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Hepatocyte Growth Factor and Hepatocyte Growth Factor Activator Release of Fibroadipogenic Progenitors in Uninjured Pharyngeal Muscles

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Abstract

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By Danbi Lim

Satellite cells (SCs) are skeletal stem cells that are quiescent in homeostatic adult muscles but are highly important for muscle regeneration upon injury. In pharyngeal muscles, SCs actively proliferate, differentiate, and fuse into muscle fibers even in an absence of injury, though the cause is unknown. Here, we concluded that pharyngeal SCs do not show a significant increase in proliferation and differentiation from limb SCs *in vitro*, indicating that external niche contributes to the activation of pharyngeal SCs more than their intrinsic abilities. In the pharyngeal environment, Fibroadipogenic progenitor cells contribute hepatocyte growth factors (HGF) and HGF activating enzymes to activate SCs. In this study, we show the possible explanation for the unique activity of pharyngeal SCs and how these distinct characteristics may contribute to their different susceptibilities to diseases like oculopharyngeal muscular dystrophy.

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Introduction

The pharynx is composed of skeletal muscles that extend from the nasal cavity into the larynx and esophagus that are essential for respiration, speech, and swallowing (Donner et al., 1985, Randolph et al., 2015). Although the pharynx and the limb muscles are both skeletal muscles, they are derived from separate embryonic origins. While craniofacial muscles, including the pharyngeal muscles, arise from craniofacial mesoderm (Noden and Francis-West, 2006), limb muscles, such as gastrocnemius muscles, arise from somites (Mootoosamy and Dietrich, 2002). These differential embryogenic origins of skeletal muscles may lead to differential susceptibilities to various muscular dystrophies. For example, the extraocular muscles (EOM) of craniofacial muscles are spared from Duchenne muscular dystrophy (DMD) (Khurana et al., 1995), but EOMs are mainly affected by oculopharyngeal muscular dystrophy (OPMD) (Victor, Hayes et al. 1962). Another hypothesis for differential susceptibility of craniofacial muscles is distinct activity of muscle stem cells, called satellite cells. Satellite cells (SCs) are skeletal muscle stem cells that reside under the basal lamina of muscle fibers (Al Tanoury et al., 2020). SCs stay quiescent in homeostatic conditions but quickly proliferate and fuse into muscle fibers upon activation through an injury (Sambasivan et al., 2011). However, previous studies have discovered that pharyngeal and extraocular muscles possess a subset of SCs that are constitutively activated even in the absence of injury (Randolph et al., 2015, McLoon et al., 2007).

Hepatocyte growth factor (HGF) is a well-known growth factor that causes SCs to proliferate, differentiate, and fuse into muscle fibers (Allen et al., 1995). HGF is secreted as an inactive form in response to muscle injury as an inactive form, pro-HGF, and is then cleaved by activators like urokinase-type plasminogen activator (uPA) to its active form (Naldini et al., 1994). Once activated, HGF binds to the cellular mesenchymal-epithelial transition factor (c-Met) and

signals to activate SCs (Czyz, 2018). HGF acts in paracrine and autocrine manner to SCs (Gal-Levi et al., 1998, Sheehan et al., 2000), suggesting that cells residing near SCs, such as fibroadipogenic progenitor cells (FAPs), influence the activation of SCs (Evano and Tajbakhsh, 2018). Accordingly, FAPs that reside in muscle fibers are known to induce activation and myogenic differentiation following muscle damage (Fiore et al., 2016). However, it is unknown how the pharyngeal microenvironment promotes increased activation of SCs.

In this study, we study whether the intrinsic or extrinsic factors impact the pharyngeal SC activation. We compare pharyngeal and gastrocnemius muscles to analyze the distinct proliferation and differentiation levels of SCs. We found that FAPs in the pharyngeal muscles are the major contributor of HGF and HGF activating enzymes in pharyngeal muscles. This study provides further insights into understanding how craniofacial SCs exhibit distinctive cellular activity.

Materials and Methods

Cell size analysis by cytospin

Pharyngeal and gastrocnemius satellite cells were isolated from Pax7 CRE^{ERT2}-tdTomato mice by enzymatic digestion (Cheung et al., 2012). Cells were centrifuged for 5 minutes with cyto-funnels onto SuperFrost Plus glass slide and fixed with 2% paraformaldehyde in PBS at room temperature for 10 minutes. Images were taken by a widefield fluorescence microscope (Echo Revolve). The size of the cells expressing tdTomato was measured using ImageJ.

SC proliferation assay by flow cytometry

To determine whether the pharyngeal MPCs have the higher proliferative ability compared to the gastrocnemius MPCs, Bromo-2'-deoxyuridine (BrdU) assays were used *in vitro*. We seeded $2x10^5$ cells of pharyngeal and gastrocnemius in collagen I-coated 6-well plates and cultured them for two days in Ham's F10 media containing 20% FBS, 100 μg/ml penicillin/streptomycin (P/S), and 25 ng/mL FGF2. The cells were then incubated for one hour in BrdU and fixed in 2% paraformaldehyde in PBS for 10 minutes at room temperature. The cells were labeled using FITC-BrdU flow kit (BD Biosciences) according to the manufacturer's instruction. $BrdU^+$ cells were detected using flow cytometry (BD Symphony A3) at Emory University School of Medicine Core Facility for Flow Cytometry.

SC differentiation and fusion index

The same number of pharyngeal and gastrocnemius MPCs were seeded in low density ($5x10^3$ cells/cm^2) to prevent cell-to-cell interaction for two days in low glucose Dulbecco's modified Eagle's medium (DMEM) with 2% horse serum (HS) and 1% P/S (differentiation media). Then, MPCs were counted and seeded in a high density ($7.5x10^4$ cells/cm^2) environment to induce fusion in differentiation media. Differentiated cells were fixed with 2% paraformaldehyde in PBS for 10 minutes at room temperature and stained with Phalloidin-iFluor 594 for 30 minutes at room temperature. Then, the nuclei were stained with 4',6-diamidino-2-pheylindole (DAPI), and the stained myofibers were mounted with Vectashield. The fusion index was calculated by quantifying the percentage of myonuclei compared to the total number of nuclei.

Protein expression of HGF by ELISA

To test for HGF from FAPs affecting the increased proliferative and differentiative abilities of pharyngeal SCs, the level of secreted HGF in FAPs media was measured by ELISA. The pharyngeal and gastrocnemius FAPs were cultured in DMEM with 20% FBS, 10% HS, and 100μg/ml P/S for two days in an identical environment, then conditioned media (CM) were collected. The number of cells for each conditioned medium were counted to normalize the number of FAPs releasing HGF. The quantity of HGF in CM was detected through ELISA using the HGF ELISA kit (Invitrogen). The samples of conditioned media were prepared using the manufacturer's instructions. The amount of HGF was determined using a standard curve that was created using known amounts of HGF.

Gene expression analysis through Real-Time qPCR

RNA was isolated from pharyngeal and gastrocnemius muscles and reverse transcribed into complementary DNA (cDNA). The amplification of cDNA was performed using 2.5 μM of each primer and Power SYBR® Green Master Mix through real-time qPCR. The cycles are as follows: denaturation at 95°C for 15 seconds, annealing and extension at 60°C for 1 minute for 35 cycles. The expression level was normalized using *Hprt* as a housekeeping gene, and fold change was quantified using ΔΔCt calculation (Livak and Schmittgen, 2001).

Statistical analysis

Statistical analysis including the normality test was performed using Prism 9.0. All of the data passed the normality test that we chose parametric analysis to determine the statistical

difference. Unpaired two-tailed Student's t-test and 1-way ANOVA were used for statistical analysis, and $p < 0.05$ was considered statistically significant. Statistical analysis methods and sample numbers are marked in the figure legends.

Results

Pharyngeal satellite cells are larger than gastrocnemius satellite cells.

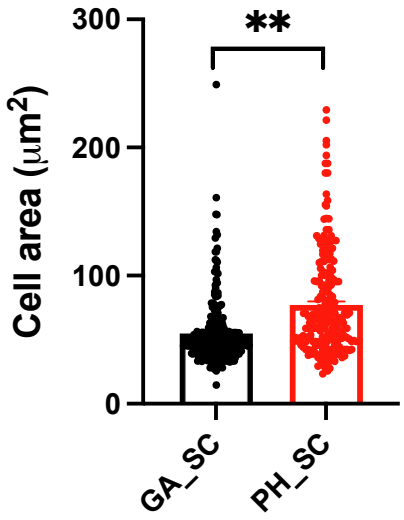


Figure 1. The average area of satellite cells. Area distribution of gastrocnemius (GA) and pharyngeal (PH) satellite cells. ** indicates statistical significance $P < 0.01$ in two-tailed student t-test comparing the average area of two populations (GA n = 6, PH n = 4).

Recent studies have found an increased activation and differentiation of pharyngeal satellite cells compared to gastrocnemius satellite cells in an absence of muscular injury (Randolph et al., 2015). Activated satellite cells have increased cell size due to their expanded metabolic needs

(Wang et al., 2017). Thus, we used Pax7 CRE^{ERT2}-tdTomato mice to label satellite cells with tdTomato red fluorescent proteins. SCs were harvested from Pax7 CRE^{ERT2}-tdTomato mice and prepared through cytospin into slide glasses. The average area of pharyngeal SCs was greater than the gastrocnemius SCs (Figure 1), indicating that the pharyngeal SCs are activated in basal muscle condition.

Proliferation and differentiation are similar for pharyngeal and gastrocnemius MPCs in vitro

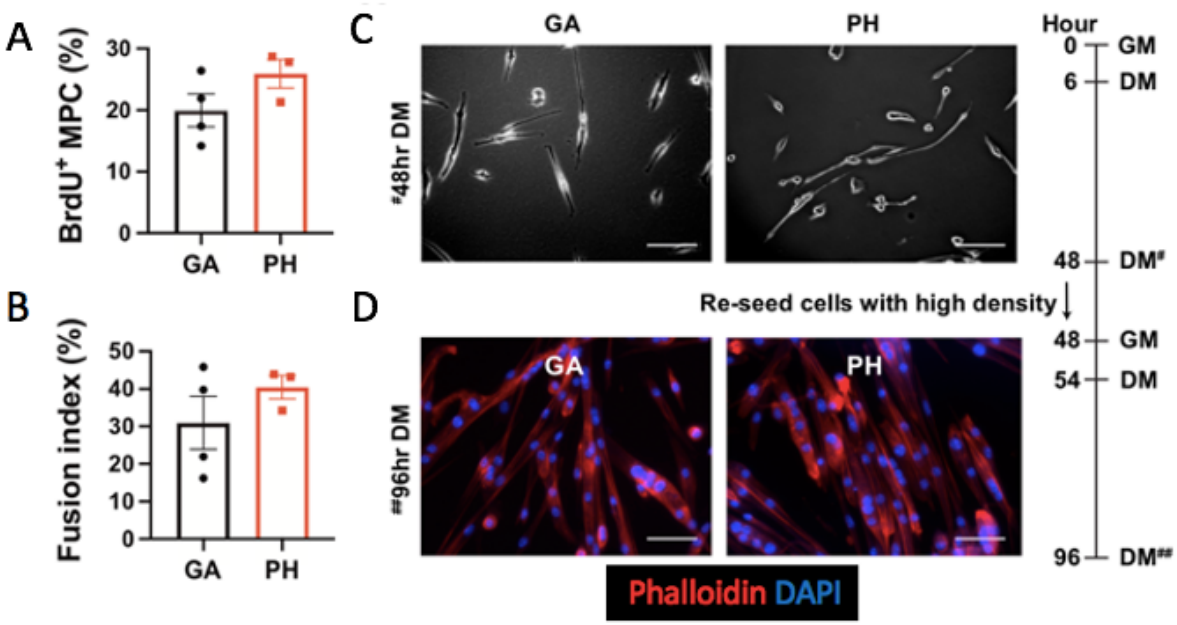


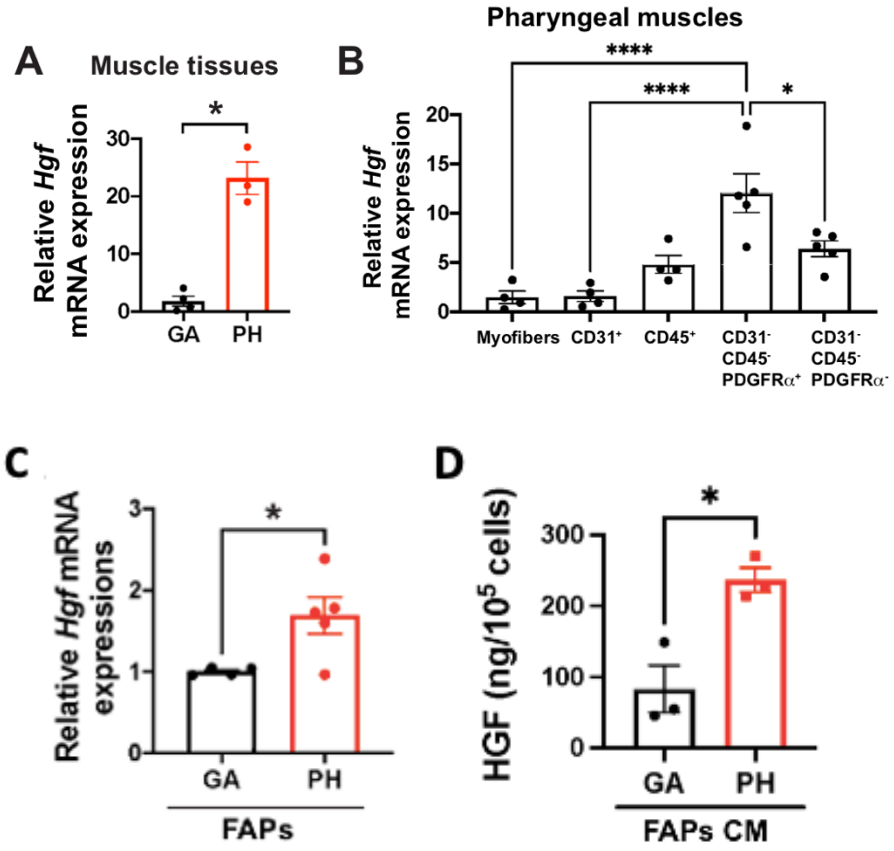
Figure 2. Pharyngeal and limb MPCs proliferate and differentiate at similar levels. **A.** Quantification of proliferation level of the gastrocnemius (GA) and pharyngeal (PH) myogenic progenitor cells (MPCs) after incubating MPCs in BrdU for one hour. $BrdU^+$ cells were quantified using flow cytometry (GA n=4, PH n=3). **B.** Percentage of fused myonuclei of the gastrocnemius (GA) and pharyngeal (PH) myogenic progenitor cells (MPCs) compared to total nuclei (GA n=4, PH n=3). **C.** Gastrocnemius (GA) and pharyngeal (PH) myogenic progenitor cells were seeded at

5 x 10^3 cells/cm^2 in growth media for 6 hours then changed to differentiation media until hour 48. Bright field images were taken after 48 hours. Scale bar = 130 µm. **D.** After 48 hours, gastrocnemius (GA) and pharyngeal (PH) myogenic progenitor cells were reseeded at 7.5 x 10^4 cells/cm^2 for 6 hours then changed to differentiation media until hour 96. Immunofluorescence staining was done after 96 hours. Scale bar = 130 µm.

In our previous study, we confirmed that the pharyngeal SCs have increased proliferation and differentiation abilities than the limb SCs *in vivo.* To determine how pharyngeal SCs show higher proliferative and differentiative ability than limb SCs, we examined the possibilities of both intrinsic and extrinsic factors that might affect SC activation. Pharyngeal SCs might intrinsically have a higher proliferation and differentiation rate than limb SCs without any intervention from the surrounding environment. To identify if pharyngeal SCs are intrinsically activated, we isolated SCs, also called Myogenic Progenitor Cells (MPCs). We compared the proliferation of pharyngeal and gastrocnemius MPCs using flow cytometry BrdU assay. BrdU is a nucleoside analog that incorporates into the cellular DNA during the S phase of cellular division, thus proliferating cells would be detected in flow cytometry as $BrdU^+$ cells. To our surprise, there was no significant difference in $BrdU^+$ pharyngeal and gastrocnemius cells ($p=0.15$) (Figure 2A). Further, we compared the differentiation potential using a two-step differentiation timeline to isolate the effect of differentiation without the proliferation rate affecting the result of differentiation (Girardi et al. 2021). We first seeded the MPCs in low density (5 x 10^3 cells/cm^2) to prevent cell-cell interaction in differentiation media for 48 hours. After two days, the same number of cells were re-seeded to high density environment (7.5 x 10^4 cells/cm^2) in differentiation media until day 4 to allow for muscular fusion and differentiation (Figure 2C, 2D). The fusion index was then calculated by

dividing the number of myonuclei, from the total number of nuclei, quantifying the percentage of cells fused. Like proliferation, there was no significant difference between pharyngeal and gastrocnemius MPCs differentiation and fusion levels (p=0.28) (Figure 2B). Thus, we concluded that the increased activation of pharyngeal MPCs were due to an extrinsic environment rather than intrinsic SC activity.

Conditioned media of pharyngeal FAPs shows increased concentration of HGF



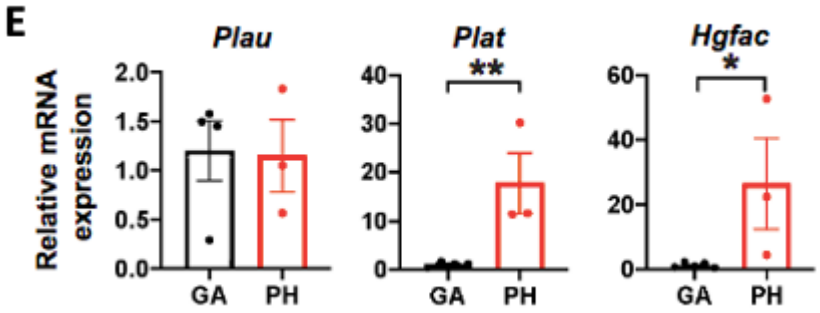


Figure 3. HGF concentration is elevated in conditioned media of FAPs. **A.** Relative expression of *Hgf* mRNA in gastrocnemius (GA) and pharyngeal (PH) muscle tissues. Statistical significance was determined using unpaired two-tailed Student's t-test. (GA n=4, PH n=3) **B.** Relative *Hgf* mRNA expression of myofibers, $CD31^{+}$, $CD45^{+}$, and $CD31^{-}/CD45^{-}$ cells of pharyngeal muscles. Statistical significance was determined using 1-way ANOVA. (n=4-5) **C.** Quantification of HGF protein expression in conditioned media (CM) of the gastrocnemius (GA) and pharyngeal (PH) fibroadipogenic progenitor (FAP) cells through ELISA. Statistical significance was determined using unpaired two-tailed Student's t-test. (GA n=4, PH n=5) **D.** Relative mRNA expression of *Hgf* of CM of the gastrocnemius (GA) and pharyngeal (PH) FAPs. Statistical significance was determined using unpaired two-tailed Student's t-test. (n=3) **E.** RT qPCR was performed to quantify the relative mRNA expression of HGF activating enzymes, urokinase-type plasminogen activator (Plau), tissue-type plasminogen activator *(Plat),* and HGF activator *(Hgfac),* normalized to hypoxanthine-guanine phosphoribosyltransferase (*Hprt),* a housekeeping gene. Statistical significance was determined using unpaired two-tailed Student's t-test. (GA n=4, PH n=5) * indicates statistical significance of $P < 0.05$.

The muscle microenvironment has been shown to contain extracellular matrix collagen, ligands, and growth factors that interact with SCs to stimulate or suppress their activation (Montarras et al., 2013). Hepatocyte growth factor (HGF) is a known growth factor that positively affects SC activity (Van Mater et al., 2015). We measured the relative mRNA level of *Hgf* in muscle tissues of the pharynx and the gastrocnemius and found a 30-fold increase in the pharynx (Figure 3A). Therefore, we sorted different types of cells that reside in pharyngeal muscles using MACs. The mRNA expression of *Hgf* was significantly increased in $CD31^{-}/CD45^{-}/PDGFR\alpha^{+}$ fraction, also known as FAPs, but not in endothelial cells ($CD31^{+}$) and hematopoietic cells ($CD45^{+}$) (Figure 3B). To confirm whether FAPs is a major source of HGF in pharyngeal muscles, we cultured FAPs from pharyngeal and gastrocnemius muscles to measure the secreted *Hgf mRNA* and HGF level in culture media. We found the culture media, also known as conditioned media (CM), of pharyngeal FAPs contained a two-fold increased level of *Hgf mRNA* transcript and HGF protein level compared to gastrocnemius FAPs (Figure 3C, 3D), confirming our hypothesis. Lastly, HGF is released in inactive pro-HGF form, which is required to be cleaved by HGF activating enzymes (Landgarf et al., 2014), such as *Plat* and *Hgfac* to be active. We investigated the activation level of HGF through quantifying the relative mRNA expression of urokinase-type plasminogen activator *(Plau),* tissue-type plasminogen activator *(Plat),* and HGF activator *(Hgfac),* normalized to hypoxanthine-guanine phosphoribosyl transferase (*Hprt)*, a housekeeping gene. The mRNA level of *Plat* and *Hgfac* were significantly higher in pharyngeal FAPs than in gastrocnemius FAPs, indicating that *Plat and Hgfac* can play an active role in converting active HGF for SC activation. In conclusion, HGF and HGF activating enzymes are released by pharyngeal FAPs, showing that FAPs play an important role in the activation of satellite cells in uninjured pharyngeal muscles.

Discussion

Satellite cells (SCs) are muscular stem cells that are quiescent in homeostatic condition in adults (Sambasivan et al., 2011). Once an injury is introduced, SCs rapidly re-enter the G_1 state from the G_0 state, differentiating and fusing into myofibers (Yin et al., 2013). Interestingly, SCs in the craniofacial muscles are constitutively activated to proliferate and differentiate without any injury (Randolph et al., 2015). We wondered if this continual activation of pharyngeal SCs is attributed to intrinsic pharyngeal SC abilities or from environmental niche factors. In this study, we found that proliferation and differentiation levels of pharyngeal and gastrocnemius SCs did not significantly differ intrinsically. Instead, the pharyngeal muscles showed an increased level of HGF and *Hgf* mRNA, indicating that cells in the pharyngeal niche can activate SCs in paracrine manner. Moreover, cultured pharyngeal FAPs showed an elevated level of HGF, *Hgf* mRNA, as well as HGF activating enzymes than gastrocnemius FAPs. We concluded that FAPs in the pharyngeal muscular niche provide HGF and HGF activating enzymes, which in turn activate SCs in homeostatic condition.

SCs in the pharynx are known to be activated in homeostatic condition without an introduction of injury, but the reason or pathway is still unknown. The activation of SCs is marked by larger cell size due to the increased energy needs to support the cytosolic organelle expansion (Rodgers et al., 2014). Accordingly, pharyngeal SCs from Pax7 CRE^{ERT2}-tdTomato mice showed increased cell size compared to gastrocnemius SCs, suggestive of activation. However, to show a definitive activation, a marker of SC activation, MyoD, expression should be examined. Our previous data showed an increased proliferation and differentiation of pharyngeal SCs compared to gastrocnemius SCs *in vivo.* To confirm if the higher proliferation and differentiation capacities of pharyngeal SCs in an intrinsic factor, we repeated a proliferation and differentiation experiment

with myogenic progenitor cells (MPCs) *in vitro,* isolating the SCs from their environmental niche. To our surprise, MPCs of the pharynx and the gastrocnemius showed similar proliferation and differentiation abilities *in vitro*. Power analyses were done on both proliferation and differentiation, showing effect sizes of d = 1.5 for both experiments. With this effect size, a sample size of 9 for each group would show a significant effect at a power of 0.8. An effect size greater than 0.8 is generally considered a large effect size. Thus, the pharyngeal SCs do not intrinsically proliferate and differentiate better than gastrocnemius SCs.

Hepatocyte growth factor (HGF) is a known activating enzyme that can positively affect SCs to proliferate and fuse into muscle fibers (Miller et al., 2000). We found an elevated level of HGF mRNA level in the pharyngeal than in the gastrocnemius tissue, suggesting that HGF is secreted into the pharyngeal muscular niche. To investigate which cell type secretes HGF in the pharyngeal niche, we used magnetic activated cell sorting to sort endothelial ($CD30^+$), hematopoietic ($CD45^+$), fibroadipogenic progenitor (FAPs) ($CD30^-/CD45^-/PDGFR\alpha^+$), and other cell types. We found that the $CD35^-/CD45^-/PDGFRa^+$ fraction, also known as FAPs, has an elevated expression of HGF mRNA. In homeostatic condition, FAPs play a role in maintenance of muscle fiber; however, in the presence of injury, FAPs assist in SC activation for muscle regeneration (Ancel et al., 2019). We cultured FAPs to ensure the elevated concentration of *Hgf* transcript level is from secreted from FAPs. The HGF protein and *Hgf* transcript level were significantly increased in pharyngeal FAPs than gastrocnemius FAPs. HGF is secreted into the extracellular matrix as inactive pro-HGF (Sisson et al., 2009). Urokinase type plasminogen activator (PLAU) is a known HGF activating enzyme that cleaves pro-HGF into an active HGF in regenerating muscles (Mars et al., 1993). Along with PLAU, other HGF activators, tissue type plasminogen activators (PLAT) and HGF activators (HGFac), were also investigated. There was

no significant difference in *Plau* transcript levels; however, the expression levels of *Plat* and *HGFac* transcripts were significantly elevated in the pharyngeal FAPs than gastrocnemius FAPs. Our data suggest that FAPs not only release HGF, but also HGF activating enzymes to induce active HGF for pharyngeal SCs to proliferated and differentiate even in homeostatic conditions.

In conclusion, our study supports that the pharyngeal niche, more specifically FAPs, can contribute HGF and HGF activating enzymes to induce SCs to proliferate in absence of injury. While the connection between FAPs and the pharyngeal niche is unclear, this study provides an insight of what might contribute to the highly activated level of pharyngeal SCs even without injury. This study is limited mostly to *in vitro* studies of FAPs. Thus, *in vivo* studies might contribute further knowledge about the role of FAPs. FAPs depleted mouse models, such as PDGFRα DTA model, might provide additional information about the *in vivo* interaction between FAPs and pharyngeal SCs.

References

Al Tanoury, Ziad et al. "Differentiation of the human PAX7-positive myogenic precursors/satellite cell lineage *in vitro*." *Development (Cambridge, England)* vol. 147,12 dev187344. 26 Jun. 2020, doi:10.1242/dev.187344

Allen, R. E., Sheehan, S. M., Taylor, R. G., Kendall, T. L., & Rice, G. M. (1995). Hepatocyte growth factor activates quiescent skeletal muscle satellite cells in vitro. *Journal of Cellular Physiology*, *165*(2), 307–312. https://doi.org/10.1002/jcp.1041650211

Ancel, S., Mashinchian, O., & Feige, J. N. (2019). Adipogenic progenitors keep muscle stem cells young. Aging, 11(18), 7331–7333. PubMed. https://doi.org/10.18632/aging.102304

Cheung, T. H., Quach, N. L., Charville, G. W., Liu, L., Park, L., Edalati, A., Yoo, B., Hoang, P., & Rando, T. A. (2012). Maintenance of muscle stem-cell quiescence by microRNA-489. *Nature*, *482*(7386), 524–528. PubMed. https://doi.org/10.1038/nature10834

Czyz, M. (2018). HGF/c-MET Signaling in Melanocytes and Melanoma. *International Journal of Molecular Sciences*, *19*(12), 3844. PubMed. https://doi.org/10.3390/ijms19123844

Donner, M. W., Bosnia, J. F., & Robertson, D. L. (1985). Anatomy and physiology of the pharynx. *Gastrointestinal Radiology*, *10*(1), 197–212. https://doi.org/10.1007/BF01893103

Evano, B., & Tajbakhsh, S. (2018). Skeletal muscle stem cells in comfort and stress. *NPJ Regenerative Medicine*, *3*, 24–24. PubMed. https://doi.org/10.1038/s41536-018-0062-3

Fiore, D., Judson, R. N., Low, M., Lee, S., Zhang, E., Hopkins, C., Xu, P., Lenzi, A., Rossi, F. M. V., & Lemos, D. R. (2016). Pharmacological blockage of fibro/adipogenic progenitor expansion and suppression of regenerative fibrogenesis is associated with impaired skeletal muscle regeneration. *Stem Cell Research*, *17*(1), 161–169. https://doi.org/10.1016/j.scr.2016.06.007

Gal-Levi, R., Leshem, Y., Aoki, S., Nakamura, T., & Halevy, O. (1998). Hepatocyte growth factor plays a dual role in regulating skeletal muscle satellite cell proliferation and differentiation. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1402*(1), 39–51. https://doi.org/10.1016/S0167-4889(97)00124-9

Girardi, F., Taleb, A., Ebrahimi, M., Datye, A., Gamage, D. G., Peccate, C., Giordani, L., Millay, D. P., Gilbert, P. M., Cadot, B., & Le Grand, F. (2021). TGFβ signaling curbs cell fusion and muscle regeneration. *Nature communications*, *12*(1), 750. https://doi.org/10.1038/s41467-020-20289-8

Khurana, T. S., Prendergast, R. A., Alameddine, H. S., Tomé, F. M., Fardeau, M., Arahata, K., Sugita, H., & Kunkel, L. M. (1995). Absence of extraocular muscle pathology in Duchenne's muscular dystrophy: Role for calcium homeostasis in extraocular muscle sparing. *The Journal of Experimental Medicine*, *182*(2), 467–475. PubMed. https://doi.org/10.1084/jem.182.2.467

Landgraf, K. E., Steffek, M., Quan, C., Tom, J., Yu, C., Santell, L., Maun, H. R., Eigenbrot, C., & Lazarus, R. A. (2014). An allosteric switch for pro-HGF/Met signaling using zymogen activator peptides. *Nature chemical biology*, *10*(7), 567–573. https://doi.org/10.1038/nchembio.1533

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods, 25(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Mars, W. M., Zarnegar, R., & Michalopoulos, G. K. (1993). Activation of hepatocyte growth factor by the plasminogen activators uPA and tPA. The American Journal of Pathology, 143(3), 949–958. PubMed.

McLoon, L., Thorstenson, K., Solomon, A., & Lewis, M. (2007). Myogenic precursor cells in craniofacial muscles. Oral Diseases, 13(2), 134–140. https://doi.org/10.1111/j.1601-0825.2006.01353.x

Miller, K. J., Thaloor, D., Matteson, S., & Pavlath, G. K. (2000). Hepatocyte growth factor affects satellite cell activation and differentiation in regenerating skeletal muscle. American Journal of Physiology-Cell Physiology, 278(1), C174–C181. https://doi.org/10.1152/ajpcell.2000.278.1.C174

Montarras, D., L'honoré, A., & Buckingham, M. (2013). Lying low but ready for action: The quiescent muscle satellite cell. *The FEBS Journal*, *280*(17), 4036–4050. https://doi.org/10.1111/febs.12372

Mootoosamy, R. C., & Dietrich, S. (2002). Distinct regulatory cascades for head and trunk myogenesis. *Development (Cambridge, England)*, *129*(3), 573–583.

Naldini, L., Vigna, E., Bardelli, A., Follenzi, A., Galimi, F., & Comoglio, P. M. (1995). Biological activation of pro-HGF (hepatocyte growth factor) by urokinase is controlled by a stoichiometric reaction. *The Journal of biological chemistry*, *270*(2), 603–611. https://doi.org/10.1074/jbc.270.2.603

Noden, D. M., & Francis-West, P. (2006). The differentiation and morphogenesis of craniofacial muscles. Developmental Dynamics, 235(5), 1194–1218. https://doi.org/10.1002/dvdy.20697

Randolph, M. E., Phillips, B. L., Choo, H.-J., Vest, K. E., Vera, Y., & Pavlath, G. K. (2015). Pharyngeal Satellite Cells Undergo Myogenesis Under Basal Conditions and Are Required for Pharyngeal Muscle Maintenance. *Stem Cells (Dayton, Ohio)*, *33*(12), 3581–3595. PubMed. https://doi.org/10.1002/stem.2098

Rodgers, J. T., Schroeder, M. D., Ma, C., & Rando, T. A. (2017). HGFA Is an Injury-Regulated Systemic Factor that Induces the Transition of Stem Cells into G(Alert). *Cell Reports*, *19*(3), 479–486. PubMed. https://doi.org/10.1016/j.celrep.2017.03.066

Rodgers, J. T., King, K. Y., Brett, J. O., Cromie, M. J., Charville, G. W., Maguire, K. K., Brunson, C., Mastey, N., Liu, L., Tsai, C.-R., Goodell, M. A., & Rando, T. A. (2014). MTORC1 controls the adaptive transition of quiescent stem cells from G0 to G(Alert). Nature, 510(7505), 393–396. PubMed. https://doi.org/10.1038/nature13255

Sambasivan, Ramkumar, et al. "Pax7-Expressing Satellite Cells Are Indispensable for Adult Skeletal Muscle Regeneration." *Development*, vol. 138, no. 17, Sept. 2011, pp. 3647–56, https://doi.org/10.1242/dev.067587.

Sheehan, S. M., Tatsumi, R., Temm-Grove, C. J., & Allen, R. E. (2000). HGF is an autocrine growth factor for skeletal muscle satellite cells in vitro. Muscle & Nerve, 23(2), 239–245. https://doi.org/10.1002/(SICI)1097-4598(200002)23:2<239::AID-MUS15>3.0.CO;2-U

Sisson, T. H., Nguyen, M.-H., Yu, B., Novak, M. L., Simon, R. H., & Koh, T. J. (2009). Urokinase-type plasminogen activator increases hepatocyte growth factor activity required for skeletal muscle regeneration. Blood, 114(24), 5052–5061. PubMed. https://doi.org/10.1182/blood-2008-12-196212

Van Mater, D., Añó, L., Blum, J. M., Webster, M. T., Huang, W., Williams, N., Ma, Y., Cardona, D. M., Fan, C.-M., & Kirsch, D. G. (2015). Acute tissue injury activates satellite cells and promotes sarcoma formation via the HGF/c-MET signaling pathway. *Cancer Research*, *75*(3), 605–614. PubMed. https://doi.org/10.1158/0008-5472.CAN-14-2527

Wang, X., Fujimaki, K., Mitchell, G. C., Kwon, J. S., Della Croce, K., Langsdorf, C., Zhang, H. H., & Yao, G. (2017). Exit from quiescence displays a memory of cell growth and division. *Nature Communications*, *8*(1), 321. https://doi.org/10.1038/s41467-017-00367-0

Yin, H., Price, F., & Rudnicki, M. A. (2013). Satellite cells and the muscle stem cell niche. Physiological Reviews, 93(1), 23–67. PubMed. https://doi.org/10.1152/physrev.00043.2011