UMCS
UNIWERSYTET MARII CURIE-SKŁODOWSKIEJ W LUBLINIE
Instytut Nauk Biologicznych, Katedra Wirusologii i. Immunologii
Prof. dr hab. Agnieszka Szuster-Ciesielska |

Lublin, 28th November 2023

Review of the doctoral thesis of MSc Mamata Savanagouder entitled "Furthering the understanding of mechanism and function of HCMV IE1 chromatin association".

Ph.D. thesis by MSc Mamata Savanagouder was undertaken in the Department of Reproductive Immunology and Pathology Institute of Animal Reproduction and Food Research Polish Academy of Science in Olsztyn under the supervision of Dr. Hab. Magdalena Weidner-Glunde.

In the presented thesis, MSc Mamata Savanagouder aims to decipher the mechanism of association of HCMV IE1 protein with the nuclear proteins. The thesis topic is also related to understanding the reason for the unique localization of IE1 protein in glioblastoma cells.

The original thesis is presented in a form broadly accepted by the Polish research system, with a structural organization that contains the following features - title page, table of contents, abbreviations in English and Polish, abstract, introduction, aims of the work, materials, and methods, results, discussion, and references. The arrangement of the thesis is apparent and well-structured, which makes it easy to follow and comprehend the main concepts.

The introduction covers a broad range of highly relevant topics for the presented results. The introduction chapter includes comprehensive summaries of current knowledge in the field. It consists of the following parts: general characteristics of the *Orthoherpesviridae* (formerly *Herpesviridae*) family with emphasis on human cytomegalovirus (HCMV), features of the immediate early 1 HCMV protein and its chromatin association, and the role of HCMV and IE1 protein in glioblastoma.

In detail, the doctoral student, after elegant characteristics of HCMV epidemiology and life cycle, describes and compares the latency mechanism of two herpesviruses – HCMV and KSHV in the context of the association of the viral early proteins with host cell chromatin. The doctoral student also emphasizes that very little is known about the mechanism of tethering and genome maintenance during HCMV latency phase since its gene expression has been challenging to characterize. However, it has been suggested that IE1 protein (particularly its variants IE1x4 and

ul. Akademicka 19, 20-033 Lublin, www.umcs.lublin.pl
tel.: +48 81 537 59 43, fax: +48 81 537 59 01
E: agnieszka.szusterciesielska@mail.umcs.lublin.pl

probably IE19) plays a role in HCMV genome maintenance in the lytic and latency phases of the virus life cycle. In the following chapters, MSc Mamata Savanagouder details the role of HCMV in glioblastoma maintenance and progression. Although HCMV is not considered a classical oncogenic virus, some evidence supports the thesis about its oncomodulatory properties, which are mediated by a few gene products, i.e., IE1, pp71, glycoprotein B, and US28. Therefore, to study IE1x4 expression and its nuclear localization during latency, the PhD candidate chose T98G and KASUMI-3 cells, broadly used as latency models for HCMV.

More precisely, the objective of the work was first to determine the localization pattern of IE1x4 in the presence of HCMV terminal repeats region to check if it is the same phenomenon as in the case of KSHV maintenance protein (EBNA1 and LANA1). The results of the first part of the work open questions about IE1 forming chromosome-associated spots (CAS) localization in glioblastoma cells. Finally, the doctoral student asked what determines two different HCMV IE1 nucleus localization patterns – chromosome painting and CAS.

An adequate experimental strategy has been set up and described clearly to reach these objectives. The Materials and Methods section is written precisely and contains all the data necessary to reproduce the experiments in another laboratory.

The student uses a combination of cellular biology, cell cultures, molecular biology, immunological techniques, and genetics to answer the clearly defined research questions. The results section is logically organized, and it is easy to follow the scientific rationale that guided the experiments. In the discussion section, the author summarizes the main findings and interprets these findings in a bigger context.

The Result section is organized into two main parts. In Part 1 student using transfected Hela and T98G cells with IE1 and IE1x4 protein confirmed "painting" chromosomes with these proteins during cell mitosis and, for the first time, indicated localization of IE1 as paired spots on some of the chromosomes. Moreover, IE1 or IE1x4 did not change their localization pattern in the presence of HCMV terminal repeat (TR). The same observation was made for HeLa cells, permissive to HCMV MRC-5 cells, and T98G cells supporting lytic and latent HCMV replication. In contrast to IE1, IE1x4 protein was not expressed during HMCV latency phase in T98G and KASUMI-2 cells. In Part 2 doctoral student presented that IE1 forms spots (CAS) on mitotic chromosomes of IE1 transfected and HCMV infected cells (but not HeLa cells). An important observation is that the same IE1 CAS can be observed in some tumor cells, i.e., THP-1 and U87MG cells. The following important observation was that HCMV IE1 spots were localized in

ul. Akademicka 19, 20-033 Lublin, www.umcs.lublin.pl
tel.: +48 81 537 59 43, fax: +48 81 537 59 01
E: agnieszka.szusterciesielska@mail.umcs.lublin.pl

the peri-centromeric regions of chromosomes. The doctoral student showed that switching from IE1 chromosome painting pattern to IE1 CAS depends on the IE1 T98G cell transfection - IE1 expression level. An interesting conclusion was made that changes in the IE1 localization pattern could indicate HCMV life cycle transition from lytic to latent.

In the discussion section, the Author summarizes the main findings and interprets these findings in a bigger context. No particular chapter is devoted to this work's conclusions, but the doctoral student's most important results are at the end of the discussion.

The Author cited 272 individual literature references, showing she has a broad overview of the field. This is crucial to interpreting the findings and embedding the obtained results in the available knowledge.

I have a few general and specific questions or comments that I would like the student to clarify during the thesis defense.

1. At present, according to ICTV taxonomy, the proper name of herpesviruses family is the *Orthoherpesviridae* (https://ictv.global/report/chapter/orthoherpesviridae/orthoherpesviridae).
2. As an oncomodulatory virus, is HCMV preferentially present only in glioma or normal neuronal cells?
3. Is this a universal mechanism of chromatin binding by IE1, as demonstrated for glioma cells, or does it occur similarly in monocytes colonized by HCMV?
4. How was the HCMV latency established in KASUMI-3 cells?
5. Ph.D. student wrote: "I observed IE1 CAS in U87MG and THP-1 cells, but not in human placental fibroblasts (HPF), neural stem cells (NSC), and HT-29 cells." – how can the student explain this phenomenon in the context of cancer and normal cells?
6. What was the source of financing for the presented research?

Final conclusion

The thesis presents several lines of high-quality research where the scientific question was clearly defined and successfully resolved. The doctoral student investigated that IE1 forms CAS in cancer cells, which is mediated by a novel chromatin binding region in IE1 protein (the core domain), with the role of this protein in latency or its establishment. The thesis is written clearly, and the

ul. Akademicka 19, 20-033 Lublin, www.umcs.lublin.pl
tel.: +48 81 537 59 43, fax: +48 81 537 59 01
E: agnieszka.szusterciesielska@mail.umcs.lublin.pl

UNIWERSYTET MARII CURIE-SKŁODOWSKIEJ W LUBLINIE
Instytut Nauk Biologicznych, Katedra Wirusologii i. Immunologii

Prof. dr hab. Agnieszka Szuster-Ciesielska |

results are embedded in the available literature. Hence, the Author has shown high scientific capabilities and well-developed technical skills.

Considering all the above, I state that the PhD thesis presented by MSc Mamata Savanagouder meets the conditions specified in Art. 187 of the Act of July 20, 2018, on academic degrees and titles in science and arts (Journal of Laws 2018, item 1668 as amended). Therefore, I recommend that the Biological Sciences Discipline Council of the Hirszfeld Institute of Immunology and Experimental Therapy of Polish Academy of Sciences admit MSc Mamata Savanagouder for the subsequent stages of the doctoral proceedings.

Sincerely yours,

Szuster-Ciesielska

Prof. dr hab. Agnieszka Szuster-Ciesielska

ul. Akademicka 19, 20-033 Lublin, www.umcs.lublin.pl
tel.: +48 81 537 59 43, fax: +48 81 537 59 01
E: agnieszka.szusterciesielska@mail.umcs.lublin.pl