Lab Visit Report

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Location: Institute for Ageing and Heallth (IAH), University of Newcastle, UK**.**
Host: Prof. J. Attems and Dr. G. Saretzki.

Funding: ISN-CAEN category A

Overview of the Lab visit

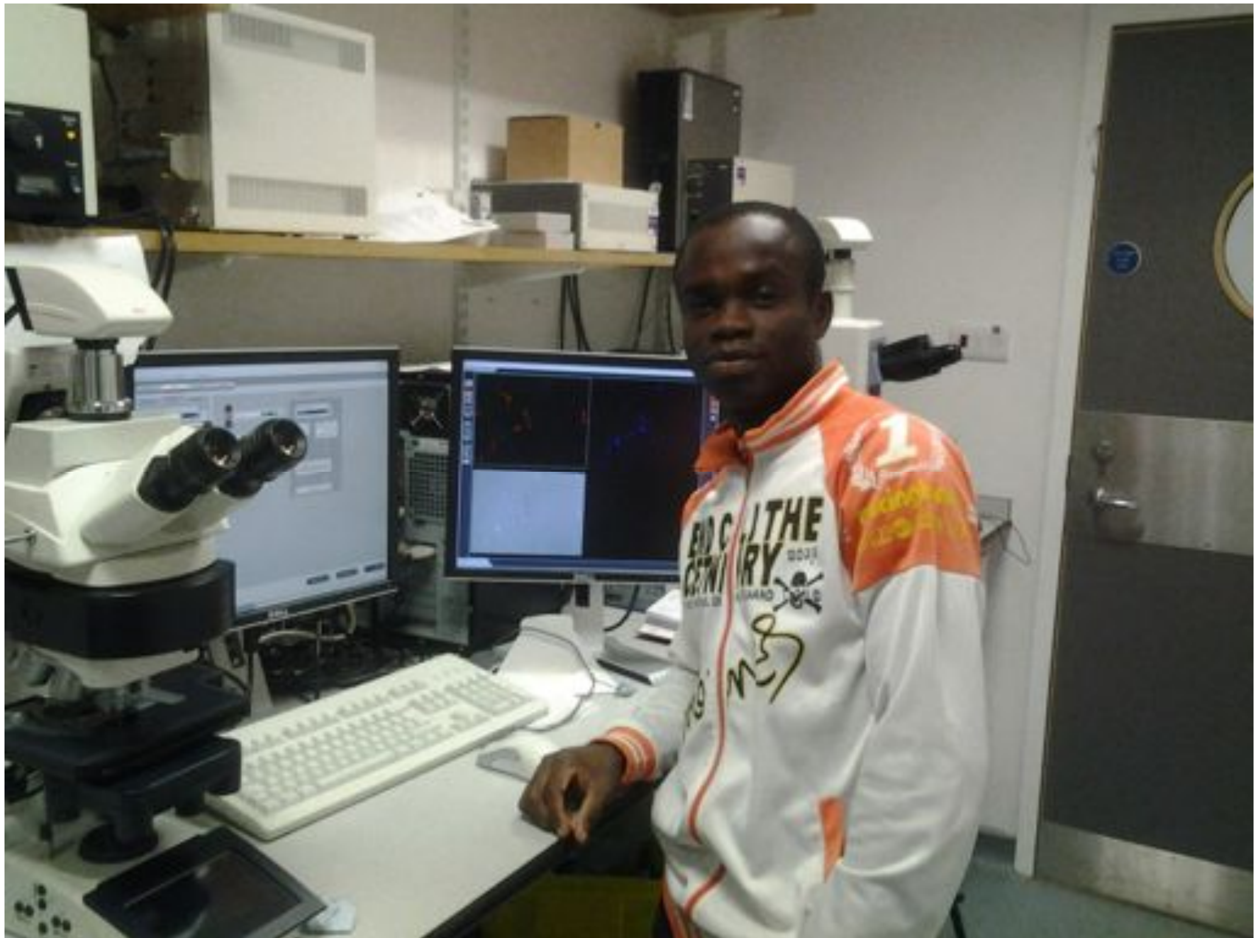
I arrived at Newcastle International Airport, UK on February 2nd, 2014 and was warmly welcomed by Dr. Saretzki. She took me to a nice apartment she reserved for my stay. On February 3rd, I officially registered at IAH as Occasional student. I was later introduced by Dr. Saretzki to some staff and her research team and subsequently given access cards to the IAH building and my office space.

Dr. Saretzki, Mr. Okojie and Prof. Attems.

I received hands-on training techniques on Immuno-fluorescence and fluorescence microscopy using brain tissue samples. I participated in one of the brain dissection sessions by Prof. Attems and subsequently sectioning using the freezing microtome to make free floating sections and paraffin embedded tissues before processing them through a series of Immunohistochemistry experimental runs using the neuronal markers AT8 to investigate neurofibrillary tangles. I also undertook procedures for Haematoxylin and Eosin, Nissl and Giemsa staining on brain tissues.

Viewing slides on fluorescence microscope

My first task was based on studying and planning the project and how to undertake it since I had limited knowledge on in-vitro studies; I deepened my knowledge and understanding from various documents related to our project as well as familiarize myself with some of the techniques that were practiced in the laboratory. This included how to seed cells, transfection, plate coating using laminin and collagen, immunofluorescence, and biochemical assays such as protein determinations by Bradford protein assay, and determination of telomerase activity (TRAP ASSAY).



Viewing slides on fluorescence microscope

The following objectives were set out to be determined in course of this study:
1. To differentiate SH-SY5Y cells seeded on laminin (10µg/ml) and collagen (10µg/ml) plated 12well plate using 10µM Retinoic acid (RA) and 10nM Tetradecanoylphorrbol-13-acetate (TPA)
2. To determine % proliferation of differentiated SH-SY5Y cells using a Ki67antibody
3. To determine the presence or absence of beta-III tubulin in differentiated SH-SY5Y cells using β-III tubulin primary antibody
4. To determine telomerase activity in differentiated SH-SY5Y cells by TRAP ASSAY
5. To double transfect SH-SY5Y cells with AT8 and shooter vectors (mitochondria and nuclear).

At the tissue/cell culture lab preparing for transfection of SH-SY5Y cells.

Our results showed that SH-SY5Y cells seeded on collagen coated plated were better differentiated than those on laminin coated plate. Based on this finding, all other experiments were subsequently conducted using only collagen coated plate. RA was far more potent in differentiating SH-SY5Y cells into mature neurons than TPA or a combination of RA+TPA. Ki67 staining showed no sign of proliferation in RA treated group after10 days unlike the other groups where there was 11.8%, 8.2%, and 15.1% proliferation for TPA, RA+TPA, and serum free medium only, respectively. β-III tubulin staining also supported this observation as all undifferentiated cells were all differentiated into mature neurons with the presence of β-III tubulin in the RA treated group unlike the other groups treated with TPA, RA+TPA, or just medium. Telomerase activity was also reduced in RA differentiated SH-SY5Y cells when compared to the other groups treated with TPA, RA+TPA, or just medium. Unfortunately, double transfection of undifferentiated cells with AT8 and shooter vectors did not work and due to time constraints we were unable to try the procedure again.

Everything went well and I left Newcastle on March 29th 2014.

At the tissue/cell culture lab checking for proliferation of SH-SY5Y cells.

Relevance to my Research

The work that I did at IAH with Prof. J. Attems and Dr. G. Saretzki; and their teams has significantly improved my knowledge and understanding of techniques in neurosciences, and will enable me not only to complete my PhD work, but will also be useful in the forth-coming years as a researcher in neuroscience.

Acknowledgments

I would like to thank CAEN and ISN for providing funding. I also want to appreciate Prof. J. Attems and Dr. G. Saretzki for their generosity as this Lab visit would not have been possible without their financial input. I would also like to thank my primary supervisor- Dr. G. Saretzki of this Lab visit for her framing, knowledge, and full support during this Lab visit, as well as her entire team (Alison, Dean, and Ihuoma) for their collaboration. I would also like to thank Glyn Nelson a postdoctoral researcher at IAH for his time in teaching me how to use the fluorescence microscope.

Dean (PhD Student), Alison (PostDoc), Kenneth (CAEN Awardee), and Gabriele (Host).