell marker Myosin VIIa (Myo7a, Proteus Biosciences Inc.) and the sensory epithelium marker Sox2 (BD Biosciences) diluted to ratios of 1:100. Secondary fluorophore-containing antibodies directed against each type of primary antibody were then applied. Coverslips were affixed to each slide and microscopy was subsequently performed. Each section was analyzed microscopically to determine the total number of vesicles within that section and the number of vesicles which contained hair cells, determined by positive immunofluorescence for the markers Myo7a and Sox2. A total of 362 sections were generated and analyzed from control aggregates and a total of 322 sections were generated and analyzed from MDL-treated aggregates.

The average number of vesicles per section (Figure 2) was 1.20 for control aggregates compared to 0.32 for MDL-treated aggregates ($p<.001$). Not all vesicles which form within the aggregates contain hair cells (Figure3). The average number of hair cell-containing vesicles per section (Figure 4) was 0.24 for control aggregates compared to 0.05 for MDL-treated aggregates ($p<.001$). The average proportion of

vesicles that were positive for hair cells within each section (Figure 5) did not differ significantly between control aggregates and those treated with MDL.

No sections from the MDL-treated aggregates demonstrated the “protruding” organoid variant, whereas sections from control aggregates demonstrated both “protruding” and “embedded” variants (Figures 6 & 7). Sox2 expression in control aggregates was primarily co-localized with Myo7a; MDL-treated aggregates however consistently demonstrated scattered Sox2 expression outside of $Myo7a^{+}Sox2^{+}$ vesicles (Figures 8 & 9).

Conclusion

Initial research design involved a coordinated set of dose-response experiments for three separate inhibitors applied once at varying time points to determine the precise concentration, time point, and ɣ-secretase inhibitor that would elicit increased yield of hair cells within the differentiation protocol. This design was planned based upon prior studies documenting the effects of Notch inhibition on otic sensory epithelia. Aggregates treated with the Notch inhibitor MDL28170 on day 8 demonstrated a significantly lower number of vesicles and a resulting lower number of hair cell-containing vesicles compared to controls. These findings are consistent with previous studies of Notch signaling in the developing inner ear.

It has been noted that Notch1 receptor activation within the Pax2+ region destined to become the otic placode causes expansion of otic placode markers.[30] The otic placode is one of various cranial placodes, which are thickened patches of ectoderm that develop into the sensory cells, neurons, and support cells of the cranial sense organs.[31-33] In the otic differentiation protocol utilized, expression of the transcription factor Pax2 is noted between day 8 and day 12. The relatively early inhibition of Notch signaling at day 8 potentially impaired expansion of sensory progenitors in the $Pax2^{+}$ otic placode through interference with the process of lateral induction.

In addition to lateral induction, Notch signaling contributes to the size of the developing otic placode through augmentation of the Wnt signaling pathway.[30] In mice, specification of the otic placode is mediated by expression of Wnt ligands including Wnt8a from the caudal hindbrain.[34-36] The role of Wnt signaling in otic specification is further supported by mouse studies in which deletion of β-catenin reduces the size of the

otic placode.[37] It has previously been shown that aggregates treated with a Wnt inhibitor display impaired vesicle production, indicating involvement of endogenous Wnt signaling in the formation of otic vesicles.[4] Based on the high degree of overlap among the Notch and Wnt pathways, the results described above suggest interference with endogenous Wnt signaling within MDL-treated aggregates and subsequently impaired otic vesicle formation.

Protruding organoids are typically present in approximately 15% of aggregates developed under original protocol conditions.[4] It has been postulated that Pax2 contributes to the thickened pseudostratified morphology of the otic placode and resultant expression of cellular adhesion molecules, facilitating placode invagination and formation of the otic vesicle.[38,39] Therefore a reduction in Pax2 expression and otic placode size in MDL-treated aggregates may also account for the complete absence of protruding vesicles.

In sum, application of MDL28170 on day 8 of the otic differentiation protocol potentially impaired organoid development by: 1) attenuating Notch-mediated lateral induction of sensory progenitors, 2) preventing Notch augmentation of Wnt-driven otic placode expansion, and 3) attenuation of the $Pax2^{+}$ region capable of invagination and otic vesicle formation. These results indicate the presence of endogenous Notch signaling within the aggregates and its influence on formation of inner ear organoids. Thus far the effects of Notch inhibition on inner ear organoid development correlate with *in vivo* embryogenesis and thereby further validate this novel model system.

Further investigation is required to determine the cause and significance of the different patterns of Sox2 expression seen between control and MDL-treated aggregates.

Within the inner ear, Sox2 is initially expressed throughout the prosensory epithelium and later localized to fully differentiated supporting cells.[12] Sox2 is also known to be involved in maintenance of pluripotency within embryonic and neural stem cells.[40] Given that undifferentiated ESCs have been shown to express Notch receptors and ligands, it is possible that Notch inhibition affected the differentiation of certain cells within the aggregates and retained them in a progenitor state.[41] Further immunohistochemical analyses may be helpful to further characterize the nature of the ectopic patches of Sox2 found in MDL-treated aggregates; for example, positive staining for β-Tubulin 3 would be consistent with nests of neural progenitor cells.

Future studies should continue use of MDL28170 concentrations near 25μM, which is efficacious based on the significant differences between control and MDL-treated aggregates. Comparison with other concentrations may be useful to establish dose-response relationships and further validate the effects of Notch inhibition. Earlier endpoint analysis will be useful to confirm and further analyze the effects of Notch inhibition on the developing otic placode. One potential extension of this data is application of Notch agonists at day 8 to determine if this augments vesicle formation and permits formation of protruding inner ear organoids. The ultimate goal for increased hair cell yield via Notch inhibition is predicated on interference with the process of lateral inhibition and not lateral induction. The above data suggest that at day 8, the region destined to become the sensory epithelium is undergoing lateral induction of sensory progenitor cells and Notch inhibition at this time point has a negative effect on organoid development. This indicates that treatment with a Notch inhibitor at a later time point is likely necessary to attenuate Notch-mediated lateral inhibition and increase the yield of

hair cells within the inner ear organoids. This later time point is likely between day 8 and day 14, when fully differentiated hair cells are noted within the organoids.

Quantification of the precise timeline, via real-time polymerase chain reaction, at which specific Notch ligands (i.e. Jag1 versus Jag2 and Delta1) are expressed may provide further insight into the precise time points to inhibit Notch signaling in order to augment the yield of organoids and hair cells generated in the 3D culture protocol.

Figures

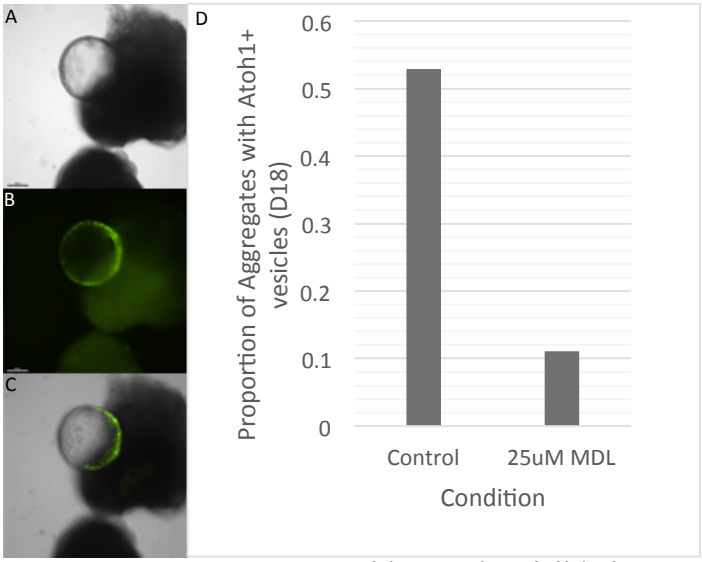

Figure 1. Inner ear organoids may be visible in aggregates floating in 3D culture. A) DIC image of day 30 aggregate with vesicle. B) Image of same aggregate demonstrating GFP expression within vesicle indicating presence of Atoh1+ hair cells. C) Overlay of GFP expression and DIC image. D) At day 18, over 50% of control aggregates displayed Atoh1+ vesicles by light microscopy as compared to 11% of MDL-treated aggregates (n= 24 aggregates per condition).

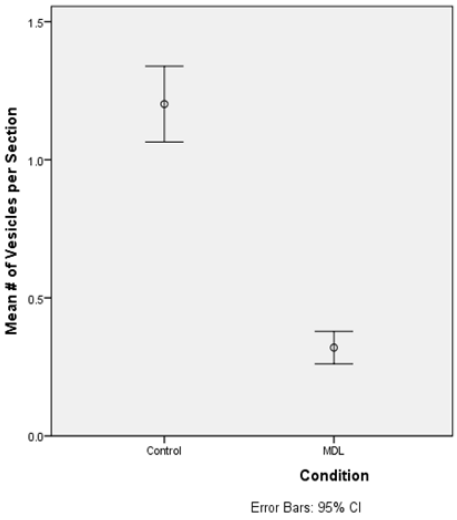

Figure 2. The mean number of all types of vesicles (those containing hair cells and those not containing hair cells) per section was significantly lower in aggregates treated with the Notch inhibitor MDL28170 ($p<.001$).

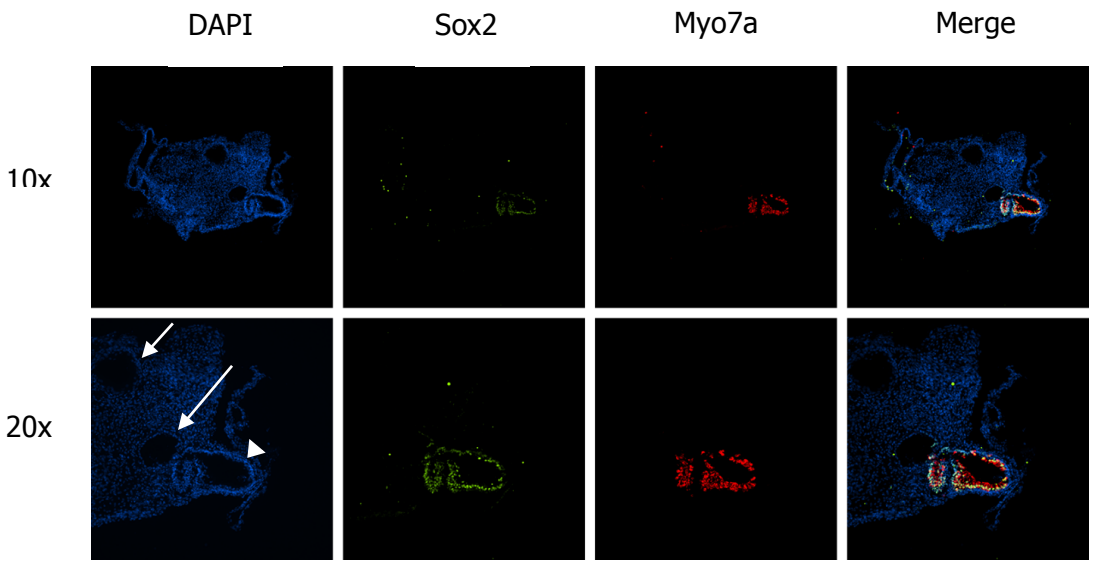

Figure 3. Immunohistochemistry of a control aggregate demonstrating vesicles which do not (arrows) and do (arrowhead) contain $Myo7a^{+}Sox2^{+}$ hair cells.

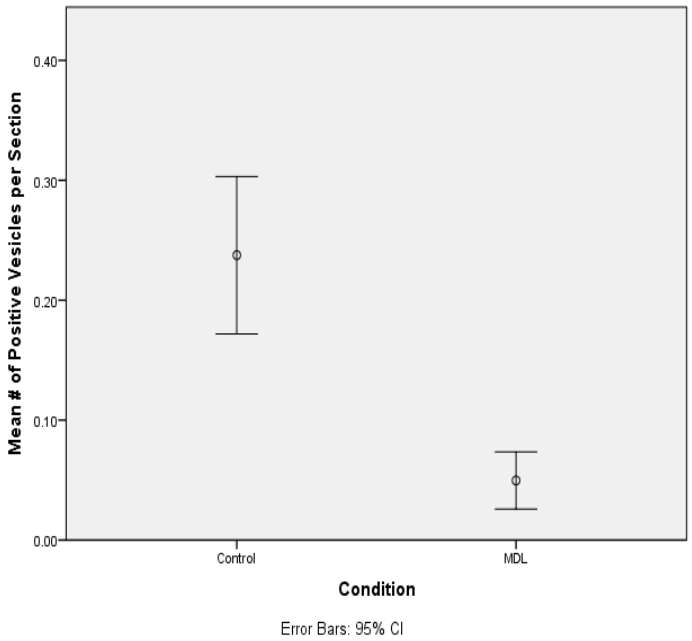

Figure 4. The mean number of vesicles containing hair cell cells was significantly lower in aggregates treated with the Notch inhibitor MDL28170 ($p<.001$).

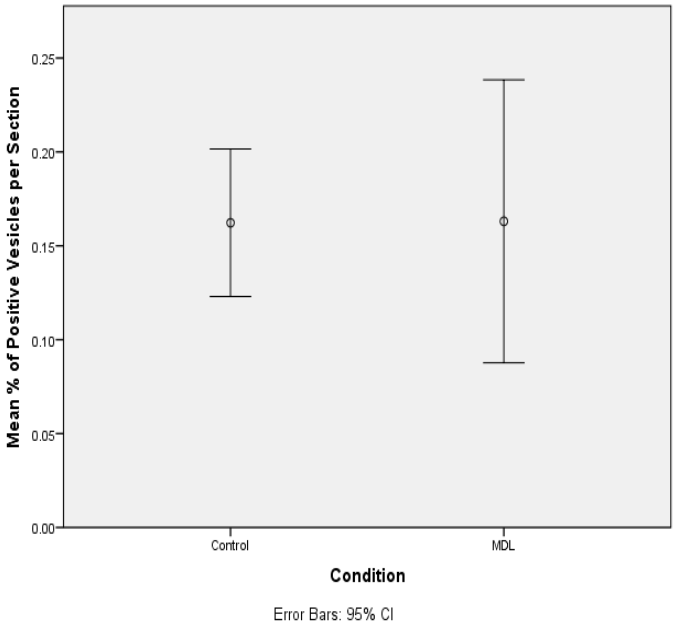

Figure 5. The mean proportion of vesicles in each section that contained hair cells did not differ significantly between conditions

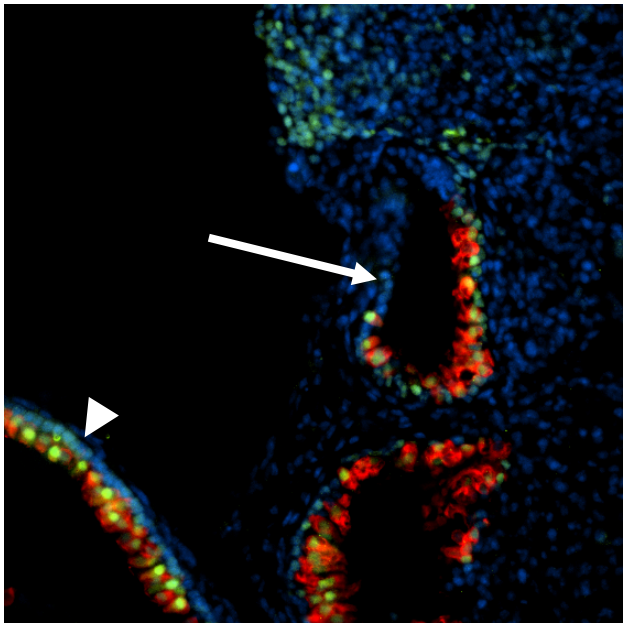

Figure 6. Immunohistochemistry of a control aggregate demonstrating the simultaneous presence of both inner ear organoid types: embedded (arrow) and protruding (arrowhead).

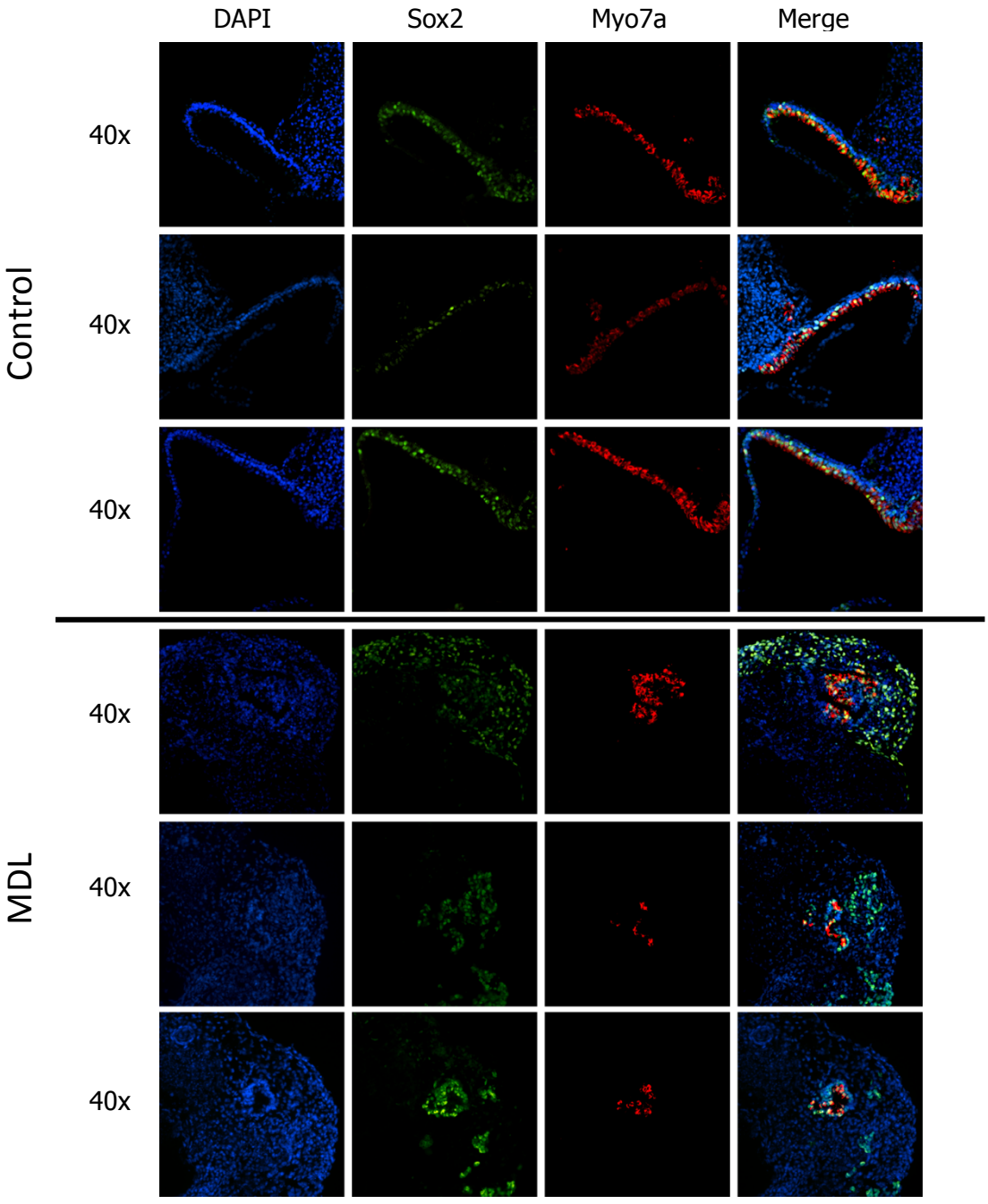

Figure 7. In contrast to control aggregates, MDL-treated aggregates never demonstrated the protruding inner ear organoid variant.

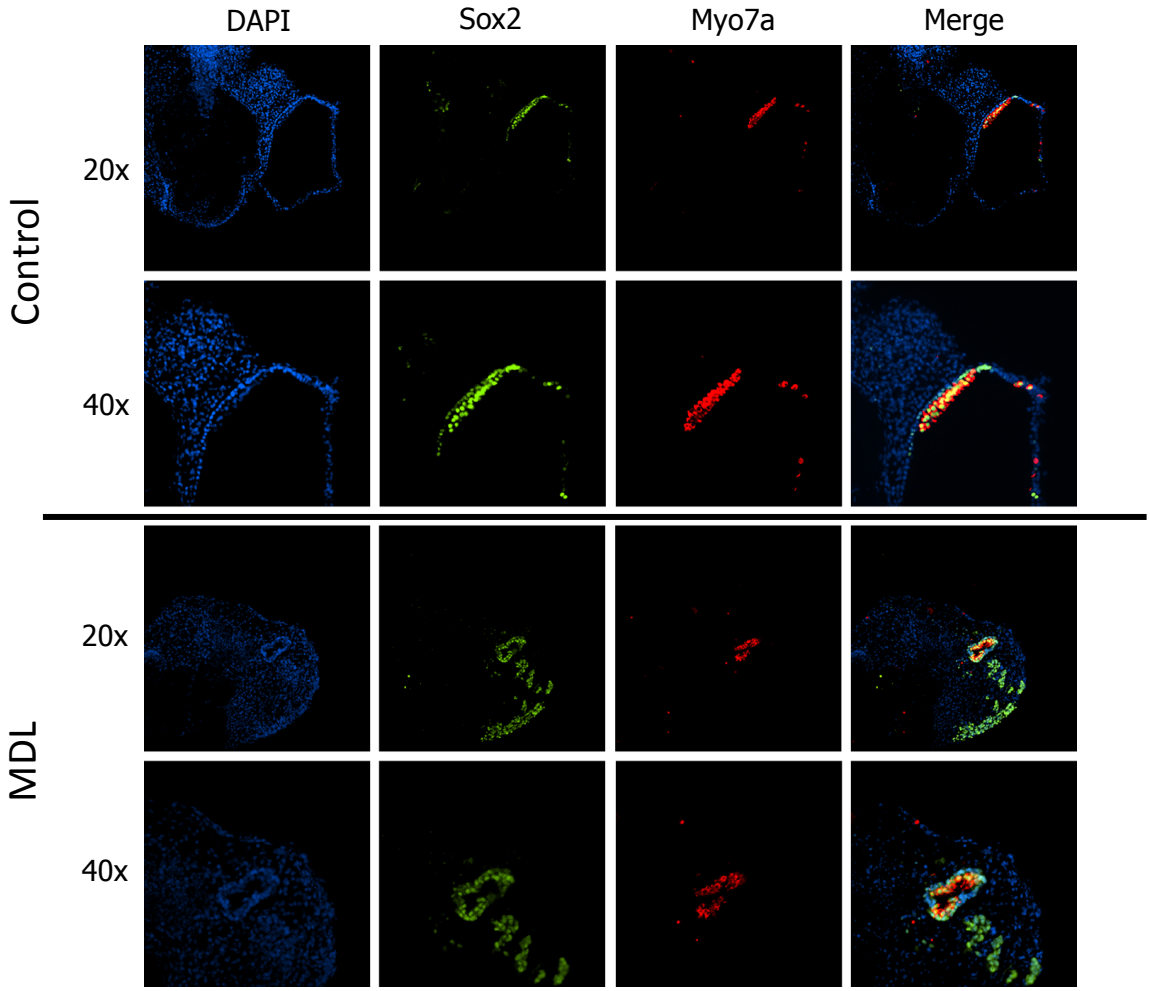

Figure 8. Sox2 expression within control aggregates was primarily co-localized with cells positive for Myo7a. In contrast, MDL-treated aggregates demonstrated scattered 'ectopic' regions of Sox2 expression in addition to co-expression with Myo7a.

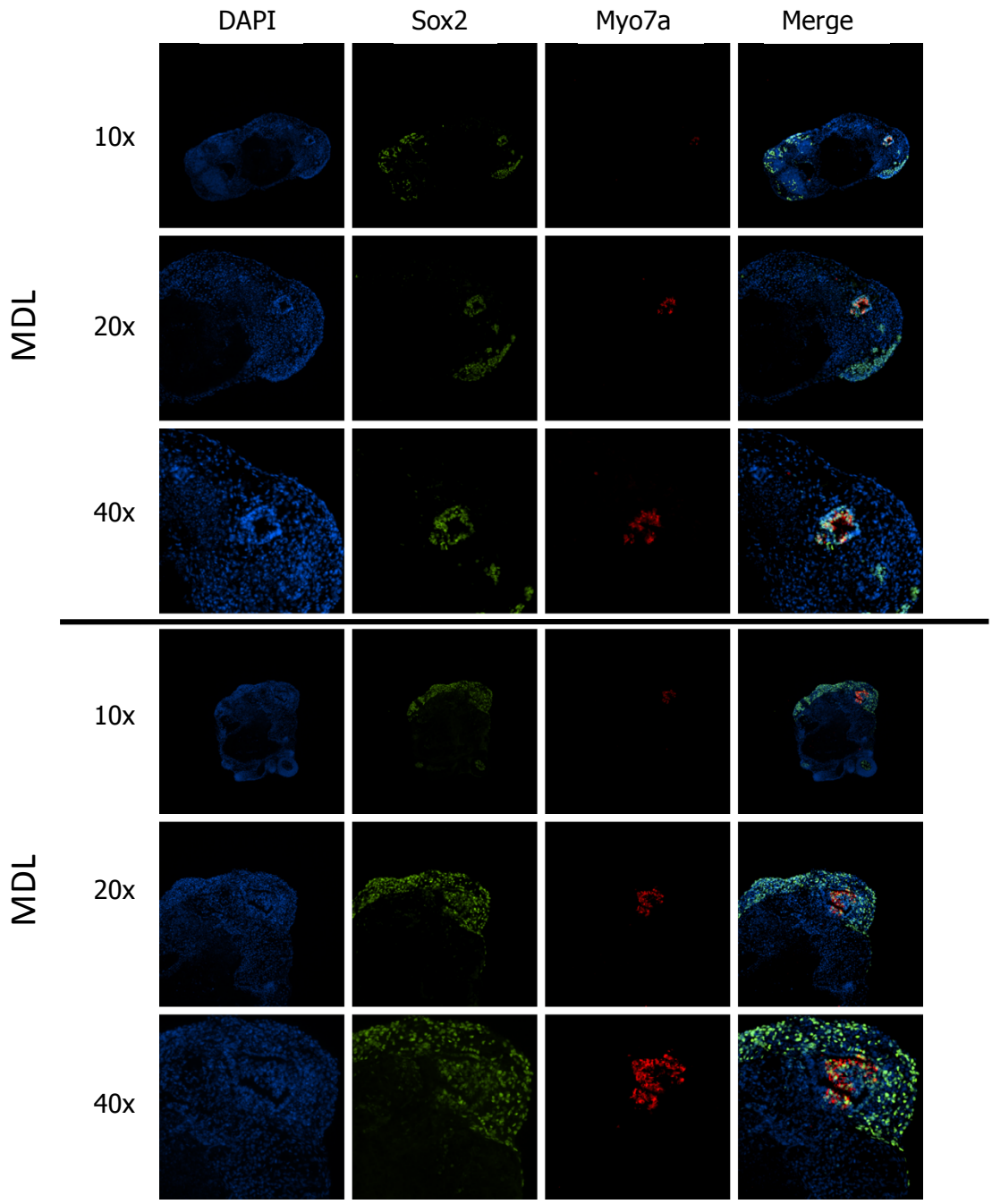

Figure 9. Representative images from two separate MDL-treated aggregates demonstrating scattered 'ectopic' regions of Sox2 expression in addition to co-expression with Myo7a within embedded inner ear organoids.

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Curriculum Vitae

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Education

- Indiana University: Indianapolis, IN (8/2011—6/2016)
  - Combined MD, MS (Translational Science)
- Indiana University: Bloomington, IN (8/2007—12/2010)
  - BA: Psychology, Minors: Biology, Chemistry, Spanish

Research & Laboratory Experience

- Prognostic factors in cutaneous squamous cell carcinoma of the head and neck: Indianapolis, IN / PI: Cecelia Schmalbach, MD, MS (2015 Sep—present)
  - Systematic review with potential meta-analysis on prognostic variables in cutaneous squamous cell carcinoma of the head & neck, with special focus on the immunosuppressed patient population.
  - Planned case series with chart review also pending to confirm importance and impact of variables identified in systematic review and contribute towards development of an accurate oncologic staging system.
- Modulation of Notch signaling pathway in 3D stem cell-derived culture of inner ear organoids: Indianapolis, IN / PI: Eri Hashino, PhD (2015 Jun—2016 May)
  - Modulation of protocol involving differentiation of embryonic stem cell aggregates into inner ear organoids (vesicles containing an epithelium with functioning mechanosensory hair cells, supporting cells, and neurons).
  - Specifically studying impact of Notch signaling pathway and effects of Notch inhibition on yield of inner ear organoids and establishing correlates with physiologic embryogenesis.
  - Carries implications for future modelling of inner ear disease states, high-throughput drug screening, and stem cell therapy.

- Endothelial Monocyte-Activating Polypeptide-II Release and its relationship to iNOS: Indianapolis, IN / PI: Matthias Clauss, PhD (2012 May—2012 Aug)
  - Conducted following acceptance into "Summer Research Program in Academic Medicine."
  - Performed research in molecular biology laboratory at Roudebush VA Medical Center. Participated in weekly seminars led by faculty on basic and translational research principles and implementation.
  - Continued proficiency in laboratory techniques (e.g. Western Blots, PCR, protein purification, and cell culture).
- Architecture of Lipid Signaling in Mouse Anterior Eye: Bloomington, IN / PI: Ken Mackie, MD (2009 Oct—2010 Dec)
  - Studied localization of endocannabinoid-related proteins and receptors.
  - Skills developed include tissue slicing/fixation, cell culture maintenance, immunohistochemistry, and confocal microscopy.

- Murine Model of Medication-Induced Autism Spectrum Disorders: Bloomington, IN / PI: Preston Garraghty, PhD (2007 Jul—2009 Sep)
    - Worked in behavioral neuroscience laboratory to validate rodent model for autism spectrum disorders.
    - Skills include rodent and primate brain and eye extraction, behavioral testing (water maze & Skinner box), and stereoscopic microscopy to assess neuronal change.

Honors & Awards

- Indiana Clinical and Translational Science Institute Research Award (2015 Jun)
- Alpha Omega Alpha Honor Medical Society, Member (2014 Aug)
- Indiana University Health West Hospital Scholarship (2011 Jul)

Leadership, Professional & Volunteer Experience

- Teacher-Learner Advocacy Committee, Member (2012 Jul—present)
    - Dean-appointed position to committee which investigates breaches in professionalism that compromise the learning environment. Responsibilities include assessment and discussion of individual cases in conjunction with other committee members to formulate recommendations for further action.
- Student Promotions Committee, Member (2014 Mar—2016 Apr)
    - Dean-appointed position to faculty committee which decides the academic standing of medical students. Responsibilities included hearing individual cases of medical students not in good academic standing and making decisions regarding dismissal or remedial action.
- IUSM OSCEs, Evaluator (2015 Jun—2015 Dec)
    - Appointed by Student Assessment Office to grade objective structured clinical examinations (OSCEs) of medical students following completion of their clerkships and also at the culmination of their third year.
- Wheeler Mission Clinic, Volunteer (2014 Mar—2015 Sep)
    - Care for homeless population of Indianapolis involving interviewing and examining patients, formulating plans, writing prescriptions, and arranging follow-up.
- Student Outreach Clinic, Volunteer (2014 Mar—2015 Aug)
    - Volunteer work in a student-run clinic. Responsibilities included care for patients, collecting laboratory samples, writing prescriptions, and arranging follow-up.
- United Kingdom Medical Student Association, Author (2015 May—2015 Aug)
    - Research and composition of articles for United Kingdom Medical Student Association Handbook, a reference text on a range of clinical topics aimed at medical students. Articles published in online handbook and mobile application.
- Institute for Healthcare Improvement IUPUI Chapter, President (2014 Jul—2015 Jul)

- Leader of newly established chapter of Institute for Healthcare at Indiana University Purdue University Indianapolis. Inter-professional group focused on increasing awareness about quality improvement and improving patient care. Worked to increase membership and incorporate patient safety and quality improvement concepts into medical curriculum.

- IUSM Gross Anatomy Course, Teaching Assistant (2014 Nov—2014 Dec)
  - Teaching assistant for head & neck portion of first year anatomy course (lecture and laboratory portions) at the Indiana University School of Medicine. Performed dissections, prosections, and instructed medical students.

Bibliography

Peer Reviewed Journal Articles

1. **Elghouche A**, Shokri T, Qin Y, Wargo S, Citrin D, Van Waes C (Epub 2016 Feb 25). "Unilateral cervical polyneuropathies following concurrent bortezomib, cetuximab, and radiotherapy for head and neck cancer." Case Reports in Otolaryngology. PMID: 27088023.
2. Franco J, **Elghouche AN**, Harris MS, Kokoska MS (Epub 2016 Mar 30). "Diagnostic delays and errors in head and neck cancer patients: opportunities for improvement." American Journal of Medical Quality. PMID: 27030690.
3. **Elghouche AN**, Lobo BC, Wannemuehler TJ, Johnson KE, Matt BH, Woodward-Hagg HK, Kokoska MS (Epub 2016 Mar 1). "Lean belt certification: pathway for student, resident, and faculty development and scholarship." Otolaryngology—Head and Neck Surgery. PMID: 26932955.
4. McDowell AL, Fransen KM, Elliott KS, **Elghouche A**, Kostylev PV, O'Dea PK, Garraghty PE. (Epub 2015 Apr 16). "Sex differences and the impact of chronic stress and recovery on instrumental learning." Neuroscience Journal. PMID: 26317113.
5. Wannemuehler TJ, **Elghouche AN**, Kokoska MS, Deig CR, Matt BH (2015 Dec). "Impact of Lean on surgical instruemtn reduction: less is more." The Laryngoscope. PMID: 26109515
6. Green LA, Yi R, Petrusca D, Wang T, **Elghouche A**, Gupta SK, Petrache I, Clauss M (2014 Feb 15). "HIV envelope protein gp120-induced apoptosis in lung microvascular endothelial cells by concerted upregulation of EMAP II and its receptor, CXCR3." American Journal of Physiology. Lung Cellular and Molecular Physiology. PMID: 24318111.

Other Articles

1. **Elghouche, A** (2015 Oct 9). "A medical student's perspective on interprofessional collaboration." Patient Safety & Quality Healthcare.

Peer Reviewed Book Chapters

1. Kim N, Harris A, **Elghouche A**, Gama W, Siesky B (In Press). Penetration enhancers in ocular drug delivery. In Yashwant P, Hirani A, Sutariya V (Ed.), Nano Bio Materials for Ophthalmic Drug Delivery. New York City: Springer.

Abstracts Accepted for Poster Presentation

1. Wannemuehler T, Lobo B, Franco J, **Elghouche A**, et al. Lean Belt Certification—Pathway for Student, Resident and Faculty Development and Scholarship. AAMC Integrating Quality Meeting; Chicago, IL (2015).
2. **Elghouche A**, Hu S, Wager-Miller J, Hutchens J, Mackie K, Straiker A. Architecture of Lipid Signaling in Mouse Anterior Eye. Gill Center for Neuroscience Research Symposium; Bloomington, IN (2010).
3. **Elghouche A**. Endothelial Monocyte-Activating Polypeptide-II Release and its Relationship to iNOS. Indiana University Summer Research Program Poster Symposium; Indianapolis, IN (2012).