Sciential

McMaster Undergraduate Science Journal

www.scientialjournal.com

Issue 1 | November 2018

TABLE OF CONTENTS

1 Editors' Letter

2 Characterizing a new species of Nematoda using genetic and morphological analyses

9 The effect of applying starch onto Arabidopsis thaliana on the feeding behaviour of Myzus persicae

16 Induced pluripotent stem cells: Acquirement, characteristics and medicinal applications

23 How our healthcare system failed during the SARS outbreak

25 Evading evasion: How phages get around the CRISPR-Cas

28 What's wrong with me? What's wrong with you? The issue of over-diagnosing ADHD in children

31 Innovative commercial and private genetic testing raises privacy and confidentiality concerns

34 Sciential Team

Layout Editor: Abhiti Kuhad

Dear Reader,

We are thrilled to welcome you to *Sciential's* debut issue. We are here to promote science communication and advance scientific inquiry by offering the McMaster community a variety of broad-scoped peer-reviewed literature pieces and publishing opportunities. Recognizing that our readers take pleasure in learning about a vast variety of topics, *Sciential* is committed to presenting interdisciplinary content as we seek to showcase works from all programs within the Faculty of Science. The journal's ultimate goal is to increase the accessibility of informative scientific works, thus contributing to an improved understanding and appraisal of scientific discourse, while providing an opportunity for readers to learn about the interesting and innovative work that is being conducted by McMaster students.

With the intention to substantially enhance your experience with the journal, we have developed a unique colour scheme representing the different types of literature pieces. You will find that all of the original research works such as student theses, independent study projects, and coursework components are represented in coral red. We hope this section will showcase the McMaster student body as a research-driven community. The academic literature reviews are represented in teal green, and offer a comprehensive overview of a particular topic. The opinion pieces, represented in light purplish blue, demonstrate a view on popular subject matter supported by peer-reviewed literature.

In this issue, you will be able to indulge in a multitude of topics covering aspects of health policy, psychology, molecular biology, and genetic testing. The issue opens with an original research by Mostafa Mohammed Elsabagh describing a new species of Nematoda, a class of invertebrate species used as trivial model organisms in scientific research. Following is the work of Ishita Paliwal et al. reporting on the change of feeding behaviour of an insect, *M. persicae*, upon varying starch exposure of the *A. thaliana* plant. The publication also features an academic literature review by Abdullah El-Sayes describing insights into stem cell acquisition, characteristics, and the notable relevance of stem cell research in modern healthcare. The issue closes with four captivating opinion pieces by Bianca Colarossi, Mostafa Mohammed Elsabagh, Tyler Redublo, and Syeda Masooma Zaidi.

The *Sciential* Team expresses sincere gratitude to the faculty and staff of the School of Interdisciplinary Sciences (SIS), including Dr. Kimberley Dej, Dr. Veronica Rodriguez Moncalvo, Dr. Katie Moisse, and the incredible Science Librarian, Abeer Siddiqui for their utmost support with the establishment and development of our initiative. We are thankful to the Science Initiative Fund (SIF) from the McMaster Science Society (MSS) for their generous funding in support of *Sciential.* We would like to acknowledge Abdullah El-Sayes and Tyler Redublo, the senior editors, for their endless effort to manage submission flow and ensure supreme quality of the published pieces. We would like to extend our appreciation to the incredible team of editors and graphic designers who have worked diligently and devoted an extensive amount of their time to bring *Sciential's* first issue to you. We genuinely hope that you enjoy your time reading the journal and will return for our future issues.

Aiman Shahid
Editor-in-Chief

Alisa Nykolayeva
Editor-in-Chief

@SCIENTIALJOURNAL

@MACSCIENTIAL

JOURNALS.MCMASTER.CA/SCIENTIAL

SCIENTIAL@MCMASTER.CA

Layout Editor: Abhiti Kuhad
Youssef El-Sayes

ORIGINAL RESEARCH

OPEN ACCESS

Characterizing a new species of *Nematoda* using genetic and morphological analyses

Mostafa Mohammed Elsabagh, Kimberley Dej, PhD

McMaster University, Biomedical Discovery and Commercialization, Class of 2020

ARTICLE INFORMATION

Received 14 October 2018
Accepted 1 November 2018

Available online:
26 November 2018

Editor:
Jennifer Williams

Layout Editor:
Athena Chang

ABSTRACT

Nematodes (Nematoda) are slim tubular worms ranging between 0.5 mm – 2 mm in length and 10 to 100 µm thick. They have effectively adapted to inhabit all regions of the Earth, but are most commonly found in soils, decomposing vegetation, and freshwater sources. *Ceanorhabditis elegans (C. elegans)*, an important member of this phylum, is a valuable model system. Owing to its small, fully sequenced genome, it is typically used to model the development of some diseases, such as neurodegenerative diseases. Nematodes are highly diverse, with over 30,000 species having not yet been described. While *C. elegans* will continue to be the primary model species, the classification of previously unknown species is valuable as it allows for study of the evolutionary pathway leading to each species, behavior and instincts, and how such animals behave as parasites. This diversity is exciting, and Drs. Kimberley Dej and Bhagwati Gupta work with students to document new species. In the laboratory, we use morphological analysis of the mouth, the pharynx, and the tail, combined with data generated by sequencing the 18S small ribosomal subunit rRNA gene to explore and document these new species. Here, we discuss how it was determined that a unique specimen collected from the Hamilton, Ontario area was found to have features of multiple genera: *Oscheius* and *Ceanoreabditis*.

Keywords: Nematode diversity, speciation, genetic analysis, sequencing, morphological analysis

INTRODUCTION

C. elegans and other nematodes continue to be major model organisms in biology, however, the known species of this family of invertebrates represents only 45% of an estimated 50,000 unique species[1]. Nematodes are diverse colonizers of microbe-rich habitats; rotting vegetation unites three of the most important lab model organisms in the same ecological niche; *S. cerevisiae, D. melanogaster, and C. elegans*. For example, analyses conducted in silico on the *C. elegans* genome have identified nearly a thousand G-protein coupled receptors which evolve at rapid rates, with evidence pointing to positive selection[1]. This great variation occurs as a result of nematodes seeking new ways to endure harsh conditions in unfamiliar environments, which is driven by natural selection[1]. *C. elegans* and other nematodes can be found to endure environments of hypoxia, osmotic stress, heat, cold, pathogens, and other toxins[1]. Logically, to understand how nematodes can successfully endure these conditions, new species need to be discovered and categorized into a

© 2018 Sciential

library. Currently, it is estimated that 30,000 species of nematode have yet to be described. The purpose of this study was to contribute to the building of this library by searching for a new species of nematode and using morphological and genetic analysis to verify its novelty.

LIFE CYCLE OF C. ELEGANS

It is important to first consider the life cycle of *C. elegans* and other nematodes. The life cycle of *C. elegans* begins with a 3.5 to 4-day embryonic developmental period, part of which occurs in the mother's uterus[2]. At this point, the nematodes exist as eggs[2]. Following this period, the eggs hatch and the resulting larvae- identical to adults with exception to their underdeveloped reproductive system- live through four stages of life, L1 to L4[2]. Each of these stages are separated by periods of lethargus and moulting. The natural environment of *C. elegans* has an influence on its development. When young larvae are exposed to environments of crowding by pheromone sensation, food depletion, and high temperature, they interrupt their developmental cycle and enter an alternative stage known as the dauer stage[1,2]. During the dauer stage, they possess reduced metabolism and increased stress resistance[2].

NEMATODE ANATOMY

Nematodes are slender creatures, and have a fairly linear morphology with several tubular tracts within them; one forms the digestive tract and the other forms the reproductive tract[1]. Morphological analysis in this study involved analysis of the mouth, pharynx, and tail regions.

Mouth

The mouth is found at the very front of the nematode and is a key characteristic involved in identifying the family of most nematodes[3]. Four distinct mouth families exist. *Rhabditidae* mouths have several protrusions at the opening of a long, narrow cylindrical tract, called a stoma[3]. *Diplogastridae*, a family which includes *Pristionchus pacificus*, contains a shorter stoma with characteristic teeth, providing it with the ability to consume live *C. elegans* when other food is scarce[3]. Finally, *Panagrolaimidae* and *Cephalobidae* have mouths where the stoma has been strengthened with hardened sclerotin[3] (Figure 1).

Pharynx

The pharynx extends from the mouth into the beginning of the digestive tract. Generally, for the most commonly studied nematode, *C. elegans*, the mouth has 4 characteristic portions: the procorpus, the bulb-like metacorpus, the isthmus, and the terminal bulb[4] (Figure 2). The functions of the metacorpus and terminal bulb are similar, in that they are responsible for transporting food, usually bacteria, into the digestive tract while also grinding it for more efficient digestion[4]. The pharynx varies between genuses, therefore it forms a reliable method of distinction between different samples that are isolated. When compared to another commonly studied soil nematode, *Oscheius tipulae*, it is found that rather than having a distinct metacorpus, the oesophageal muscles are spread out from the procorpus through to the terminal bulb[4].

Tail

The tail is unique in that it is the only source of sexual dimorphism occurring outside of the internal anatomy of nematodes[3]. Most *C. elegans* worms are born as hermaphrodites, which means that they have both male and female reproductive systems. Males develop as a result of non-disjunction of the sex chromosomes during meiosis, which is a rare event[3]. The male tail is different from a hermaphrodite's by having a fan-like projection at the end of the tail[3]. The tail is where the nematode's body tapers in an asymmetrical manner, and contains the rectum[1,3]. For morphological analysis, the tail can be used to distinguish between species by comparing the side which the rectum exits to[3].

MATERIALS AND METHODS

Collection and Decontamination

Samples of decomposing vegetation were collected from different regions of the McMaster University campus in Hamilton, Ontario. Most samples were collected from the McMaster community garden. The community garden contains many different ecological niches within which a diverse collection of nematode species may live. This, in turn, would improve the likelihood of finding a new species, and so therefore provided a preferred place of collection.

© 2018 Sciential

Portions of collected specimens were then transferred into 50 mm petri dishes with Nematode Growth Media (NGM) seeded with Escherichia coli. The animals were then kept at room temperature (20 °C to 25 °C) and serially transferred to new plates to decontaminate them from mites, molds, and fungi.

Clonal Colony Formation

Out of the 9 different samples, animals at adult stage were selected and placed as individuals into new NGM plates to grow into new clonal colonies. As known from their lifecycle, selection of adults allows for both gonochoristic and hermaphroditic animals to be bred, as the potential fertilization event has already occurred[5]. The worms were observed and kept at room temperature and success was determined if they produced a clonal colony with a high number of individuals (50+ per entire 50 mm dish).

Reproductive Methods

Out of 5 different clonal colony formation attempts, a single clonal colony was selected for further testing and work within the study: sample M.1.1.1. Several young animals, preferably between the L2 and L4 stage, were selected from the M.1.1.1 clonal colony and transferred to individual NGM plates and observed for clonal colony formation according to the previous criteria of 50+ individuals per 50 mm dish. Young worms were selected to eliminate the chance of a fertilization event from occurring, in the case that the species was gonochoristic. Formation of a clonal colony indicates that the species is not gonochoristic, which introduces the possibility for a hermaphroditic or parthenogenetic species[6].

Morphological Analysis

Nomarksi microscopy was used for the morphological analysis. To prepare the worms for observation, the WormAtlas agar pad protocol was followed[7]. A solution of 5% agarose in PBS was first melted, from which a drop was dispensed onto a glass microscope slide. A second slide was laid upon the drop of agarose solution to flatten it. Then, a drop of 10 mM NaN3 solution was dispensed onto the agarose flat pad after the flattening slide was removed. 5-10 animals were placed onto the drop of NaN3. Once the animals were motionless, a glass coverslip was added above the worms and the now prepared slide was observed at 20x, 40x and 60x objective magnification using Nomarski prisms.

Genetic Analysis

Adult worms were first digested in proteinase K. Polymerase chain reaction amplified the genomic content of the samples and was followed by separation of cell contents by gel electrophoresis to yield pure genomic DNA. A third-party DNA sequencing service (MOBIX Lab) returned the gene sequence obtained by Sanger-sequencing. The sequence was then compared to known sequences using the National Library of Medicine Blastn service.

RESULTS AND DISCUSSION

MORPHOLOGICAL ANALYSIS

Mouth

When the mouth of sample M.1.1.1 was compared to common mouth types of different nematode families, it was found that it showed very strong resemblance to the mouths of the *Rhabditidae*: a long and narrow stoma, preceded by protrusions at the front of the mouth which form the opening/closing mechanism of the mouth (Figure 3).

Pharynx

Knowing that the sample M.1.1.1 is likely a *Rhabditid*, the study of pharynxes was narrowed to members of that family. Comparison with *C. elegans* was performed initially as it was the most common. It was found that the pharynx of M.1.1.1 clearly resembles those of the *Oscheius genus,* as it lacks the metacorpus of a *Caenorhabditid* (Figure 4).

Tail

The final criterion for the morphological analysis of M.1.1.1 was comparing its tail to that of two other species, specifically *Ceanorhabditis elegans* and *Oscheius tipulae*. These two were chosen from insights generated from the pharyngeal analysis, as it was not anticipated that the tail would provide specific categorization abilities earlier in the study. When compared, it was found that M.1.1.1 had two features of its tail that were common to that of *C. elegans*. The tail of M.1.1.1 was moderately long (80 µm), with two faces; one linear and the other curved. The rectum and its opening were on the straight/linear face of the tail, a feature that is found in *C. elegans*. When examining

© 2018 Sciential

images of the tail of *O. tipulae*[8], it was clear that the rectum's position on the tail was reversed, which discounted support for M.1.1.1's inclusion into the *Oscheius genus* (Figure 5).

REPRODUCTIVE METHODS & GENETIC ANALYSIS

When individuals of the M.1.1.1 sample were placed independently on new seeded NGM dishes, it was found that they consistently produce full and healthy clonal colonies with nearly 50+ individuals by 72 hours (over 7 attempts). This provides evidence for the claim that M.1.1.1 is likely hermaphroditic. When the 18S small ribosomal subunit rRNA gene was sequenced and alignment generated, results returned very high (99%) query coverage with the 18S small ribosomal subunit rRNA gene of *C. briggsae* (Figure 6).

CONCLUSION

The purpose of this study was to use morphological and genetic analysis to characterize an unknown specimen of nematode from Hamilton, Ontario. Examination of the mouth indicated support for a *Rhabtididae* family. The genuses *Ceanorhabditis* and *Oscheius* were contenders from examination of the rectum and pharynx respectively. Considering the genetic similarity to *C. briggsae* and the hermaphroditic nature of M.1.1.1, we believe this sample is a variant of the *Oscheius* genus with deep influence from the *Ceanorhabitis* genus, a novel finding when consulting known phylogenies. However, additional studies into this unknown specimen is necessary, in order to conclude that it is indeed novel and not a consequence of genetic variability.

ACKNOWLEDGEMENTS

This study would not have been possible without the exceptional guidance, mentorship, and help of supervisor, Dr. Kimberley Dej. Thanks are extended to Mr. Ryan Belowitz, for his advice during analysis and for providing the necessary instruction and facilities for Nomarski/DIC microscopy. Thanks are also extended to Dr. Bhagwati Gupta and PhD candidate Avijit Mallick for providing additional microscopy facilities and instruction on nematode morphology. Finally, we appreciate the exceptional work by the third-party DNA sequencing service, MOBIX.

Refer to next page for appendix

REFERENCES

(1) Félix M-A, Braendle C. The natural history of Caenorhabditis elegans. Curr Biol. 2010.

(2) Byerly L, Cassada RC, Russell RL. The life cycle of the nematode Caenorhabditis elegans. Dev Biol [Internet]. 1976;51(1):23–33. Available from: http://www.sciencedirect.com/science/article/pii/0012160676901196

(3) Barrière A, Félix M-A. Isolation of C. elegans and related nematodes. WormBook. 2006;1–9.

(4) Mango SE. The C. elegans pharynx: a model for organogenesis. In: WormBook: The Online Review of C elegans Biology. The C. elegans Research Community; 2007.

(5) Corsi A, Wightman B, Chalfie M. A Transparent window into biology: A primer on Caenorhabditis elegans. In: WormBook: The Online Review of C elegans Biology [Internet]. 1st ed. Pasedena; 2005. Available from: https://www.ncbi.nlm.nih.gov/books/NBK299460/

(6) Kiontke K, Fitch DHA. The phylogenetic relationships of Caenorhabditis and other rhabditids. WormBook. United States; 2005 Aug;1–11.

(7) Driscoll M. Mounting Animals for Observation with Nomarski DIC Optics [Internet]. Wormatlas. 2009. Available from: http://www.wormatlas.org/agarpad.htm

(8) Félix M-A. Oscheius tipulae. In: WormBook: The Online Review of C elegans Biology. The C. elegans Research Community; 2006.

© 2018 Sciential

APPENDIX

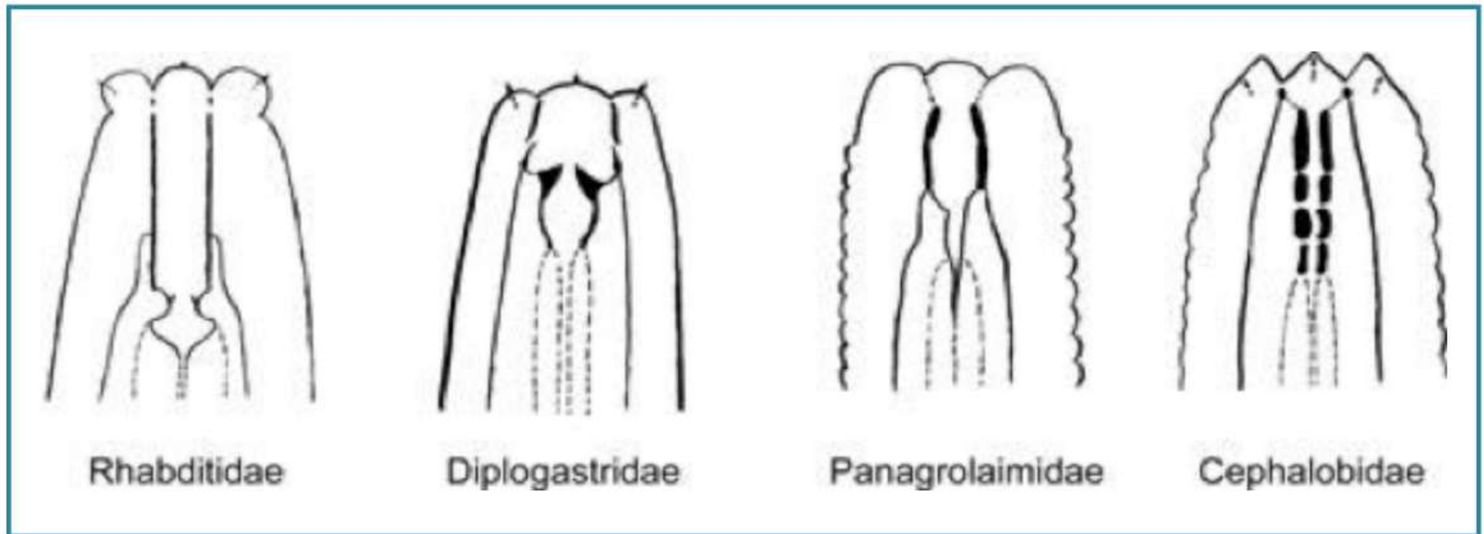


Figure 1 - Four nematode mouth families that form as a guide to examination of the mouth[3].

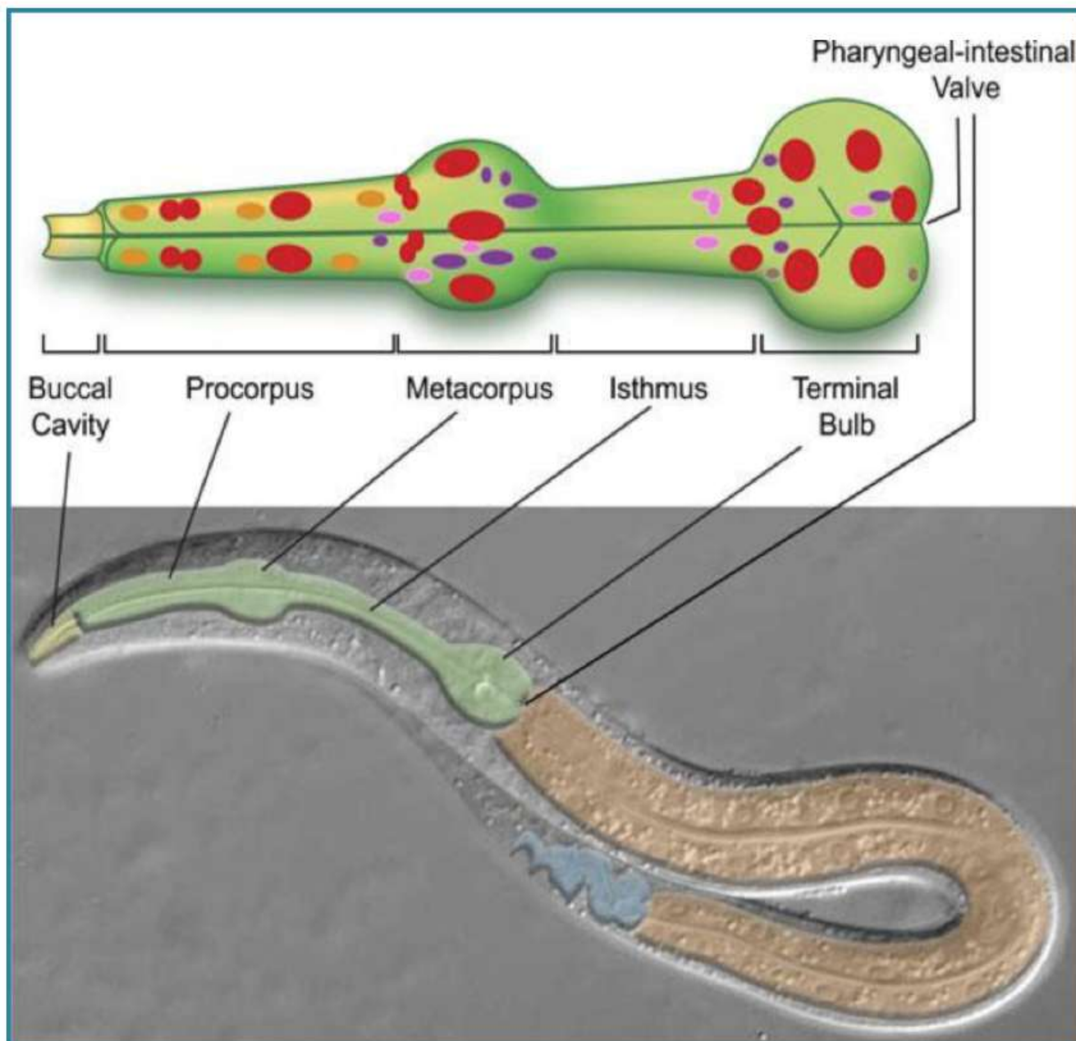


Figure 2 - The pharynx of the commonly studied nematode, *C. elegans*. There are four distinct regions of the pharynx, each of which are essential to transportation of food into the digestive tract[4].

© 2018 Sciential

A

Rhabditidae

Diplogastridae

Panagrolaimidae

Cephalobidae

B

40 µm

C

Figure 3 - Morphological Analysis of the mouth. (A): The four different mouth families of nematodes. (B) The mouth of sample M.1.1.1 at 60x oil immersion objective, with arrowheads highlighting the front mouth protrusions. (C) The mouth of *C. elegans,* which is very similar in structure to that of M.1.1.1.[3].

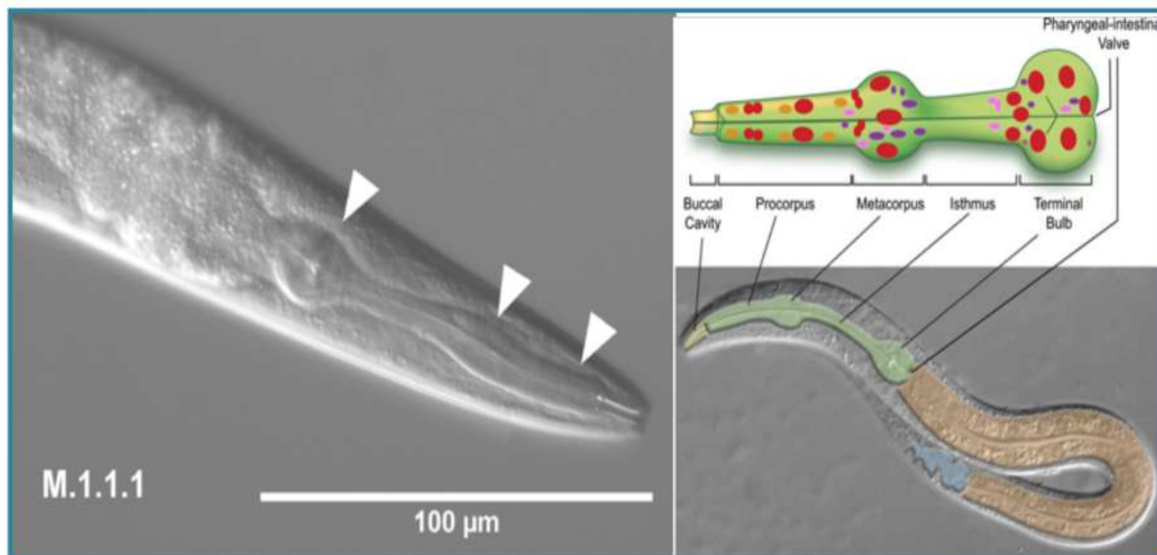


Figure 4 - Comparison of the pharynx of sample M.1.1.1. to the pharynx of *C. elegans*, a member of the same family. It is clear that the sample M.1.1.1. lacks the metacorpus which would have been found at the middle arrowhead. Right image[4].

© 2018 Sciential

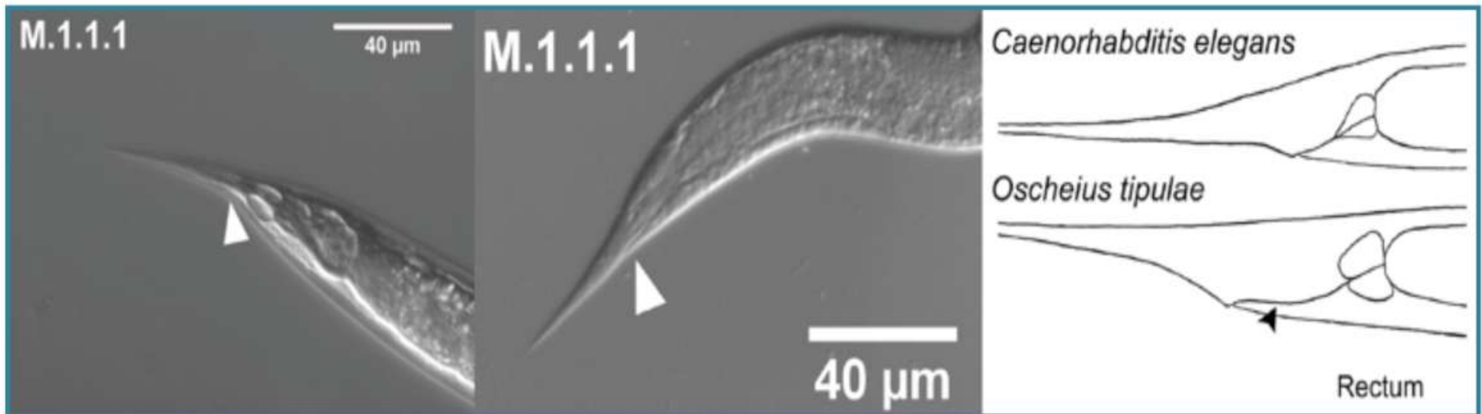


Figure 5 - Comparison of the tail of sample M.1.1.1. (two left-most images) with known morphological reproductions of C. elegans and O. tipulae. Right-most image[8].

Caenorhabditis plicata
56 leaves
5 leaves
Caenorhabditis sp. 20 KK-2011
5 leaves
Caenorhabditis japonica
Caenorhabditis japonica
15 leaves
Caenorhabditis sp. JU727
Caenorhabditis sinica
Caenorhabditis sp. 16 KK-2011
Caenorhabditis brenneri
Nematoda sp. SCN4
Caenorhabditis nigoni
Caenorhabditis remanei
Caenorhabditis briggsae
Caenorhabditis briggsae
4 leaves
Caenorhabditis sp. JLR-2009
unknown
Caenorhabditis briggsae

Figure 6 - A tree representing the distance of the alignment results of the 18S small ribosomal subunit rRNA gene. M.1.1.1. appears to have some genetic relation to *C. briggsae*.

© 2018 Sciential

ORIGINAL RESEARCH

OPEN ACCESS

The effect of applying starch onto *Arabidopsis thaliana* on the feeding behaviour of *Myzus persicae*

Ishita Paliwal, Caitlin Reintjes, Pamela Schimmer, Mary Anne Schoenhardt, Jasmine Yang

McMaster University, Honours Integrated Science, Class of 2021

ARTICLE INFORMATION

Recieved 17 October 2018
Accepted 11 November 2018

Available online:
26 November 2018

Editors:
Amama Khairzad
Bianca Colarossi
Gabrielle Gonsalves

Layout Editor:
Youssef El-Sayes

ABSTRACT

It is well known that plant-animal systems interact in many complex ways, and each organism must adapt and develop mechanisms to best survive in their given conditions. While much is understood about the plant *Arabidopsis thaliana* and the aphid *Myzus persicae*, additional research must be conducted to gain more knowledge about the interactions between the two species. As a defence mechanism, in response to aphid feeding, *A. thaliana* converts sucrose into starch. Due to a lack of sucrose, there is less feeding by *M. persicae*. However, it has not yet been shown if these aphids are able to detect an increase in starch and recognize this as a deterrent to feeding. To test this, varying concentrations of potato starch were applied mechanically to *A. thaliana* (n=36) and the effect on aphid population size and plant health was analyzed. The research team found that *M. persicae* do not detect higher starch levels on *A. thaliana* as an indicator that nutrient availability on the plant is limited. Instead, it was found that on all but one plant, high starch concentration was a factor in plant deterioration. Thus, the research team advises against using starch as an organic pesticide. The findings of this study are significant as they will contribute to a better understanding of the organisms that threaten plant health, which will prove to be useful in the maintenance of various food crops.

Keywords: *Arabidopsis*, *Myzus*, aphid, starch, feeding, herbivory

INTRODUCTION

Myzus persicae, commonly referred to as the green peach aphid, is one of the most economically important crop pests worldwide[1]. They are generalist herbivores[2] that are globally distributed and have a host range of over 400 different species[1]. In addition to damaging its host plant through feeding, *M. persicae* is a very strong viral vector which can cause the plant to sustain viral damage[3,4]. The main method in controlling *M. persicae* has been using chemical insecticides. The widespread use of these insecticides, aside from being harmful to the environment, has led aphids to develop resistance to many of them[1,5].

When *M. persicae* feeds, it inserts its stylus into the phloem of the plant, effectively wounding it. As a response to the aphid, plants have developed various

© 2018 Sciential

mechanisms of defense. In *A. thaliana*, one of these mechanisms involves the conversion of sucrose to starch in plant leaves[6,7]. This process is controlled by the enzyme 9 trehalose-6-phosphate synthase 1 (TPS11 9), which promotes the accumulation of starch. *A. thaliana* upregulates the gene that codes for this protein after attack by *M. persicae*[6]. *M. persicae* infestation on *A. thaliana* has also been shown to upregulate the gene responsible for synthesizing ADP-glucose, the sugar that is donated to a growing starch chain. Similar studies have been conducted suggesting that these effects are also seen in tomato plants[2]. In addition, *M. persicae* numbers were found to be lower on plants that were mutant for starch synthase III, which caused starch to be hyper-accumulated. From these findings, it was suggested that starch accumulation in *A. thaliana* deters feeding by *M. persicae*[8,6].

A. thaliana is frequently used in laboratory studies as a model since it is easy to grow and has a fast reproduction cycle[9,10]. For the purpose of this experiment, *A. thaliana* and *M. persicae* are used as a model system to determine how starch applied to the plants will affect the feeding behaviour of *M. persicae*. Since glucose level will not be altered in any of the plants, it is possible to attribute any feeding behaviour changes to the increase in starch levels. Starch will be applied by coating *A. thaliana* with varying concentrations of potato starch. This procedure was chosen since both potato starch and starch produced by *A. thaliana* as a defence mechanism have rounded, discoid granules and appear similar in conformation (although potato starch granules are larger)[9,11,12]. In vivo, potato starch has been found to cause a decreased level of probing from *M. persicae* when compared with a control, also supporting the use of potato starch as a treatment[8].

If the levels of starch on *A. thaliana* have a negative effect on *M. persicae*, it is expected that lower numbers of *M. persicae* will be observed. When under high levels of stress and in environments, *M. persicae* will start to reproduce to create alate morphs. If not under stress, *M. persicae* will be wingless; however, if they are in a stressful environment, their offspring will have wings, allowing for movement to a new location. A complete life cycle of *M. persicae* can occur in 10-12 days, meaning that these changes can be easily and quickly observed[13,14]. If any alate forms are observed on *A. thaliana*, this will also show that *M. persicae* are not receiving enough nutrients, and act as an indicator that the starch has affected their ability to feed.

The null hypothesis for this experiment is: "Different concentrations of starch coatings on the exterior of *A. thaliana* will not affect the feeding behaviour of *M. persicae.*" Conversely, the corresponding alternative hypothesis for the research question is: "Covering *A. thaliana* plants in varying concentrations of starch will have an effect on the feeding behaviour of the green peach aphid." The research team predicts that as starch concentration increases, there will be a negative effect on aphid population. The findings of this experiment may contribute to the development of a novel and effective insecticide specific to *M. persicae* that is natural and easy to obtain. This may be used to decrease the usage of commercial pesticides, which are more harmful to the environment and which *M. persicae* have developed a resistance towards[1]. Furthermore, these findings could promote the development of similar insecticides for economically significant crops such as canola, which is also consumed by *M. persicae*[15].

MATERIALS AND METHODS

Obtaining the Study System

A. thaliana seeds that were dried and sealed in envelopes in 2014 were suspended in 1.5 mL of distilled water kept in Eppendorf tubes. Suspensions were placed in the refrigerator to mimic the conditions of springtime, optimizing growth and maintaining natural conditions. Pots were prepared by packing soil and pouring water on top of, as well as underneath the soil, until it was completely soaked. After refrigeration, seeds were placed onto a plate and two seeds were placed in each pot of soil using a micropipette. One was planted in the middle of the pot and the other was planted near the side, as the *A. thaliana* seeds have 70% success rate of establishment. If any of the seeds did not grow, they would be removed from the pot. If two of the seeds succeeded in growing within the same pot, the roots of one plant were dug up and transferred into a pot without any plants. In the end, each pot had one plant. Plants were subjected to 14 hours of light per day for three weeks and then 10 hours of light per day afterwards. Following the third week of growth, fertilizer was applied across the top to prevent damage to the roots. A batch of 500 aphids were ordered from two federal labs. One is the lab of Jean-Philippe Parent, Research Scientist in Entomology, Agriculture and Agri-Food Canada, Government of Canada, Vineland, ON. The other is Agriculture and Agri-Food Canada/Agriculture et Agroalimentaire Canada, Frederic-

© 2018 Sciential

Fredericton Research and Development Centre. These aphids were inoculated on *A. thaliana* in domed carriers. In the end, there was a batch stock of 2000-3000 aphids.

Manipulating the Study System

Four stock solutions of Clubhouse potato starch dissolved in water were prepared in four labelled spray bottles. The solutions were divided into a control (0%), low (10%), medium (30%), and high (50%) concentrations of potato starch, created by weighing 0 g, 20 g, 60 g, and 100 g of starch respectively and dissolving each sample in 200 mL of water. *A. thaliana* plants in the rosette phase (n=36) were divided into four groups of nine plants per treatment. For each treatment, plant samples were sprayed five times from a distance of approximately 48 cm from the plant. During this time, the plants were also rotated to ensure that they were entirely covered with the starch solution on both sides of the leaves and on the stem. Solutions were mixed constantly by vigorously shaking the bottle in between sprays to ensure even distributions of the starch.

A stratified random design was achieved by numbering plant samples from 1-36 with plants 1-9 corresponding to the control (red tape), 10-18 corresponding to 30% starch solution (green tape), 19-27 corresponding to 50% starch solution (blue tape), and the 28-36 corresponding to 10% starch solution (yellow tape). Three covered trays contained 12 plants each. Using an online random number generator, a random sequence of numbers from 1-12 were generated. The first three numbers in the sequence corresponded to the positions of the control plants, the next three numbers correspond to low concentrations, the following three numbers corresponded to the medium concentration, and the last three numbers corresponded to the high concentration. Figure 1 is the layout of the position of plants on each tray; three plants were placed per row, with a total of four rows. When placing the plants, care was taken to ensure that they were evenly spaced across the tray so that none of the plants were touching, to reduce the likelihood of aphids transferring between plants within a tray.

Plants were left to dry for an hour before *M. persicae* inoculation. Using a probe, aphids were taken from a variety of plants within a stock tray and two aphids were inoculated on each plant. While inoculating, aphids were placed on top of two leaves on opposite sides of the plant, and aphid colour was recorded.

1	2	3
4	5	6
7	8	9
10	11	12

Figure 1 - Layout of plant position numbers for each of the three trays.

Data Collection

The number of living aphids on each plant, the location of the aphids on the plant, the colour of the aphids, the height of each plant, as well as observations on plant health were then recorded on days one, four, six, eight, and eleven. On each of the data collection days, two researchers counted and measured the aforementioned traits of the same plants. Results were compared to reach a consensus in order to maintain accuracy of any observations made. Days one, four, six, eight and eleven were chosen for collecting data and recording observations. This was to maintain approximately equal spacing between data collection days after inoculation. Between data collection times, trays were covered with a translucent plastic lid to prevent aphids from escaping.

Statistical Methods

A two-way ANOVA and a post-hoc Tukey test were used to analyze the data. The number of aphids counted was transformed on a log(n+1) scale for analysis purposes and analyzed in the software R (R v.3.4.4; The R Foundation for Statistical Computing, 2018).

The differences found between the average number of aphids in 0%, 10%, 30%, and 50% starch concentration levels were measured using a two-way ANOVA, comparing number of aphids with concentration levels, with a covariance analysis on time and an interaction with trays. Testing with a tray interaction would indicate whether or not a block effect is present, despite using a stratified random design. The interaction between starch concentration and time was shown to be insignificant in its effect on aphid number ($F_{15,190}$=0.23, P=0.98), and therefore a three-way ANOVA was not used. To gain a better understanding of our data, a post-hoc Tukey test was conducted to determine which level(s) in our treatment groups of starch concentration and trays caused significant differences between the means of *M. persicae* population size.

© 2018 Sciential

RESULTS

QUALITATIVE ANALYSIS

Trends between the varying concentrations of starch

Plants with solutions of 0% and 10% starch concentration mainly experienced a decrease, then increase in aphid number, with a few cases of disappearance. Plants with solutions of 30% starch concentration also experienced a similar trend of decrease, followed by an increase in aphid number. Finally, plants with solution of 50% starch concentration primarily demonstrated cases of aphid disappearance.

Plants 4 and 21 from the 0% and 50% starch concentration solutions were initially inoculated with red aphids. However, throughout the 11-day period red aphids appeared on two different plants with 0% starch concentration solution and on one plant with 10%, 30% and 50% starch concentration solutions each (excluding plants 4 and 21). Most plants had aphids that were initially found under leaves, which eventually relocated to the plant stems. In addition, a majority of plants from all treatment groups had yellowing leaves. On plants sprayed with 30% and 50% starch concentration solutions, there was greater deterioration and moulding of leaves. In terms of plant height, all four treatment groups experienced an initial growth period, which was then followed by a subsequent decrease.

Trends between the trays

The first, second and third trays experienced 17%, 50%, and 42% of cases of aphid disappearance respectively. The first and third trays only contained one plant with red aphids, whereas the second tray contained three plants, each with their own population of aphids. Overall, most aphids progressed from being on leaves to stems. The second and third trays' plant samples experienced yellowing of their leaves, but also exhibited budding. Conversely, the first tray's plant samples demonstrated an equal spread in terms of overall plant health. An initial increase followed by a decrease in plant height was prominent in all trays. Furthermore, the disappearance of aphids and plant health was similar in the second and third trays.

QUANTITATIVE ANALYSIS

In terms of aphid population, time ($F_{5,199}$=5.5386, P<0.001), starch concentration ($F_{3,199}$=3.4180, P<0.05), and trays ($F_{2,199}$= 9.7554, P<0.001) were all significant independent variables. A two-way analysis of variance was performed and there was a significant effect of starch concentration and tray on aphid population ($F_{6,199}$=2.1920, P<0.05). The post-hoc Tukey HSD test shows the specific differences as described below.

For time, there were significant differences in overall aphid population between days 11 and 1 (P<0.001), days 11 and 4 (P<0.01), and days 11 and 6 (P<0.05). Whether the data was qualitatively compared by categorizing concentrations or by trays, the general trend was a decrease in population from day 0 to day 1 and then an overall increase in aphid number by day 11 (Figure 2).

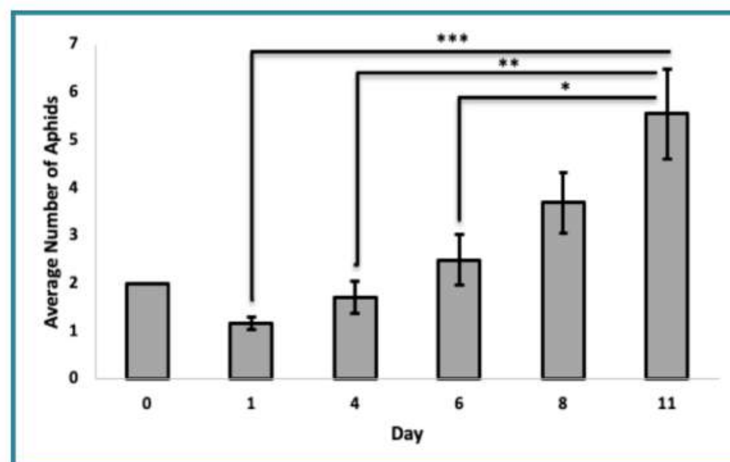


Figure 2 - Average number of aphids on each day of data collection. Error bars represent the standard error of the mean (SE). SE= ± 0, 0.129099445, 0.341823103, 0.523268118, 0.635983733, 0.940655331 for days 0, 1, 4, 6, 8, and 11, respectively. There were significant differences in aphid numbers between days 11 and 1 (***P<0.001), days 11 and 4 (**P<0.01), and days 11 and 6 (*P<0.05). After the decrease in population from days 0 to 1, there was a steady increase in aphid number until day 11.

The significant differences in aphid population due to concentration were between the 50% and 10% (P<0.05) as well as the 50% and 30% (P<0.05) starch concentration solutions (Figure 3).

There was a significant difference (P<0.001) in aphid population between the second and first trays, as well as the third and first trays. This was observed qualitatively for cases involving aphid disappearance in both the second and third trays (50% and 42%, respectively) and for the first tray (17%). In addition, overall plant health

© 2018 Sciential

remained relatively consistent, and appeared similar between the second and third trays. On the other hand, a wider range of overall plant health was seen in the first tray samples (Figure 4).

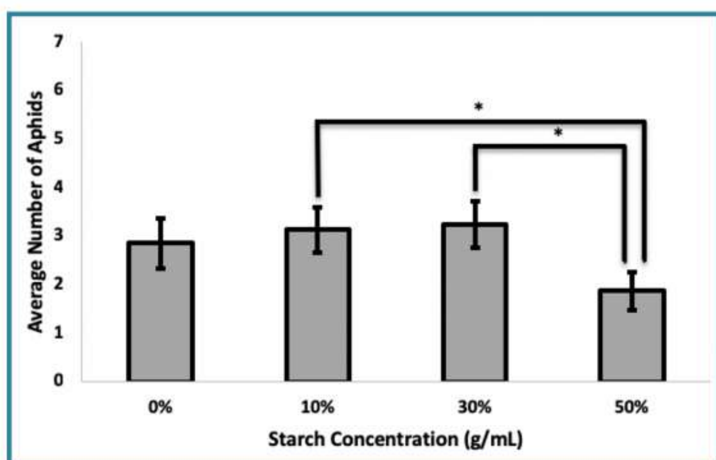


Figure 3 - Average number of aphids for each treatment of starch concentration (g/mL). Error bars represent standard error of the mean (SE). SE= ± 0.526321443, 0.469210122, 0.48667441, 0.392136452 for concentrations 0%, 10%, 30%, and 50%, respectively. There was a significant difference (*P<0.05) in aphid population between starch concentrations of 50% and 10% as well as the 50% and 30%. A significant decrease in aphid number was observed following treatment of the 50% starch concentration.

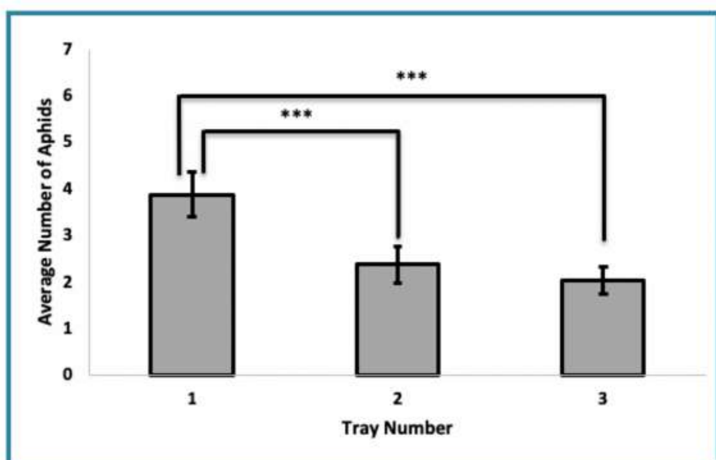


Figure 4 - Average number of aphids on each tray. Error bars represent standard error of the mean (SE). SE= ± 0.489944088, 0.390895783, 0.298953247 for tray numbers 1, 2, and 3 respectively. There was a significant difference (***P<0.001) in aphid population between the second and first trays and the third and first trays. There were significantly more aphids on the first tray than the second and third tray.

Out of all of the samples observed, the most significant differences of aphid population were seen for the 10% and 30% starch solutions in the first tray. There were also significant differences between the 30% and 50% concentrations in the third tray, as well as the 10% concentration in the first tray (P<0.05; P<0.001), along with a difference between the 50% concentration in the third tray and 30% concentration in the first tray (P<0.01). Lastly, there were significant differences between the controls in the second tray and 10% and 30% concentrations of starch in the first tray (P<0.01; P<0.05).

DISCUSSION

The goal of this study was to determine whether *M. persicae* would detect high levels of starch concentrations on a plant and thus be deterred from feeding on it. Statistical analysis and observations of the collected data showed that differing levels of starch have an effect on *M. persicae* abundance. From this experiment's results, there was no strong indication that the starch directly deterred the aphids from feeding on the plants. Alternatively, the *A. thaliana* plants treated with 10% and 30% starch concentrations unexpectedly showed higher numbers of *M. persicae* than the control plants. These results suggest that *M. persicae* do not interpret increased levels of starch on a plant as a signal of it having limited nutrients. It is also possible that *M. persicae* simply cannot detect increased levels of starch on a plant.

Based on qualitative observations, it is worth noting that plant health was visibly worse on the samples treated with 30% and 50% starch solutions. A possible explanation for this is that the starch solution- which dried as clumps- blocked the plants from sunlight, thus inhibiting photosynthesis and stunting their growth. Based on these observations, the samples of *A. thaliana* coated with 50% starch solution showed a general decrease both in the *M. persicae* population size, and in plant health. This led us to believe that these negative effects were likely not a cause of *M. persicae* feeding, but rather a consequence of blocked sunlight. Furthermore, the control and 10% starch solution groups showed increases in *M. persicae* population size near the end of the observation period. However, the extent of plant health deterioration was not as great in these two samples, as was observed for the 30% and 50% starch treatment groups.

© 2018 Sciential

It is important to recognize that feeding by *M. persicae* on *A. thaliana* did still play a role in deteriorating plant health. Some signs of this in the samples that showed high levels of feeding are i) plant leaf yellowing and curling from losing moisture or by toxins that may have been injected by *M. persicae* and ii) blackened plant mass and growth of sooty mold fungi, which originate from "honeydew", a sticky substance produced by *M. persicae* during feeding[16]. The former was seen in all samples except for plant number 10, which was in the control treatment. As such, there does not seem to be a strong relationship between starch concentrations applied onto the plant, and the amount of feeding by *M. persicae*. Interestingly, in the case of the latter, most moldy plants observed were found to be under the 30% and 50% starch solution treatment. Since no definitive relationship can be determined from such information, it is proposed that the blockage of sunlight by the opaque clumps of starch likely contributed to the plant deterioration seen in the 30% and 50% starch treatment samples.

Looking at the data, a trend can be seen whereby a higher number of *M. persicae* existed on the 10% and 30% plants than on the 50% plant and the control. While it was predicted that higher starch concentrations would decrease the numbers of *M. persicae* feeding on *A. thaliana*, the opposite occurred. That is, samples with higher starch concentrations actually increased aphid population for the intermediate treatments, compared to the control. If it is true that the starch negatively impacted *A. thaliana*, then it is possible that the carbohydrate reduced the plant's defense capabilities, which would explain the increase in *M. persicae* numbers seen on the 10% and 30% starch concentration plants. The 50% concentration may have negatively affected the plant to a degree that caused it to have lower levels of sucrose, thus reducing the food available for *M. persicae* and causing a subsequent decrease in the aphid population.

Some possible limitations of this study include the sample size, tray locations, and ability to apply starch uniformly across the entire plant. In terms of sample size, only nine plants were studied per treatment group. In the future, the design of this experiment may be improved upon by increasing sample sizes, to better control for any variance between individual samples that may have impacted the number of aphids that were seen on each plant. These variations include differences in leaf surface area and number of leaves on each plant. Pertaining to tray location, the trays were placed under a window to allow for the exposure to sunlight, which was necessary for growth. However, due to the limited size of the window, it is possible that all trays did not receive equal or optimal sunlight, which may have contributed to plant deterioration. Lastly, applying the starch via a spray bottle allowed for consistent spraying of all plants. However, the starch dried in droplets, meaning that the exterior of the plant was not covered evenly at all times. This could be ameliorated by using different methods of starch application, such as using a brush, as well as periodically spreading the starch overtime. This could be performed to ensure that the entire plant is thoroughly and evenly covered at all times.

CONCLUSION

From these observations, the research team rejects the null hypothesis. Covering *A. thaliana* plants in varying concentrations of starch did appear to have an effect on aphid feeding behaviour, as depicted by the significant differences between aphid number in 50%-10% and 50%-30% starch concentration. However, since there was no clear trend between increased starch concentration and aphid numbers on *A. thaliana*, these findings show that the motivation of creating a less environmentally harmful, starch-based pesticide would not be successful. Nonetheless, the results from the study indicate that there was a significant difference in the aphid population between differing starch concentrations, and that potato starch may have an effect on *A. thaliana* health. With more investigation into the potential of starch as a natural pesticide, this compound may prove to be a useful resource in reducing crop damage. Similar principles can be applied to more economically important plants, such as canola, in order to develop insecticides that would deter aphids, and trigger plant defences in a similar way.

Further studies should be performed to corroborate the findings by the research team. As such, the team would suggest replicating this experiment over a longer period of time, with a higher sample size and more treatment groups. Additionally, future researchers could mechanically apply the potato starch to the plants, so that the starch is spread out. This will help to avoid clumping, and the subsequent blockage of photosynthesis.

© 2018 Sciential

ACKNOWLEDGEMENTS

Our research group would like to sincerely thank Dr. Chad Harvey for his continuous feedback and statistical recommendations. We are grateful to Russ Ellis and Genevieve van Wersch for setting up and preparing the labs, which were crucial in conducting the research. Additionally, we are thankful to Noah Houpt for his recommendations on methodology and support throughout the data collection process.

REFERENCES

(1) Bass C, Puinean AM, Zimmer CT, Denholm I, Field LM, Foster SP, et al. The evolution of insecticide resistance in the peach potato aphid, Myzus persicae. Insect Biochem Mol Biol. 2014 Aug;51:41–51.

(2) Louis J, Shah J. Arabidopsis thaliana-Myzus persicae interaction: shaping the understanding of plant defense against phloem-feeding aphids. Front Plant Sci. 2013;4:213.

(3) Matthews, R.E.F. 14- Relationships Between Plant Viruses and Invertebrates. Plant Virology (Third Edition). 1991. Academic Press, San Diego, pp. 520–561.

(4) Harmel N, Létocart E, Cherqui A, Giordanengo P, Mazzucchelli G, Guillonneau F, et al. Identification of aphid salivary proteins: a proteomic investigation of Myzus persicae. Insect Mol Biol. 2008 Apr;17(2):165–74.

(5) Guedes RNC, Smagghe G, Stark JD, Desneux N. Pesticide-Induced Stress in Arthropod Pests for Optimized Integrated Pest Management Programs. Annu Rev Entomol. 2016;61:43–62.

(6) Louis J, Singh V, Shah J. Arabidopsis thaliana-Aphid Interaction. Arabidopsis Book. 2012;10:e0159.

(7) Lemoine R, La Camera S, Atanassova R, Dédaldéchamp F, Allario T, Pourtau N, et al. Source-to-sink transport of sugar and regulation by environmental factors. Front Plant Sci. 2013;4:272.

(8) Campbell BC, Jones KC, Dreyer DL. Discriminative behavioral responses by aphids to various plant matrix Polysaccharides. Entomologia Experimentalis et Applicata. 1986 May 1;41(1):17–24.

(9) Meinke DW, Cherry MJ, Dean C, Rounsley SD, Koornneef M. Arabidopsis thaliana: A Model Plant for Genome Analysis | Science [Internet]. [cited 2018 Oct 30]. Available from: http://science.sciencemag.org/content/282/5389/662

(10) Rhee SY, Beavis W, Berardini TZ, Chen G, Dixon D, Doyle A, et al. The Arabidopsis Information Resource (TAIR): a model organism database providing a centralized, curated gateway to Arabidopsis biology, research materials and community. Nucleic Acids Res. 2003 Jan 1;31(1):224–8.

(11) Streb S, Zeeman SC. Starch Metabolism in Arabidopsis. Arabidopsis Book [Internet]. 2012 Sep 24 [cited 2018 Oct 30];10. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3527087/

(12) Tomasik P. Specific Physical and Chemical Properties of Potato Starch. (1):12.

(14) Capinera, JL. Green Peach Aphid - Myzus persicae (Sulzer) University of Florida. [Internet]. [cited 2018 Oct 17]. Available from: http://entnemdept.ufl.edu/creatures/veg/aphid/green_peach_aphid.htm

(15) PestFax Issue 21, 14 September 2018 [Internet]. [cited 2018 Oct 17]. Available from:https://www.agric.wa.gov.au/newsletters/pestfax/pestfax-issue-21-14-september-2018

(16) Aphids Management Guidelines--UC IPM [Internet]. [cited 2018 Oct.16]. Available from: http://ipm.ucanr.edu/PMG/PESTNOTES/pn7404.html

© 2018 Sciential

ACADEMIC REVIEW

OPEN ACCESS

Induced Pluripotent Stem Cells: Acquirement, Characteristics and Medicinal Applications

Abdullah El-Sayes

McMaster University, Honours Life Sciences, Class of 2020

ARTICLE INFORMATION

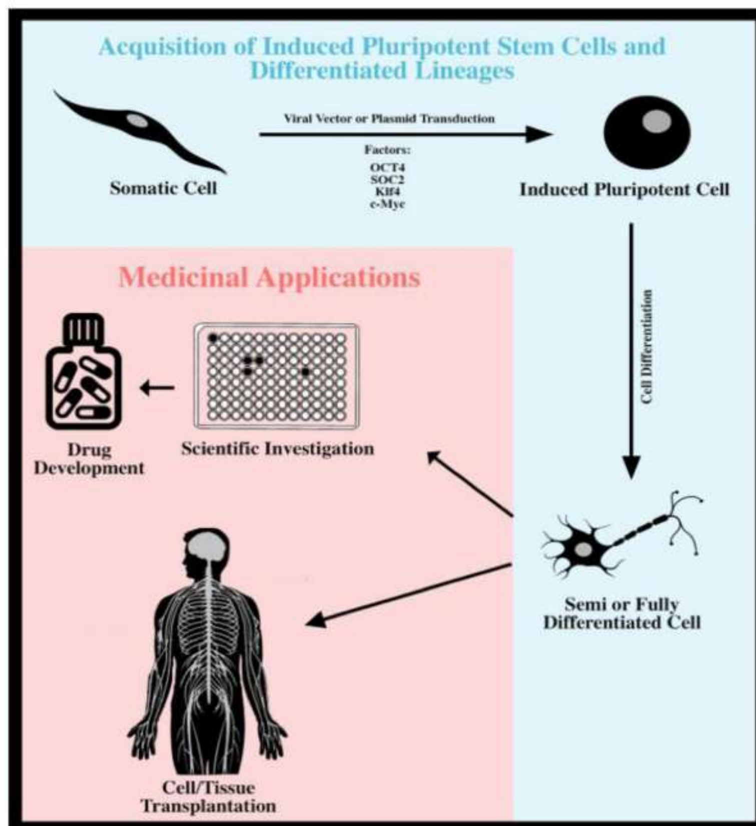
Received 8 October 2018
Accepted 8 November 2018

Available online:
26 November 2018

Editors:
Bianca Colarossi
Gabrielle Gonsalves

Layout Editor:
Athena Chang

GRAPHICAL ABSTRACT



iPS: Induced pluripotent stem - ES: Embryonic stem - ICM: Inner cell mass
ALS: Amyotrophic Lateral Sclerosis - LQTS: Long QT Syndrome

ABSTRACT

The isolation of human embryonic stem cells in 1998 has since fueled the ideology that stem cells may eventually be used for human disease therapies as well as the regeneration of tissues and organs. The transformation of somatic cells to a pluripotent state via somatic nuclear transfer and embryonic stem cell fusion brought the scientific community nearer to understanding the molecular mechanisms that govern cellular pluripotency. In 2006, the first induced pluripotent stem (iPS) cell was reported, where a mouse somatic cell was successfully converted to a pluripotent state via transduction of four essential factors. This cellular breakthrough has allowed for robust scientific investigations of human diseases that were once extremely difficult to study. Scientists and pharmaceuticals now use iPS cells as means for disease investigations, drug development and cell or tissue transplantation. There is little doubt that scientific progress on iPS cells will change many aspects of medicine in the next couple of decades.

Keywords: Pluripotent, stem, cells, genetics, iPS.

© 2018 Sciential

INTRODUCTION

Induced pluripotent stem cells are relatively novel in the scientific literature, however, their use in clinical science has been immense. The first iPS cells were developed from murine organisms in 2006[1] and later in humans in 2007[2]. These pluripotent cells are created from an organisms' somatic cells by transducing four essential factors required for pluripotency. Once iPS cells are yielded, they can differentiate into different cell lineages for scientific investigations or potential organismal transplantation. The benefit of employing iPS cells is the avoidance of host immune rejection after transplantation, as the newly produced cells carry the same genome and characteristics of an individual's regular somatic cells. Current research focuses on using iPS cells to create novel therapeutics for common human diseases such as Amyotrophic Lateral Sclerosis and Congenital Long QT Syndrome. Using similar techniques, stem cells similar to iPS cells have also shown therapeutic potential for treating human cancers. The purpose of this review is to depict the acquisition, characteristics, and medicinal application of iPS cells. Specifically, this paper will discuss the following:

1. Stem Cells
2. Acquisition and Characteristics of iPS cells
3. iPS Cell Medical Therapeutics
4. iPS-Cell-Alike Medical Therapeutics

STEM CELLS

Stem cells have three characteristics that define their identity. First, stem cells are capable of self-renewal that entails the capability of symmetrical divisions, where both daughter cells have stem cell characteristics. This usually occurs during development, after a stem cell transplantation, or after an insult to an existing stem cell pool[3]. The second characteristic of stem cells is that they are able to yield differentiated progeny. Depending on the potency of the cells, differentiated descendants may be from one, or a combination, of the ectoderm, mesoderm or endoderm germ layers. Third, stem cells must be capable of populating tissue in vivo[3]. This phenomenon, however, is still under scientific investigation.

All mammals are derived from a totipotent stem cell that can give rise to embryonic and extraembryonic tissue. During the process of embryogenesis, descendent cells- usually the cells of the inner cell mass (ICM)- lose their potency and become pluripotent, giving rise to only embryonic tissue[3]. Eventually, cells of the ICM differentiate to become constituents of different tissue types and stem cells for different tissues. These tissue stem cells are deemed multipotent or unipotent and may only be differentiated as one particular cell type. Whether all tissues have resident stem cells is a topic still under debate in the scientific literature. For instance, it remains unresolved whether true stem cells reside in adult heart muscle in postnatal life, or in the pancreas, among other tissues[3].

INDUCED PLURIPOTENT STEM CELLS

Pluripotent stem cells have the capability of differentiating into all three germ layers: ectoderm, mesoderm and endoderm. In 2006, Takahashi and Yamanaka discovered a method for yielding pluripotent cells from organismal somatic cells (Refer to section 4.1). This was first created using murine animals, but one year later, in 2007, Yu and colleagues identified key factors for inducing pluripotent cells in human somatic cells. This finding is immense as it allows scientists to obtain easily accessible somatic cells from an organismal model or patient, transform it to a pluripotent state and thereafter differentiate it to another cell type. Examples of this include transforming blood cells to peripheral neural sensory cells, which are nearly impossible to obtain from human patients due to potential neuronal damage and ethical reasons[4].

ACQUISITION OF PLURIPOTENT STEM CELLS

Somatic Nuclear Transfer

The somatic cell nuclear transfer experiment was the first to show that differentiated cells are capable of becoming pluripotent. In 1960, it was demonstrated that implanting an albino *Xenapus laevis* nucleus into a brown *Xenapus laevis* enucleated oocyte was able to generate a clone of the donor brown frog[5]. Hence, a nucleus of a differentiated cell is capable of undergoing changes that entails a pluripotent state to eventually give rise to a fully developed organism. The reprogramming of the cell occurs 24-48 hours after the implantation of the nucleus into the enucleated oocyte. A similar experiment has also been conducted with sheep somatic nuclear transfer giving rise to a clone famously known as Dolly[6].

© 2018 Sciential

Fusion of Somatic Cells with Embryonic Stem Cells

Another method of transforming somatic cells to a pluripotent state is to fuse differentiated cells with embryonic stem (ES) cells[7]. Similar to the somatic cell nuclear transfer experiment, molecular changes occur to allow differentiated cells to become pluripotent. Studies have found that ES cell fusion with differentiated cells activated OCT4 expression in somatic cells[8], which is a gene essential for pluripotency. The exact mechanism of this transformation, however, is not fully understood.

INDUCED PLURIPOTENT STEM CELL GENOME EXPRESSION

Factors Needed for Murine Models

Studies have identified certain genes highly expressed in embryonic stem cells of murine organisms. A total of 24 factors were identified including: b-catenin, c-Myc, STAT3, S33Y-b-catenin[9], T58A-c-Myc[10], STAT3-C[11], and Grb2DSH2[12]. When these 24 factors were transduced in differentiated somatic cells, they effectively transformed the cells into undifferentiated murine stem cells. However, further investigation proved that not all 24 factors are needed and only genes c-Myc, Klf4, SOX2, and OCT3/4[1] are necessary for the induction of induced pluripotent stem cells from differentiated mouse fibroblast cells. iPS cell colonies did not form when either OCT3/4 or Klf4 were removed. The removal of SOX2 resulted in only a few cell colonies, and the removal of c-Myc caused cell colonies to emerge but without ES-cell-like morphology[1]. Therefore, if any one of these 4 factors are removed, cells fail to transform into iPS cells, hence all four gene factors are deemed necessary.

Factors Needed for Humans

There are differences between mouse fibroblast cells and human cells. In mice, c-Myc promotes pluripotency, however, in human cells c-Myc causes death and differentiation of ES cells[13]. Hence, a different combination is needed to initiate the transformation of somatic cells to iPS cells. These factors are OCT4, SOX2, NANOG, and LIN28[2]. Removal of OCT4 and SOX2 eliminates the formation of iPS cell colonies[2]. Meanwhile, overexpression of NANOG shows a 200-fold increase in reprogramming efficiency, and LIN28 has a consistent but modest effect on the reprogramming of cells[2].

GENOME MODIFICATION TECHNIQUES

The first method used to transform somatic cells into iPS cells was performed using viral transduction with the reprogramming factors. Viruses commonly used include retrovirus and lentivirus. This method poses risks as it may lead to insertional mutagenesis, tumour formation and unpredictable genetic dysfunction[14]. Instead, recent studies have shown that short-term overexpression of the transcription factors required for the 4 factors using plasmids, or using transposons that are subsequently removed by a Cre-Lox system, are adequate to transform differentiated cells to pluripotent cells[15]. Specifically, studies have demonstrated that repeated transfection of two expression plasmids, one containing the complementary DNAs of OCT3/4, SOX[2], and Klf4 and the other containing the c-Myc cDNA, into mouse embryonic fibroblasts resulted in iPS cells without evidence of plasmid integration[14]. Also, these cells produced teratomas when transplanted into mice and contributed to adult chimeras, thus proving their pluripotent cell state[14].

Furthermore, cell penetrating peptides (CPP) may be used to overcome the cell membrane barrier during the cellular reprogramming process. CPPs contain a high concentration of arginine and lysine amino acids that allow associated peptides to surpass the cell membrane[16]. Studies have shown that red fluorescent protein fused to 9 arginine (a form of CPP), injected into COS7 cells and human newborn fibroblasts successfully surpassed the cell membrane and entered the cytoplasm. Therefore, CPP provides a plausible mechanism to deliver factors for transcription of the four essential genes into cells[17]. Other studies have also concluded that chemical agents, BIX-01294 and BayK8644, cause histone modifications, particularly methylation and acetylation modulation of the OCT4 and Klf4 genes to improve the transformation of somatic cells to pluripotent cells[18]. Therefore, many safe and secure methods exist for the formation of induced pluripotent stem cells from somatic cells, which yields great advantages for clinical applications.

ASSAY FOR STEM CELL CULTIVATION

To determine if the cultivated cells are indeed pluripotent, an assay is employed. A potent example of this in

© 2018 Sciential

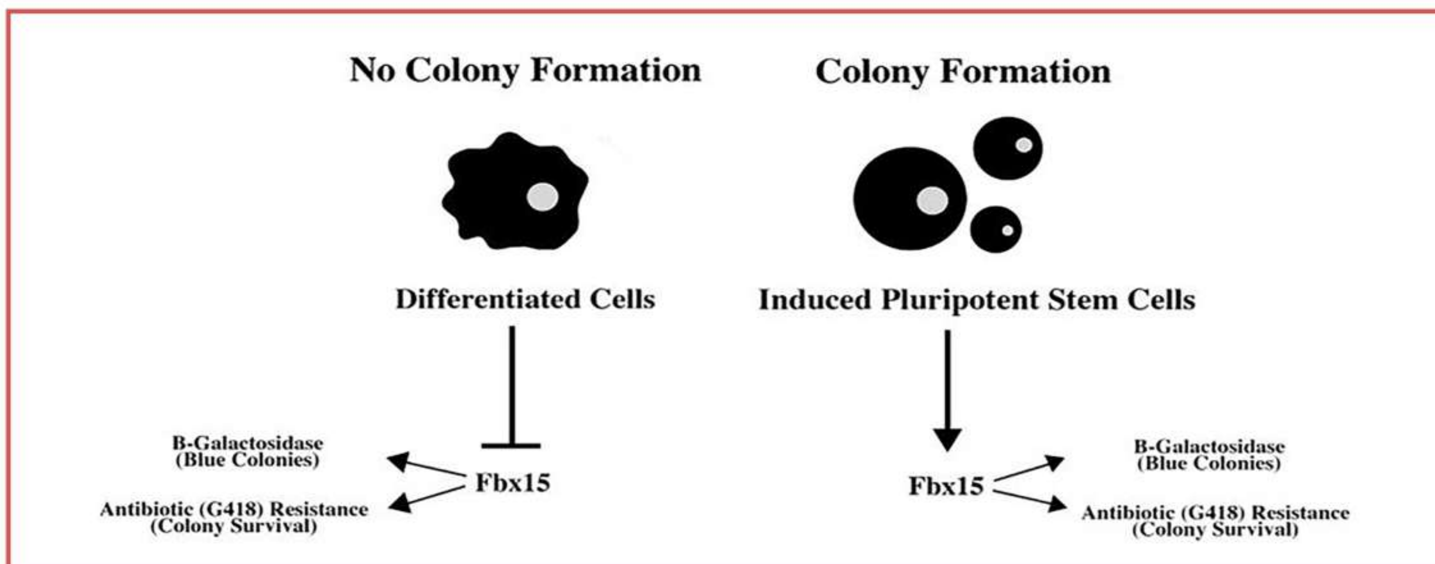


Figure 1 – Formation of colonies occurs only for pluripotent stem cells as they transcribe Fbx15 with the ßgeo cassette, a fusion including the ß-galactosidase and neomycin resistance genes. Pluripotent stem cell colonies appear blue and have embryonic stem cell-like characteristics.

-cludes an antibiotic resistance assay. ßgeo cassette of a fusion of ß-galactosidase and neomycin resistance genes is inserted into the Fbx15 gene by homologous recombination[1]. Hence, by cultivating cell cultures in G418 antibiotic conditions, only stem cells will survive and will be illuminated blue in colour[1] (due to the ß -galactosidase insertion into the Fbx15 gene) (Figure 1.0). Stem cells homozygous for the ßgeo knock-in construct ($Fbx15^{bgeo/bgeo}$) were resistant to extremely high concentrations of G418 (up to 12 mg/ml), whereas somatic cells derived from $Fbx15^{bgeo/bgeo}$ mice were sensitive to normal concentrations of G418 (0.3 mg/ml) as these differentiated cells do not express the Fbx15 gene[1].

COMPARISON TO EMBRYONIC STEM CELLS

Doubling Time and Genetic Markers

Most cultivated iPS cells demonstrate morphologies similar to that of embryonic stem cells. These characteristics include round cellular shape, large nuclei and a scant cytoplasm[1]. The doubling time of these cultivated pluripotent stem cells was also similar to those of ES cells at approximately 17.0 hours[1]. Reverse transcription PCR demonstrated further similarities of iPS cells and ES cells. Particularly, iPS cells were found to transcribe OCT3/4, NANOG, E-Ras, Cripto, Dax1, Zfp296[19], Fgf4[20], Myb, Kit, Gdf3, and Zic3 which are deemed gene markers and highly expressed among ES cells[1]. Overall, these characteristics confirm that iPS cells are similar but not identical to ES cells.

Teratoma Formation

Another test that is employed to determine if the obtained iPS cells are pluripotent involves the implantation of iPS cells into immunosuppressant mice and the subsequent observation for teratoma formation. Several of the implanted iPS cell colonies proved that the injected cells formed benign tumours of all three germ layers including: neural tissues, cartilage, and columnar epithelium[1]. However, some teratomas only differentiated to the endoderm and ectoderm layers, and other tumours did not differentiate at all[1]. These observations effectively establish that most, but not all, of the colonies transform into a pluripotent state.

Embryoid Bodies and Differentiation

Cell cultures can also be tested for the formation of embryoid bodies and differentiation. When grown in tissue culture dishes, embryoid bodies from iPS cells containing the 4 factors promoting pluripotency effectively attached to the bottom of the dish and began differentiation[1]. Immunostaining tested positive for the formation of the ectoderm (using βIII tubulin), mesoderm (using α-smooth muscle actin) and endoderm (using α-fetoprotein) germ layers[1]. However, cells without the 4 necessary factors failed to form embryoid bodies and did not differentiate[1].

© 2018 Sciential

MEDICINAL AND THERAPEUTIC APPLICATIONS OF INDUCED PLURIPOTENT STEM CELLS

Applications for Autologous Transplantations

Implantation of ES cells into patients for tissue replacement or repair poses significant consequences in terms of bodily immune responses. Patients would require lifelong immunosuppressive therapy[21]. However, if patient somatic cells are used to derive tissue-specific stem cells, immunosuppression would not be needed as the inserted cells would be the patients' own cells. One drawback to this method of disease resolution is that autologous iPS cells would have the potential genetic mutations that underlie the existing disease. This, however, can be overcome with in situ repairs of the defected genome using homologous recombination[21]. Another concern regarding the implantations of iPS cells into patients is the potential for the iPS cells to form teratomas in the body. A plausible solution to this is to inject differentiated and descendent multipotent or unipotent stem cells into patients, in order to prevent tumour development.

Applications for Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder where motor neuron loss in the spinal cord and motor cortex leads to paralysis and death[22]. Glia cells from ALS animal models have shown to produce factors that are toxic to motor neurons[23]. A study conducted by Dimos and colleagues, 2008, demonstrated that somatic fibroblast cells of elderly ALS patients may be transformed into iPS cells for pathology investigation. Specifically, an 82-year-old patient was used for this study. This patient was heterozygous for the L144F dominant allele of the superoxide dismutase gene resulting in a slowly progressing form of ALS[25]. Primary skin cells isolated by biopsy from the patient had factors KLF4, SOX2, OCT4, and c-MYC transduced into it by means of vesicular stomatitis virus glycoprotein pseudotyped Moloney-based retroviruses[24]. Some of these differentiated skin cells transformed into iPS cells and demonstrated nearly complete silencing of viral SOX2 and KLF4. Nevertheless, some expression of viral OCT4 and c-MYC persisted[24]. The embryoid bodies formed from these iPS cells were treated with an agonist of the sonic hedgehog signal-ling pathway and retinoic acid to induce neuronal differentiation[24]. This experiment was successful and motor neuron differentiation took place with the patient's exact genotype[24]. These cells may then be used to study the physiology and anatomy of the dysfunctional cells or alter the genome to make it functional and transplant the cells into the body for repair. However, some limitations exist for this protocol. First, non-viral techniques (as discussed in Genome Modification Techniques) must be employed for clinical trials to begin, as the use of viral vectors poses health risks. Second, patient genome defects must be understood, or successfully mitigated, for successful therapeutic applications[24].

Congenital Long QT Syndrome

Congenital long QT syndrome (LQTS) is a disease classified into 12 subtypes, all in which share a common feature of delayed repolarization, a prolonged QT interval in the electrocardiogram, and a life-threatening polymorphic ventricular tachycardia known as torsade de pointes[26]. There is currently a lack of in vitro sources for human cardiomyocytes, thus an inability to model the disease in humans[27]. However, by utilizing iPS cells, LQTS may now be modelled in vitro to better understand the disease and potentially derive therapeutics. Recently, scientists have isolated dermal fibroblasts from a 28-year-old woman with a diagnosis of familial type-2 LQTS due to a missense mutation in exon 9 of the KCNH2 gene that encodes the pore-forming region of potassium channels, hence leading to a significant reduction of the delayed-rectifier potassium current[27]. From these dermal cells, transduction of SOX2, Klf4 and OCT4 was committed with retroviral vectors. All of the iPS cells showed ES cell-like morphologies and expressed the pluripotency gene markers: NANOG, SSEA4, OCT4 and TRA-1-60[27]. These iPS cells are then differentiated into the cardiac lineage, confirmed by the presence of cardiac-specific transcription factors: NKX2-5 and genes MLC2V, MYH6 and MYH7[27]. From these differentiated cells, it was found and modelled that action-potential duration prolongation occurred in LQTS differentiated cardiomyocytes relative to control cells[28]. Therefore, iPS cells have provided a foundation for understanding human pathologies specific to the cardiac system. These studies have proven that if cells are not easily obtainable or isolated, iPS cells may be used to induce the creation of one cell type from another, in order to better understand diseases and to potentially develop therapeutics.

© 2018 Sciential

CELL DIFFERENTIATION WITHOUT USE OF INDUCED PLURIPOTENT STEM CELLS

Although common transformations of one cell identity-type to the other (e.g. blood cells to neurons) makes use of the iPS cell state, there are methods that only achieve multipotent intermediate cells rather than pluripotent. In certain contexts, this situation is favoured as it limits the potential for cells to differentiate into undesired or malignant cell lineages. The method of transformation, however, is similar to that of the pluripotent state discussed previously. In this conversion process, OCT4 is preferentially expressed but levels of SOX2 and NANOG expression remain relatively low[29]. This method will transform the donor cell to a stem-cell state, however, pluripotency will not be achieved.

CONCLUSION

Overall, the scientific community has expanded its knowledge of stem cells and their applications over the past few decades. There is little doubt that this field of research will continue to grow, and develop into new avenues that will ultimately advance human health. The identification of the four factors necessary to induce somatic cells to a pluripotent state was a scientific breakthrough that led the authors to acquire a Nobel Prize in 2006. However, there is more work to be conducted. Perhaps further research on this topic may lead researchers to find ways to create human organs in the lab for autologous transplantation. Likewise, stem cell investigations may uncover an underlying malignant pathway that is necessary for tumour formation, allowing us to intercept the tumours before they form. Although the advances in stem cell research have been vast thus far, there is still much more to learn in this field of science.

ACKNOWLEDGEMENTS

This work did not receive funding. There are no conflicts of interests.

REFERENCES

(1) Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. cell. 2006 Aug 25;126(4):663-76.

(2) Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II. Induced pluripotent stem cell lines derived from human somatic cells. science. 2007 Dec 21;318(5858):1917-20.

(3) Verfaillie C. Pluripotent stem cells. Transfusion clinique et biologique. 2009 May 1;16(2):65-9.

(4) Lee JH, Mitchell RR, McNicol JD, Shapovalova Z, Laronde S, Tanasijevic B, Milsom C, Casado F, Fiebig-Comyn A, Collins TJ, Singh KK. Single transcription factor conversion of human blood fate to NPCs with CNS and PNS developmental capacity. Cell reports. 2015 Jun 9;11(9):1367-76.

(5) Elsdale TR, Gurdon JB, Fischberg M. A description of the technique for nuclear transplantation in Xenopus laevis. Development. 1960 Dec 1;8(4):437-44.

(6) Campbell KH, McWhir J, Ritchie WA, Wilmut I. Sheep cloned by nuclear transfer from a cultured cell line. Nature. 1996 Mar;380(6569):64.

(7) Ying QL, Nichols J, Evans EP, Smith AG. Changing potency by spontaneous fusion. Nature. 2002 Apr;416(6880):545.

(8) Do JT, Schöler HR. Nuclei of embryonic stem cells reprogram somatic cells. Stem cells. 2004 Nov;22(6):941-9.

(9) Sadot E, Conacci-Sorrell M, Zhurinsky J, Shnizer D, Lando Z, Zharhary D, Kam Z, Ben-Ze'ev A, Geiger B. Regulation of S33/S37 phosphorylated β-catenin in normal and transformed cells. Journal of cell science. 2002 Jul 1;115(13):2771-80.

(10) Chang DW, Claassen GF, Hann SR, Cole MD. The c-Myc transactivation domain is a direct modulator of apoptotic versus proliferative signals. Molecular and cellular biology. 2000 Jun 15;20(12):4309-19.

(11) Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao Y, Pestell RG, Albanese C, Darnell Jr JE. Stat3 as an oncogene. Cell. 1999 Aug 6;98(3):295-303.

© 2018 Sciential

REFERENCES

(12) Miyamoto Y, Yamauchi J, Mizuno N, Itoh H. The adaptor protein Nck1 mediates endothelin A receptor-regulated cell migration through the Cdc42-dependent c-Jun N-terminal kinase pathway. Journal of Biological Chemistry. 2004 Aug 13;279(33):34336-42.

(13) Sumi T, Tsuneyoshi N, Nakatsuji N, Suemori H. Apoptosis and differentiation of human embryonic stem cells induced by sustained activation of c-Myc. Oncogene. 2007 Aug;26(38):5564.

(14) Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. Science. 2008 Nov 7;322(5903):949-53.

(15) Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. Science. 2008 Nov 7;322(5903):945-9.

(16) El-Sayed A, Futaki S, Harashima H. Delivery of macromolecules using arginine-rich cell-penetrating peptides: ways to overcome endosomal entrapment. The AAPS journal. 2009 Mar 1;11(1):13-22.

(17) Kim D, Kim CH, Moon JI, Chung YG, Chang MY, Han BS, Ko S, Yang E, Cha KY, Lanza R, Kim KS. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell stem cell. 2009 Jun 5;4(6):472-6.

(18) Shi Y, Desponts C, Do JT, Hahm HS, Schöler HR, Ding S. Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. Cell stem cell. 2008 Nov 6;3(5):568-74.

(19) Mitsui K, Tokuzawa Y, Itoh H, Segawa K, Murakami M, Takahashi K, Maruyama M, Maeda M, Yamanaka S. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. cell. 2003 May 30;113(5):631-42.

(20) Yuan H, Corbi N, Basilico C, Dailey L. Developmental-specific activity of the FGF-4 enhancer requires the synergistic action of Sox2 and Oct-3. Genes & development. 1995 Nov 1;9(21):2635-45.

(21) Hanna J, Wernig M, Markoulaki S, Sun CW, Meissner A, Cassady JP, Beard C, Brambrink T, Wu LC, Townes TM, Jaenisch R. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. Science. 2007 Dec 21;318(5858):1920-3.

(22) Pasinelli P, Brown RH. Molecular biology of amyotrophic lateral sclerosis: insights from genetics. Nature Reviews Neuroscience. 2006 Sep;7(9):710.

(23) Di Giorgio FP, Carrasco MA, Siao MC, Maniatis T, Eggan K. Non–cell autonomous effect of glia on motor neurons in an embryonic stem cell–based ALS model. Nature neuroscience. 2007 May;10(5):608.

(24) Dimos JT, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, Chung W, Croft GF, Saphier G, Leibel R, Goland R, Wichterle H. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science. 2008 Aug 29;321(5893):1218-21.

(25) Ferrera L, Caponnetto C, Marini V, Rizzi D, Bordo D, Penco S, Amoroso A, Origone P, Garrè C. An Italian dominant FALS Leu144Phe SOD1 mutation: genotype-phenotype correlation. Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders. 2003 Sep 1;4(3):167-70.

(26) Goldenberg I, Horr S, Moss AJ, Lopes CM, Barsheshet A, McNitt S, Zareba W, Andrews ML, Robinson JL, Locati EH, Ackerman MJ. Risk for life-threatening cardiac events in patients with genotype-confirmed long-QT syndrome and normal-range corrected QT intervals. Journal of the American College of Cardiology. 2011 Jan 4;57(1):51-9.

(27) Itzhaki I, Maizels L, Huber I, Zwi-Dantsis L, Caspi O, Winterstern A, Feldman O, Gepstein A, Arbel G, Hammerman H, Boulos

© 2018 Sciential

OPINION PIECE

OPEN ACCESS

How Our Healthcare System Failed During the SARS Outbreak

Bianca Colarossi

McMaster University, Honours Life Sciences, Class of 2019

ARTICLE INFORMATION

Recieved 31 October 2018
Accepted 14 November 2018

Available online:
26 November 2018

Editors:
Gabrielle Gonsalves
Caitlin Reintjes

Layout Editor:
Athena Chang

ABSTRACT

Severe Acute Respiratory Syndrome (SARS) was an active pandemic in the spring of 2003, ravaging places such as Hong Kong and Canada. In Ontario, the healthcare system was extremely unprepared, hence resulting in a multitude of deaths, in which many were healthcare professionals. In contrast, Vancouver took the necessary precautions leading up to the outbreak, and the benefits of this can be seen in their low death toll. In the future, the Ontario healthcare system needs to learn from these mistakes by preparing personal protective equipment and educating healthcare professionals on proper infectious disease control protocol. This is a call to action for the Ontario healthcare system.

Keywords: SARS, infectious disease control, Canada, healthcare

INTRODUCTION

In the spring of 2003, the world was in a global crisis. Severe Acute Respiratory Syndrome affected places such as Hong Kong, China, Singapore, and Canada. Specifically, in Ontario, the public health system failed to recognize the severity of the disease, putting hundreds of healthcare professionals and civilians at risk. This sheer lack of unpreparedness fueled the fire that spread across Ontario that spring, putting those working on the front line under viral attack.

DISCUSSION

Ontario's Response to the Outbreak

The World Health Organization (WHO) reports that over 250 Canadians were infected with SARS, with 49 confirmed deaths by the time the pandemic was called off in July of 2003[1]. Of these cases, 45% were healthcare workers who were not given the protection they needed. Healthcare workers were asked to save personal protective equipment (PPE) - such as masks, gloves, and isolation gear- for when a truly confirmed case of SARS was present. Workers in Toronto were not being told to treat every unidentified case as if it were SARS[2]. This led to the spread of the deadly disease across hospital floors and from worker to worker. The city of Toronto was not prepared for the speed at which the disease spread, thus failed to provide their workers with the protection they needed and had a right to.

A nurse at Mount Sinai Hospital recounts her experience with SARS. Susan Sorrenti was an intensive care nurse who was working with a transplant patient with a suspected case of pneumonia[3]. She feared that the patient was presenting with symptoms of SARS and was confused as to why he was not being quarantined. Nurse Sorrenti was informed that masks, isolation gear, and other PPE was in short supply and was only to be used in confirmed SARS cases. This transplant patient later went on to infect Nurse Sorrenti and 6 other people at Mount Sinai Hospital that day[3].

© 2018 Sciential

Vancouver's Response to the Outbreak

In contrast, the city of Vancouver was prepared when their first case of SARS touched down and employed various tactics to control the spread. Learning from past pandemics, Vancouver had been increasing awareness and preparedness for years. They created an online bulletin to ensure that news about any infectious disease spread was made public as quickly as possible. An alert was sent out long before their first infected patient touched down, ensuring that citizens knew the signs and symptoms to look for among their friends and family[4]. This vigilance limited the opportunity for the disease to spread at all in the city.

Patient 0 landed in central Vancouver after a trip to Hong Kong with his wife, presenting with symptoms that were consistent with those released on the online bulletin. From the airport, him and his wife went directly to their family doctor, who referred them immediately to their local hospital and patient 0 was quarantined immediately[4]. Health care workers were given proper personal protection equipment, and none were infected[2,4]. Vancouver proves that it was possible to limit the effects of the disease if the right precautionary steps were taken.

Implications and Solutions

The way that Toronto health officials handled the outbreak of SARS was abysmal. Justice Archie Campbell writes"[SARS] was a disaster waiting to happen because the province's public health system had been badly neglected..."[2]. This negligence put thousands at risk and cost the lives of many. I believe that in Toronto, every case needed to be treated as if it were SARS. If isolation gear was in low supply, more needed to be ordered. Materials around the hospital needed to be used as make-shift equipment if necessary, as anything is better than going into the situation completely unprotected. Nurses and doctors needed infectious disease control training. They must know how to handle a situation when a severely contagious disease is spreading uncontrolled around the city. Most importantly, it is necessary that doctors and nurses understand their rights. They have a right to refuse work in unsafe situations; this includes the right to protect themselves and their families against the deadly diseases found in hospitals each and every day. The main issue that the healthcare system had was waiting for SARS to reach Canada before attempting to control it. As the disease tore across places like Hong Kong, the Canadian healthcare system watched, hoping it never made it and did not prepare for an outbreak. This extra preparation - spreading the word to civilians, stocking up PPE, and educating healthcare workers on the spread of the disease and how to protect themselves - would have made a world of difference, drastically dropping the death toll that SARS left behind.

CONCLUSIONS

This is a call to action. Future outbreaks need to be contained before they escalate to the level seen during the SARS pandemic and healthcare workers need to be the first protected. They are the first line of defense and put their lives at risk every day to save people they do not even know. How can nurses and doctors properly perform at work when they are constantly looking over their shoulders, fearing the spread of another deadly disease with little to no support from the healthcare system? Something needs to change, and this change needs to be made before the next pandemic hits Canada.

ACKNOWLEDGEMENTS

A special thank you to Dr. Katie Moisse for her continued support and creative opinions during the writing of this piece.

REFERENCES

(1) WHO | Cumulative Number of Reported Probable Cases of Severe Acute Respiratory Syndrome (SARS) [Internet]. Who.int. 2018 [cited 30 October 2018]. Available from: http://www.who.int/csr/sars/country/en/

(2) Report: Gov't failed health workers during SARS [Internet]. CTV News Toronto. 2007 [cited 30 October 2018]. Available from: https://toronto.ctvnews.ca/report-gov-t-failed-health-workers-during-sars-1.223196

(3) Dempsey A. SARS, 10 years later: One family's remarkable story | The Star [Internet]. thestar.com. 2013 [cited 30 October 2018]. Available from: https://www.thestar.com/news/insight/2013/03/0 2/sars_10_-years_later_one_familys_remarkable_story.html

(4) Skowronski D, Petric M, Daly P, Parker R, Bryce E, Doyle P et al. Coordinated Response to SARS, Vancouver, Canada. Emerging Infectious Diseases. 2006;12(1):155-158.

© 2018 Sciential

OPINION PIECE

OPEN ACCESS

Evading Evasion: How Phages get around CRISPR-Cas

Mostafa Mohammed Elsabagh

McMaster University, Biomedical Discovery and Commercialization, Class of 2020

ARTICLE INFORMATION

Recieved 14 October 2018
Accepted 11 November 2018

Available online:
26 November 2018

Editors:
Ishita Paliwal
Caitlin Reintjes

Layout Editor:
Youssef El-Sayes

ABSTRACT

The CRISPR-Cas9 system has paved the way for realising gene-editing, but its main weakness lies in its potential for off-target effects. Studies into phages reveal that they express "anti-CRISPR" proteins which if harnessed, could provide us with the solution to this lack of control.

Keywords: Phage, CRISPR-Cas, anti-CRISPR, antimicrobial resistance

INTRODUCTION

Today, the CRISPR-Cas system has become the most promising source of enthusiasm in biotechnology. It has had a fair share of attention with wide-ranging, controversial discussions of gene-editing. While the CRISPR-Cas system may be the gene-editing tool we had hoped for, we should not overlook the prospect of off-target effects. Writing in Nature Microbiology, Hynes et al.[1] reports how this problem may soon be surmounted by studying how phages evolved a method to evade CRISPR-Cas.

WHAT IS CRISPR?

The CRISPR-Cas system is a bacterial defence mechanism against a prophage, the integrated genome of an invading phage[2]. It accomplishes this by recognising the foreign DNA sequence and cleaving it from the bacterium's genome[2]. The CRISPR-Cas is a riboprotein complex, made up of a crRNA, which recognises foreign DNA sequences by binding to them, and Cas, an endonuclease which cleaves DNA[2]. Scientists have been able to modify the crRNA to cut any sequence they choose, the essence of gene-editing.

CRISPR's PROBLEMS

However, the CRISPR-Cas system may occasionally cleave sequences they are not intended to. This occurs despite the absence of full complementarity, and for biological research and gene therapy, these off-target effects are a serious concern[3]. In human cell studies, up to five mismatches can occur between the target and crRNA without a noticeable change in editing activity[3]. As a result, many scientists have been developing methods to detect off-target effects[3]. However, modification of the guide RNA to lower mutation rates can cause a decrease in binding or cuts by Cas in intended regions[3]. Hence, Hynes et al.[1] looked to another avenue to overcome this issue.

© 2018 Scientiał

SEARCHING FOR ANTI-CRISPRs

While bacteria battle phage infections with the CRISPR-Cas system, many phages possess genes coding for anti-CRISPR (Acr) proteins to inhibit CRISPR-Cas[1]. Hynes and colleagues sought to find Acr's in phages targeting *Streptococcus thermophilus*, a member of the same genus of the original source of CRISPR-Cas. Using the phage-first method, they screened phages for their ability to bypass bacterial immunity conferred by CRISPR-Cas[1]. *S. thermophilus*, is frequently challenged by phages to produce phage-resistant variants[1]. This is analogous to "immunizing" them against strains of phage. It was observed that some phages did not result in immunized bacteria, and thus research was focused on five of these phages[1].

These candidates looked promising, but failure of CRISPR-Cas in the bacteria is not the only possible reason for their death. It could be that the phages had taken over the bacteria faster than CRISPR's ability to defend against them[1]. Hence, Hynes and colleagues created a new strain of *S. thermophilus* containing genes common to the five phages, such that its CRISPR-Cas system would readily prevent invasion from any of these phages. Despite this, one phage: D4276, was consistently successful in destroying this strain[1]. This was the candidate Hynes was looking for.

Having narrowed down the search, Hynes and colleagues created strains of *S. thermophilus*, each with acrIIA5, a novel Acr. The effect of this gene was a six-fold increase in sensitivity[1], indicating high efficacy. Hynes and colleagues attempted to challenge the Acr-bearing strain against other unrelated phages, to which it was immune, and they observed sharp increases in sensitivity[1]. The findings were clear and promising: acrIIA5 is an effective anti-CRISPR. Hynes and colleagues are now working on the analysis of acrIIA5's mechanism of action and structure[4].

ARE ANTI-CRISPRs THE RIGHT PATH?

While their discovery illustrates evolution resulting from phage-host interaction, Acr's could be essential in the development of several biotechnological applications. This includes gene-editing, as Acr's will allow for modulation of CRISPR-Cas systems. Studies in human cells have shown that with a correct time delay, adding an Acr can lead to a significant reduction in off-target edits by Cas9, while retaining target specificity, leading to improved accuracy in gene-editing[5,6].

It is natural to rush towards Acr's as a solution to the off-target effects. However, an area of exploration with low activity is antimicrobial resistance by bacteria. CRISPR-Cas evolved in bacteria to destroy invading phages to prevent plasmid integration. However, studies have shown that some bacterial species lose this ability if it impedes acquisition of beneficial bacterial DNA, including those coding for antimicrobial resistance[6]. In fact, some lineages do not even possess a CRISPR-Cas system[6]. Therefore, if some lineages benefit from the absence of this system, it may be possible that a bacterial strain with a CRISPR-Cas system acquiring an Acr could improve its ability to attain and integrate plasmids from antimicrobial-resistant neighbours. This is alarming when we consider a field of CRISPR-Cas antimicrobials. This endeavor harnesses CRISPR-Cas gene-editing to remove genes conferring antimicrobial-resistance in bacteria[7]. If, through the increased use of Acr's in one application, strains of bacteria acquire these Acr genes to evade CRISPR-Cas antimicrobials, will we have closed a newly opened avenue in the battle against antimicrobial-resistance? It becomes clear that additional research into these phenomena is necessary and that Acr's will provide us with great power, but clearly, with great power comes great responsibility.

© 2018 Sciential

REFERENCES

(1) Hynes, A. P. et al. An anti-CRISPR from a virulent streptococcal phage inhibits Streptococcus pyogenes Cas9. Nat. Microbiol. 2, 1374–1380 (2017).

(2) Jiang, F. & Doudna, J. A. CRISPR – Cas9 Structures and Mechanisms. Annu.Rev.Biophys 46, 505–529 (2017).

(3) Peng, R., Lin, G. & Li, J. Potential pitfalls of CRISPR/Cas9-mediated genome editing. FEBS J. 283, 1218–1231 (2016).

(4) Hynes, A. P. et al. Widespread anti-CRISPR proteins in virulent bacteriophages inhibit a range of Cas9 proteins. Nat. Commun. 9, 1–10 (2018).

(5) Shin, J. et al. Disabling Cas9 by an anti-CRISPR DNA mimic. Sci. Adv. 3, (2017).

(6) Pawluk, A., Davidson, A. R. & Maxwell, K. L. Anti-CRISPR: discovery, mechanism and function. Nat. Rev. Microbiol. 16, 12 (2017).

(7) Pursey, E., Sünderhauf, D., Gaze, W. H., Westra, E. R. & van Houte, S. CRISPR-Cas antimicrobials: Challenges and future prospects. PLoS Pathog. 14, e1006990 (2018).

© 2018 Sciential

OPINION PIECE

OPEN ACCESS

What's Wrong With Me? Whats Wrong With You? The Issue of Over-Diagnosing ADHD in Children

Tyler Redublo
McMaster University, Honours Life Sciences, Class of 2019

ARTICLE INFORMATION

Recieved 17 October 2018
Accepted 11 November 2018

Available online:
26 November 2018

Editors:
Ishita Paliwal
Ashwinie Sivakumar

Layout Editor:
Youssef El-Sayes

ABSTRACT

Historically, the field of mental health has been shrouded in controversy and conflict. The problems associated with diagnosing mental illnesses are still prevalent today, and this process becomes even more complicated when assessing children, who have yet to develop mature social skills and cognitive functioning. Attention-deficit hyperactivity disorder (ADHD) is one of the mental health conditions that is diagnosed using the Diagnostic and Statistical Manual of Mental Disorders (DSM). Overwhelming support from the primary literature suggests that the current procedures of diagnosing ADHD - which begin during childhood - allow for a high degree of subjectivity, inconsistency, and uncertainty. For these reasons, the issue of over-diagnosing ADHD in children has become more significant, and more plausible than ever before. By outlining the key factors that contribute to this problem, certain modifications can be made to improve the ADHD diagnostic procedures for future applications. These changes can increase the accuracy of mental health assessments, thus minimizing the number of false positive diagnoses of ADHD in children worldwide.

Keywords: ADHD, children, mental, health, overdiagnosis, DSM

INTRODUCTION

Attention-deficit hyperactivity disorder (ADHD) is described as "a persistent pattern of inattention and/or hyperactivity-impulsivity that interferes with functioning or development"[1]. ADHD is the most common mental disorder among children and teenagers (5.9%-7.1%), and the prevalence rates of ADHD diagnoses of this cohort have been increasing steadily over time[2-4].

What mechanism or explanation can be used to justify these findings? Has there truly been an increase in the number of children with ADHD over time? Many researchers in the field of mental health do not believe so, and instead suggest the possibility that a large majority of these ADHD diagnoses are false positives. That is, many children diagnosed with ADHD do not actually suffer from the disorder. Perhaps these observations are not representative of increases in ADHD prevalence. Instead, they are a portrayal of the underlying problems with the current mental health assessment system of children.

© 2018 Sciential

FACTORS THAT CONTRIBUTE TO ADHD OVER-DIAGNOSIS IN CHILDREN

DSM Criteria and Diagnostic Procedures

The problem begins with the procedures in which healthcare professionals diagnose ADHD in children, and the definition of the condition itself. The Diagnostic and Statistical Manual of Mental Disorders (DSM) provides guidelines for diagnosis and criteria of each type of mental illness. Each condition is described by a standardized set of symptoms[5]. On the surface, the premise and design seem simple. However, this is far from the reality. The main problem with the DSM is the presence of bias, and inconsistency during assessment. For instance, one of the criteria for "hyperactivity and impulsivity" associated with ADHD is: "Often talks excessively"[1]. "Excessively" is a subjective term; one professional's interpretation of an excessive behaviour may not be the same for another. This is problematic considering that even the slightest difference in understanding of DSM criteria can result in a misdiagnosis of a mental disorder.

In addition, ADHD diagnosis begins at a young age, so clinicians receive information about a child's behaviour from individuals who regularly interact with and observe them (parents, teachers)[6]. This may further contribute to false positive ADHD diagnoses because the accounts from these informants may be uninformed and introduce a higher degree of subjectivity. Consider the following finding: studies show that teachers are more likely than parents to report disruptive behaviour in school-age children[7]. However, their reports might be biased based on the circumstances in which they make their observations. For instance, if a classroom is predominantly filled with introverted students, a teacher may be more likely to report an individual student as hyperactive, if they do not follow the norms established by their peers. Even if the student does not suffer from ADHD, their teacher's reports may play a deciding factor in the diagnostic process.

Comorbidity

Another factor associated with over-diagnosing ADHD in children is the issue of comorbidity; the simultaneous presence of two or more conditions. It was found that up to 75% of children diagnosed with ADHD also meet criteria for other mental health disorders[8]. This is because many symptoms of ADHD are also considered characteristic of other disorders (e.g. attention deficits are commonly seen with anxiety and depression)[9]. This means that the observed symptoms may not be indicators of ADHD, but something else entirely.

Unique Developmental Trajectories

The last, and arguably most sensitive, factor that contributes to over-diagnosis of ADHD in children is the fact that many of the DSM's defining traits of ADHD are merely a part of a child's normal cognitive development. Take the following criteria: "Often has difficulty waiting their turn", and "Often unable to play or engage in leisure activities quietly"[1]. These features are often demonstrated by many young children because they have not yet fully matured or adapted to society and its norms. Everybody learns at a different rate, and perhaps some of these individuals require more time to understand acceptable behaviours and mindsets. However, this is not necessarily evidence of a mental disorder, and these individuals do not deserve to suffer the social and psychological consequences of such a label.

LOOKING AHEAD

Mental health is unlike any other form of physiological equilibrium; it is often referred to as a spectrum because the line between healthy and unhealthy is blurred. Unfortunately, there are no current methods of correctly diagnosing ADHD with absolute certainty. Even the DSM itself has been significantly modified overtime because experts in the field are constantly changing the ways that mental health is defined and understood[10].

Moving forward, the responsibility of this issue rests primarily on the shoulders of the health care system, and the contributors of the DSM. One potential change is to include more specific criteria and guidelines of mental health disorders, thus establishing concrete signs and symptoms, so that less of the diagnostic process is left to interpretation. For instance, the aforementioned ADHD criteria "often talks excessively" could be improved by measuring tangible aspects of speech, such as the amount of time that the individual

© 2018 Sciential

speaks during conversations with a clinician. Another suggestion is to refrain from assessing a child's mental health until a certain age (unless presented with an extreme case), in order to promote their development and maturity.

The procedures used to diagnose ADHD in children today are filled with uncertainties and inconsistencies, but it is currently the best that science has to offer. All that is known for certain is that the mental health of children around the world must be treated with the utmost respect, and care. One misdiagnosis can lead a young child into thinking that there is something "wrong" with them. When in reality, it was their health care system and professionals that wronged them.

ACKNOWLEDGEMENTS

This work did not receive any funding, and there were no conflicts of interests involved in the writing process.

REFERENCES

(1) Diagnostic and statistical manual of mental disorders: DSM-5. Arlington, VA: American Psychiatric Association; 2013.

(2) Willcutt EG. The Prevalence of DSM-IV Attention-Deficit/Hyperactivity Disorder: A Meta-Analytic Review. Neurotherapeutics. 2012;9(3):490–9.

(3) Increasing prevalence of parent-reported attention-deficit/hyperactivity disorder among children-United States, 2003 and 2007. PsycEXTRA Dataset. 2010; 59(44): 1439-1443.

(4) Schwarz A, Cohen S. ADHD Seen in 11% of U.S. Children as Diagnoses Rise. The New York Times. 2013Mar31.

(5) Shapse SN. The diagnostic and statistical manual of mental disorders. 2008.

(6) DuPaul GJ, Power TJ, Anastopoulos AD. ADHD rating scale-IV: checklists, norms, and clinical interpretation. New York: Guilford Press; 1998.

(7) Scahill L, Schwab-Stone M. Epidemiology of Adhd in School-Age Children. Child and Adolescent Psychiatric Clinics of North America. 2000;9(3):541–55.

(8) Barkley RA. Attention-Deficit Hyperactivity Disorder, A Handbook for Diagnosis and Treatment. 3rd ed. New York: Guilford Press; 2005.

(9) Sciutto MJ, Eisenberg M. Evaluating the Evidence For and Against the Overdiagnosis of ADHD. Journal of Attention Disorders 2007;11(2):106-113.

(10) Carpenter WT, Tandon R. Psychotic disorders in DSM-5 Summary of changes. Asian Journal of Psychiatry. 2013;6(3):266–8.

(11) Rutter M, Cox A, Tupling C, Berger M, Yule W. Attainment and Adjustment in Two Geographical Areas: I--The Prevalence of Psychiatric Disorder. The British Journal of Psychiatry. 1975 Jan;126(6):493–509.

© 2018 Sciential

OPINION PIECE

OPEN ACCESS

Innovative Commercial and Private Genetic Testing Raises Privacy and Confidentiality Concerns

Syeda Masooma Zaidi
McMaster University, Honours Life Sciences, Class of 2020

ARTICLE INFORMATION

Recieved 13 October 2018
Accepted 11 November 2018

Available online:
26 November 2018

Editors:
Caitlyn Wilcken
Ashwinie Sivakumar

Layout Editor:
Youssef El-Sayes

ABSTRACT

Obtaining information about your genes can be as easy as swabbing your cheek for DNA testing. Companies that offer direct-to-consumer genetic testing with saliva have the authority to collect and share personal data as well as test results from their clients. However, patients want their personal information to be protected and although these companies ask for consent before sharing information with third-party sources, companies have the right to use client data to initiate research or improve their business. Genetic testing companies need to respect their clients and understand that they are paying for a service which deals with sensitive information that individuals may not want collected and stored.

Keywords: DNA testing, privacy, data, companies, genetics, ancestry

INTRODUCTION

Individuals are now turning towards companies such as 23andMe and Ancestry to learn more about their family history and health screening. 23andMe is also known for their direct-to-consumer genetic testing with saliva that can report whether the individual carries genetic indicators associated with health conditions such as celiac disease, late-onset Alzheimer's disease, and Huntington's disease[1]. Many individuals that use these companies are fascinated by their results. However, a majority of individuals are unaware that once they have sent their DNA, it is stored in the company's database. Thus, the company now owns the right to the data.

TERMS AND CONDITIONS

Commercial and private genetic testing companies should not be permitted to collect and share personal data or DNA test results of their clients. The privacy policy of Ancestry states, "Once our laboratory partner has produced your DNA Data, the DNA and saliva (also referred to as "biological samples") are stored so that they can be available for future testing"[2]. Furthermore, 23andMe states in their privacy statement that they "do use and share aggregate information with third parties in order to perform business development, initiate research, send you marketing emails and improve services"[3]. Although these statements are available for everyone to view, a CBC article regarding privacy implications argues that these policies are time-consuming and tedious to read. Therefore, individuals accept the terms

© 2018 Sciential

and conditions of the service without changing the default privacy settings[4].

ACCESS TO PERSONAL GENOMIC DATA

A CBC article from 2018 mentions that Canadians are protected by the Genetic Non-Discrimination Act. Therefore, companies are not permitted to share DNA testing or results with insurers or employers[4]. However, Canadians who purchase DNA kits in other countries are not protected by the Canadian Act and they must follow the rules of the country in which the kit is made[4]. Additionally, the National Human Genome Research Institute allows researchers to access genetic and individual research data upon request. Access includes databases of genotypes and phenotypes, the National Database for Autism Research, and The Cancer Genome Atlas[5]. In the United States, these databases are under "controlled access". Nonetheless, some data is readily accessible while keeping the identities anonymous.

A MacLean's article from 2017 states that customers are under no impression that they have consented to the collection and storage of their private information and results[6]. Moreover, at any time, this information can be sold to third parties such as pharmaceutical or insurance companies for targeting individuals with marketing schemes[6]. Another article published by CNN in 2016 makes an appalling disclosure regarding a team of American and Israeli scientists who could reconstruct the identity of individuals who provided anonymous genetic samples simply through readily available databases on the internet[7]. Researchers analyzed data of approximately 1.28 million people and discovered that they can be tracked through open genealogical databases[7]. Through extensive examination, researchers were able to determine the individual's age, place of residence and other factors[7]. An article from CBC also states that if people use their Facebook or Twitter accounts to sign into 23andMe or Ancestry, the companies will collect profile data, age range, friends and followers if their privacy settings are not restricted[4].

CONCLUSION

Genetic testing websites house an individual's most sensitive information that is often shared unknowingly. A majority of users click the "I Agree" option and are unaware of the hidden details in the terms of use. Although it is highly unlikely to ban these websites from using client data without consent, nor storing and sharing it, these companies must be transparent about how exactly they handle sensitive information. The terms and conditions are usually written by lawyers which may not provide transparency to costumers and thus, it should be written with the general audience in mind. Most often, users do not consider changing the default privacy settings or are unaware that they are able to. Companies should take responsibility to ensure that people can select the most private options available. Information collected by these companies is not only risky for the individual obtaining the test, but for their family, relatives, and children.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Dr. Katie Moisse and Learning Support Librarian Abeer Siddiqui for their continuous encouragement, support and guidance in writing this piece.

© 2018 Sciential

REFERENCES

(1) DNA genetic testing & analysis - 23andMe Canada [Internet]. How it Works - 23andMe. 23andMe; [cited 2018Sep28]. Available from: https://www.23andme.com/en-ca/

(2) Your privacy [Internet]. Ancestry. [cited 2018Sep28]. Available from: https://www.ancestry.ca/cs/-legal/privacystatement

(3) Privacy and data protection - 23andMe Canada [Internet]. How it Works - 23andMe. 23andMe; [cited 2018Sep28]. Available from: https:/ www.23andme.com/en-ca/privacy/

(4) Colbert Y. The privacy implications of DNA testing kits that can 'alter your life' | CBC News [Internet]. CBCnews. CBC/Radio Canada; 2018 [cited 2018Sep28]. Available from: https://www.cbc.ca/news/canada/nova-scotia/dna-testing-privacy-terms-and-conditions-1.4508488

(5) Privacy in genomics [Internet]. National Human Genome Research Institute (NHGRI). 2015 [cited 2018Sep28]. Available from: https://www.genome.gov-/27561246/privacy-in-genomics/

(6) Munro D. The dangers of mail-in genetic testing [Internet]. Macleans.ca. Macleans.ca; 2017 [cited 2018Sep28]. Available from: https://www.macleans.ca/society/the-dangers-of-mail-in-genetic-testing/

(7) Caplan A. Opinion: Get ready for the risks of genetic testing [Internet]. CNN. Cable News Network; 2016 [cited 2018Sep28]. Available from: https://www.cnn.com/2013/03/12/opinion/-caplan-dna-testing/index.html

© 2018 Sciential

SCIENTIAL TEAM

Editors in Chief

Aiman Shahid
Honours Life Sciences, 2020

Alisa Nykolayeva
Honours Life Sciences Co-Op, 2021

Creative Director

Youssef El-Sayes
Honours Life Sciences, 2021

Graphic Designers

Abhiti Kuhad
Honours Life Sciences, 2020

Athena Chang
Honours Life Sciences, 2019

Senior Advisors

Dr. Kimberley Dej
School of Interdisciplinary Sciences

Dr. Veronica Rodriguez Moncalvo
School of Interdisciplinary Sciences

Dr. Katie Moisse
School of Interdisciplinary Sciences

Abeer Siddiqui
Science Librarian

Senior Editors

Abdullah El-Sayes
Honours Life Sciences, 2020

Tyler Redublo
Honours Life Sciences, 2019

Graduate Editors

Jennifer Williams
Department of Kinesiology, PhD Candidate

Undergraduate Editors

Amama Khairzad
Honours Life Sciences, 2020

Ashwinie Sivakumar
Honours Life Sciences, 2019

Bianca Colarossi
Honours Life Sciences, 2019

Caitlin Reintjes
Honours Integrated Science, 2021

Caitlyn Wilcken
Medical Radiation Sciences, 2020

Gabrielle Gonsalves
Honours Life Sciences, 2021

Ishita Paliwal
Honours Integrated Science, 2021

Communications Coordinator

Hope Michael
Honours Life Sciences, 2019

COVER PAGE PHOTOGRAPH CREDIT:

YOUSSEF EL-SAYES

Image of a tree branch with a dried leaf attached during the dormant season

FUNDING YOUR IDEAS
sif
SCIENCE INITIATIVE FUND

ISSN 2562-1483
9 772562 148018